Occurrence of *Streptococcus* and *Candida* Species and Salivary pH in Patients Wearing Complete Denture

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**Background:** Saliva has many essential functions in the maintenance of oral health. Salivary components interact with the oral microbial communities and hence control the composition of the oral Microflora.

**Objectives:** To examine the carriage of *Streptococcus* and *Candida* species in the oral cavity of complete denture wearers and non-denture wearers. To assess the pH of saliva.

**Materials and Methods:** 30 completely edentulous patients (15 complete denture wearers, 15 non-denture wearers) aged between 45-80 years were included in the study. Saliva samples were collected and was inoculated into Mutanssanguis agar and Sabouraud’s dextrose agar and incubated at 37°C for 24 hours. The number of colony forming units (CFU) were identified and counted. The pH of saliva was examined using pH strips (Qualigens).

**Results:** No significant differences were detected in the proportion of *Streptococcus* species and the incidence rate of *Candida* species in patients with (20%) and without (6.7%) complete denture were comparable. The mean value of saliva pH was 6.93 and 7.1 in complete denture wearers and non-denture wearers respectively.

**Conclusion:** The most dominant microorganism was *Candida* species. No significant differences observed in the prevalence of *Streptococcus* species in the oral cavity of patients with and without complete denture. There was a decrease in saliva pH of complete denture wearers.

**Keywords:** *Streptococcus, Candida, Complete denture, Saliva.*
INTRODUCTION

The oral cavity is a moist environment which is kept at a relatively constant temperature (34 to 36°C) and a pH close to neutrality in most areas and thus supports the growth of a wide variety of microorganisms. The microbial flora of the oral cavity is rich and extremely diverse. This diversity is due to the fact that the mouth is composed of a variety of bacteria, viruses and fungi supplied with a wide range of different nutrients. In addition, this environment is affected by the pH and oxygen levels of saliva, salivary antimicrobial agents, microbial interactions and salivary flow. Dentures also provide a protected habitat, especially beneath the fitting surface, which results in colonization and growth by a range of bacteria and yeasts. The tissue surface of a maxillary denture in healthy patients was shown to be highly colonized by a variety of bacteria and yeasts. Many studies provide evidence that a tissue surface of a maxillary complete denture is particularly susceptible to microbial colonization. This may be due to the barrier created by the denture that prevents salivary cleansing that enhances adherence of microorganisms. Adhesion of microorganisms to oral mucosa and fitting surface of the denture is a prerequisite for the formulation of denture plaque that may lead to the development of denture stomatitis. Microbial colonization on the acrylic surface is always preceded by the adsorption of an acquired pellicle. The composition of the mature denture plaque is dependent on the primary binding between pioneer bacteria and the acquired pellicle. It was found that bacteria predominate in early plaque formation. It was also proved that microbes such as Candida spp., Streptococcus spp. and Staphylococcus spp. have a major role in the development of denture stomatitis.

Other major influences on the oral microflora are nutrients, pH and the integrity of the host defences. In this respect, saliva plays a major role in determining whether the resident oral microflora has a beneficial or destructive relationship with the host. The buffering action of saliva ensures that the pH of the mouth is maintained around neutrality, which favours the growth of the majority of bacteria associated with oral health. In the oral cavity, the pH is maintained near neutrality (6.7 to 7.3) by saliva. The pH or hydrogen ion concentration of an environment affects microorganisms and microbial enzymes directly and also influences the dissolution of many molecules that indirectly influence microorganisms.

The objectives of the study were to examine the carriage of Streptococcus spp. and Candida spp. in the oral cavity of complete denture wearers and non-denture wearers. pH of saliva was also assessed.

MATERIALS AND METHODS

The participants for the study were selected from the Out Patient Department of Department of Prosthodontics, A.B. Shetty Memorial Institute of Dental Sciences. Thirty completely edentulous patients were selected out of those 15 were complete denture wearers while 15 non denture wearers. Subjects aged between 40 – 70 years, having no active infection in the mouth, presently wearing complete acrylic resin dentures for more than one year, with healthy oral mucosa and a good general health were included in this study. Subjects with a history of major salivary glands pathology or extirpation, serious illness, medically compromised, reduced intelligence or mentally challenged were excluded.

The study was approved by the Bioethics committee of A.B. Shetty Memorial Institute of Dental Sciences. All the subjects gave their informed consent for participation in the study. The patients were informed not to drink and eat for two hours before the collection of the saliva sample.

Collection of saliva samples

Unstimulated whole saliva was collected from all subjects by direct expectoration into a sterile container during a 5 minute period. Saliva samples were processed within 2-4 hrs of collection (Figure 1).

Microbiological procedures

Saliva samples were inoculated onto Mutanssanguis agar plates and Sabouraud’s dextrose agar plates for the isolation of Streptococcus species and Candida species respectively. Mutanssanguis agar plates were incubated in an atmosphere of 10% CO₂ at 37 degree Celsius for 24 hours (Figure 2). The organisms were then identified by their colony...
numbers of colony forming units (CFU) were determined (Figure 5).

Examination of pH of saliva

pH of saliva was examined using pH strips (Thermo Fisher Scientific India Pvt. Ltd., Qualigens) (Figure 6).

Statistical analysis

Incidence rate of Streptococcus and Candida species in complete denture wearers and non-denture wearing patients were presented as frequency and percentage. Student t-test was used to compare the mean values of pH in complete denture wearers and non-denture wearing patients. Probability value of less than 0.05 was considered significant.

RESULTS

Table 1 shows the incidence rate of Streptococcus and Candida species in complete denture wearers and non-denture wearing patients. It was observed that no significant differences were detected in the proportion of Streptococcus species in the saliva between that of complete denture wearers and non-denture wearers. Candida colonies were isolated in 3/15 (20%) patients wearing complete denture and 1/15 (6.7%) patients without complete denture.

Table 2 shows the comparison of mean values of pH in complete denture wearers and non-denture wearing patients. It was observed that there is a decrease in pH of the saliva of complete denture wearers when compared with that of non-denture wearing patients. But this difference was not statistically significant (P = 0.087).

DISCUSSION

The composition of the oral micro flora varies at different surfaces within the mouth because of the respective physical and biological morphology (Figure 3). Gram staining and standard biochemical tests. Sabouraud’s dextrose agar plates were incubated aerobically at 37 degree Celsius for 24 hours (Figure 4). The Candida species were identified by colony morphology and Gram staining. The total

Table 1 Incidence rate of Streptococcus and Candida species in Complete denture wearers and Non-denture wearers

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td></td>
<td>Denture (n=15)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Candida</td>
<td>3 (20)</td>
</tr>
</tbody>
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properties of each site. A number of relevant factors influence the micro flora of the oral cavity such as age, changes in dietary habits (eg: due to impaired saliva flow), medication, disease and denture wearing\(^\text{11}\).

A significant proportion of the adult populations wear complete or partial dentures. The factors associated with tooth loss—dental caries, loss of periodontal support, a history of dentoalveolar trauma, a history of dental care—are additive over time, thus denture wearing is more associated with an older population\(^\text{10}\). Complete denture prosthesis function within the oral cavity in an environment which contains various types of pathogenic and non-pathogenic organisms. However well and efficient the complete denture prosthesis may be fabricated, if it accumulates plaque and bacterial growth in the mouth during function and if this is not eliