Prevalence of Candidal Carriage Rate in Denture Wearers and Evaluation of the effect of Whole Unstimulated Salivary Flow Rate and pH of Saliva on their Carriage Rates

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ABSTRACT
Objectives: To assess the prevalence of Candida carriage rate in denture wearers and to evaluate the effects of local factors and habits of tobacco and betelnut on Candida carriage rate.

Study design: 60 edentulous subjects and 60 dentulous subjects were evaluated. Standard methods for culture, salivary flow rates and pH determination were used. Statistically analysis was carried out using Students unpaired "t" test, 'Chi' square test, z-test, ANOVA F-test and Tukey multiple comparison test.

Results: Highest prevalence of Candida carriage rate was shown by group 1 (63.33%). The mean whole unstimulated salivary flow rate (p < 0.01) and pH (p < 0.05) were less in the subjects showing positive candida culture. Significant (p < 0.05) increase in prevalence of Candida carriage rate was found in subjects wearing prosthesis and having habits.

Conclusion: The prevalence of Candidal carriage rate in healthy individuals increased in the presence of prosthesis and decreased salivary flow rate and pH.

Keywords: Complete denture, Removable dentures, Candida, Whole unstimulated salivary flow rate, pH of saliva.

INTRODUCTION
Candida, a yeast like fungus is one of the members of oral microflora found in approximately 1/4th of the normal healthy individuals. Wearing a prosthesis/appliance predisposes a person to various alterations in the oral environment, altering the normal oral microbial equilibrium and also acts as a reservoir for yeast, predisposing the person to be a carrier or can infect the compromised mucosa causing denture stomatitis. Alterations in the oral environment can also be caused by local factors like salivary flow rate and salivary pH and various addictive habits of tobacco and betelnut. Studies have been done to prove this, most of which gave ambiguous results.

Because of various inconsistencies in the literature, the present study was planned to assess the prevalence of Candida carriage rate in saliva of complete denture (CD) wearers and removable partial denture (RPD) wearers, to evaluate the effect of local factors: Salivary flow rate (unstimulated) and salivary pH, and the effect of the habit of tobacco and betelnut on Candida carriage rate.

MATERIALS AND METHODS
The protocol of this study was approved by the institutional ethical committee of Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha, in accordance with the ethical guidelines prescribed by the central ethical committee on Human Research. With the exception of those having any of the predisposing conditions for candidiasis, in this cross-sectional study, 120 healthy subjects were evaluated without any age or sex bar. Subjects divided into 4 groups as follows: 1. Group 1: Included 30 edentulous subjects wearing removable CD. 2. Group 2: Included 30 age and sex matched edentulous subjects not wearing removable CD (control group). 3. Group 3: Included 30 dentulous subjects wearing RPD. 4. Group 4: Included 30 age and sex matched dentulous subjects not wearing RPD (control group).

Selection criteria for prosthesis wearers (CD/RPD) were those wearing good fitting and clean prosthesis for at least one year. In case of RPD wearers subjects wearing upper or upper and lower prosthesis were included. Selection criteria for dentate subjects (RPD wearers/not wearing RPD) were those having good oral hygiene, minimal/no gingival inflammation, good periodontal condition and no carious lesion. Subjects having habit of smoking/chewing tobacco/chewing betelnut more than once daily for more than one year were included. The medical history, clinical examination and necessary investigations were carried out. Oral hygiene index simplified (OHIS) was performed in dentate patients to assess oral hygiene status, and prosthesis cleanliness was assessed with erythrosine dye (Plaksee, Plaque disclosing
solution, ICPA Health Products LTD, Ankleshwar, India) to disclose denture plaque.5

Oral rinse for Candida culture, which is as sensitive as imprint culture technique,6 was collected in early morning or early afternoon.7 Sabouraud’s dextrose agar containing chloramphenicol and actidione was used for inoculation. This was then incubated for 48 hours at 37°C in the incubator.8 Candida, colonies were confirmed by gram staining.9 Colony count was done using digital colony counter (MC Dalal and Co, Chennai, India). A subject was considered to be a carrier of Candida, if one or more colonies were found on any plate. The total number of colonies obtained from saliva was converted to colony-forming units (CFU) per ml of oral rinse.9 A small portion of an isolated colony of the yeast was later tested for germ tube.9 Candida species were identified by carbohydrate assimilation test for which HiCandida Identification Kit (HIMEDIA, India) was used.2

Saliva collection was done between 9.00 am and 12.00 noon to avoid diurnal variations.10 Each subject was requested not to drink, eat, smoke or perform oral hygiene procedures two hours before and during entire study. Saliva collection was done by the spitting method.11 After collection, the whole unstimulated salivary flow rate (WUSFR) was measured and expressed in ml/1 minute pH of the saliva was immediately measured by the digital pH meter (El Instruments, Parwanoo, India) within 15 to 20 seconds.12

All variables from the study were statistically analyzed for the mean values, standard deviations, and “p” values. Evaluation of results and statistically analysis was carried out using Students unpaired “t” test, ‘Chi’ square test, Z-test, ANOVA F-test and Tukey Multiple comparison test. The data was analyzed using SPSS 10.0 package.

RESULTS

Total 42 subjects out of 120 showed positive candida culture and a prevalence rate of 35%. Individual groups had following prevalence rates: Group 1 = 63.33% (19/30), group 2 = 0% (0/30), group 3 = 43.33% (13/30) and group 4 = 33.33% (10/30). Highest mean count of the mean candidal colony forming units (CFU) per ml of oral rinse was found in group I (289.47 ± 124.25).

Inter group comparisons of prevalence of Candida show statistically significant (p < 0.001) difference in-between all groups except in-between group 3 and group 4 (Table 1).

Mean whole unstimulated salivary flow rate (WUSFR) and pH of all the groups (group 1 SFR = 0.35 ± 0.08 ml/min and pH = 6.69 ± 0.33, group 2 SFR = 0.39 ± 0.07 ml/min and pH = 6.90 ± 0.31, group 3 SFR = 0.36 ± 0.08 ml/min and pH = 6.75 ± 0.33 and group 4 SFR = 0.38 ± 0.08 ml/min and pH = 6.81 ± 0.33) did not show statistically significant variations. The mean WUSFR [Table 2 (p < 0.01)] and pH [Table 3 (p < 0.05)] in all the four groups was less in the subjects showing positive candida culture as compared to the subjects not showing positive candida culture.

Subjects with various habits showed the following candida prevalence rate: Smoking tobacco (ST) = 45.45% (15/33), smokeless tobacco (SLT) = 28.57% (8/28) and betelnut chewing

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of subjects</th>
<th>+ ve Sub.</th>
<th>Prevalence %</th>
<th>Z</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 vs</td>
<td>30</td>
<td>19</td>
<td>63.33%</td>
<td>7.24</td>
<td>S, p &lt; 0.001</td>
</tr>
<tr>
<td>Group 2</td>
<td>30</td>
<td>00</td>
<td>00.00%</td>
<td>1.46</td>
<td>NS, p &gt; 0.05</td>
</tr>
<tr>
<td>Group 3 vs</td>
<td>30</td>
<td>13</td>
<td>43.33%</td>
<td>2.74</td>
<td>S, p &lt; 0.05</td>
</tr>
<tr>
<td>Group 4</td>
<td>30</td>
<td>10</td>
<td>33.33%</td>
<td>3.87</td>
<td>S, p &lt; 0.001</td>
</tr>
<tr>
<td>Group 1 vs</td>
<td>30</td>
<td>19</td>
<td>63.33%</td>
<td>2.55</td>
<td>S, p &lt; 0.05</td>
</tr>
<tr>
<td>Group 3</td>
<td>30</td>
<td>13</td>
<td>43.33%</td>
<td>4.78</td>
<td>S, p &lt; 0.001</td>
</tr>
</tbody>
</table>

+ ve sub.: subjects showing positive Candida culture, Z: Z-test value, S: significant, NS: nonsignificant.

Table 2: Comparisons of WUSFR of subjects showing positive candidal culture and subjects not showing positive candidal culture

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1(30)</th>
<th>Group 2(30)</th>
<th>Group 3(30)</th>
<th>Group 4(30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ ve (19)</td>
<td>– ve (11)</td>
<td>+ ve (0)</td>
<td>– ve (30)</td>
<td>+ ve (13)</td>
</tr>
<tr>
<td>Mean WUSFR (ml/min)</td>
<td>0.32 ± 0.03</td>
<td>0.47 ± 0.05</td>
<td>0.39 ± 0.07</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>t-value</td>
<td>5.98</td>
<td>3.47</td>
<td>3.40</td>
<td>3.40</td>
</tr>
<tr>
<td>p-value</td>
<td>0.00, S, p &lt; 0.01</td>
<td>0.00, S, p &lt; 0.01</td>
<td>0.00, S, p &lt; 0.01</td>
<td>0.00, S, p &lt; 0.01</td>
</tr>
</tbody>
</table>

WUSFR: whole unstimulated salivary flow rate, + ve: subjects showing positive candidal culture, – ve: subjects not showing positive candidal culture S: significant.
Prevalence of Candidal Carriage Rate in Denture Wearers.....

(BNC) = 33.33% (10/30), whereas subjects without any habit showed a prevalence rate of 31.03% (9/29). Table 4 (p < 0.05) shows various comparisons between subjects having habits, wearing prosthesis and those having none. The mean WUSFR and pH of saliva of subjects having habits and subjects without any habits did not show statistically significant difference.

Out of 42 positive isolates of candida culture, 36 isolates were identified as C. albicans (85.71%), 4 as C. tropicalis (9.52%) and 2 as C. pseudotropicalis (4.76%) by the HiCandida™ Identification Kit.

DISCUSSION

The prevalence of candidal carriage rate in CD wearers was 63.66%, which is consistent with the findings of previous studies.13,14 Studies also show comparable findings of prevalence of candidal carriage in RPD wearers4 and non-RPD dentate subjects15 in the present study. The type of prosthesis, i.e. complete palatal coverage in the case of CD and partial coverage in some cases of RPD, might influence the yeast colonization.16 This factor might be responsible for the significant difference between groups I and III of the present study from the others.17 The prosthesis encourages the presence and growth of candida, thus increasing the carriage rate, 4,17 and has been shown by the increased prevalence of candidal carriage in RPD/CD wearers than that of non-RPD/CD wearers in the present study.

Whole unstimulated salivary flow rate (WUSFR) was evaluated in this study, as the biting efficiency of each group was different and hence the stimulated salivary flow rates by mechanical stimulation in each of the groups could not be comparable with those of the others as it has been shown that a number of teeth might affect the secretion rate.18 Also stimulated SFR by standard methods of chemical stimulation (by citric acid) could not be done, as this would interfere with the accuracy of pH measurement of saliva.

In the present study, the mean whole unstimulated salivary flow rates and mean pH of the 4 groups were well within the normal ranges, i.e. 0.30 to 0.50 ml/min and 6.5 to 7.5 respectively.19 The major buffer of saliva is bicarbonate, whose concentration is directly linked to the flow rate of the saliva.19 Similar positive correlation of WUSFR and pH of saliva is found in the present study and is consistent with the findings of Dreizen et al.19 Findings of the present study suggest that decreased WUSFR and pH leads to increased colonization of Candida and hence increase in the prevalence of candidal isolation, which is consistent with the previous studies.19,20 It has been reported that susceptibility to oral C. albicans infection can partially be predicted by the whole unstimulated salivary flow rate.21

Young et al 19 have stated that C. albicans is an active producer of acid from a number of carbohydrates and also grow abundantly in media like tomato juice agar, which is already acidified from other sources. Thus, it is clear from the above that there is a cause and effect relationship between pH and Candida. Olsen I et al.22 and Samaranayake LP23 have stated that the possible effects on Candida, of a diet rich in carbohydrates, apart from the above, are enhanced adhesion of Candida to epithelial cells and acrylic denture surfaces.

Table 3: Comparisons of pH of subjects showing positive candidal culture and subjects not showing positive candidal culture

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 (30)</th>
<th>Group 2 (30)</th>
<th>Group 3 (30)</th>
<th>Group 4 (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pH ± SD</td>
<td>+ ve (19) 6.47 ± 0.13</td>
<td>– ve (11) 7.06 ± 0.23</td>
<td>+ ve (0) 6.90 ± 0.35</td>
<td>– ve (30) 6.54 ± 0.08</td>
</tr>
<tr>
<td>t-value</td>
<td>9.07</td>
<td>–</td>
<td>3.57</td>
<td>3.97</td>
</tr>
<tr>
<td>p-value</td>
<td>0.00</td>
<td>S, p &gt; 0.05</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4: Comparison of candidal carriage prevalence between subjects having habit and wearing prosthesis, subjects having habit but not wearing prosthesis and subjects not having any habit and not wearing prosthesis

<table>
<thead>
<tr>
<th>Variable</th>
<th>ST + ve (n)%</th>
<th>Inference</th>
<th>SLT + ve (n)%</th>
<th>Inference</th>
<th>BNC + ve (n)%</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB with prosthesis vs HB without prosthesis</td>
<td>11 (17) 64.70%</td>
<td>Z = 14.3, S, p &lt; 0.05</td>
<td>6 (13) 46.15%</td>
<td>Z = 2.02, S, p &lt; 0.05</td>
<td>8 (16) 50%</td>
<td>Z = 2.31, S, p &lt; 0.05</td>
</tr>
<tr>
<td>HB without prosthesis vs HB without prosthesis</td>
<td>4(16) 25%</td>
<td>2 (15) 13.33%</td>
<td>2 (15) 13.33%</td>
<td>NA</td>
<td>2 (14) 14.28%</td>
<td>Z = 0.07 NS, p &gt; 0.05</td>
</tr>
<tr>
<td>HB without prosthesis vs NHB and without prosthesis</td>
<td>4 (16) 25%</td>
<td>Z = 0.86 NS, p &gt; 0.05</td>
<td>2 (15) 13.33%</td>
<td>NA</td>
<td>2 (15) 13.33%</td>
<td>Z = 0.07 NS, p &gt; 0.05</td>
</tr>
</tbody>
</table>

Regarding correlation of prevalence of smokers with that of nonsmokers, conflicting results have been given. Few studies have given nonsignificant difference,7,14,24 while others reported a higher prevalence of 70% in smokers as compared to nonsmokers.20 Oliver DE and Shillitoe EJ7 have stated that cigarette smoke provides nutrition for C.albicans. The related species of C.tropicalis, C.guilliermondi and C. pulcherrima have inducible enzyme systems, which allow them to replicate using polycyclic aromatic hydrocarbons as their source of carbon and energy. Cigarette smoke is composed to a large extent of similar hydrocarbons.7 This relationship between cigarette smoke and Candida is particularly important as the enzyme system in question can increase the carcinogenic activity of hydrocarbons. Prevalence of CCR in smokers (45.45%) in the present study correlates with the findings of Bastian RJ and Reade PC,24 although increased prevalence of CCR in smokers (45.45%) in the present study correlates with smoking and tobacco chewing/betelnut chewing with that of betelnut. In the present study, no statistically significant difference in the pH of betelnut chewers and non-chewers was reported.20 They stated that the change in pH might be due to other components like lime, tobacco, etc. used along with betelnut. In the present study, no statistically significant difference was found in the WUSFR and pH of subjects having habit of smoking/tobacco chewing/betelnut chewing with that of subjects not having any habit, which is consistent with the findings of other studies.10

C. albicans are highly resistant to lysozyme, and therefore, in individuals with reduced salivary flow, there is a rise in the prevalence of more lysozyme-resistant Candida species.21 This very well correlates with the present study findings.

Although yeasts constitute a minor part of the total microflora, the role of yeast antigens and toxins of denture plaque are significant factors, which may lead to initiation and maintenance of denture-induced stomatitis. Thus, the primary therapeutic measure is to reduce plaque formation on the denture by mechanical or chemical plaque control. Also efforts to increase the salivary flow rate and institution of alkaline substances have therapeutic potential in the treatment or prophylaxis of oral candidosis in addition to antifungal therapy.

REFERENCES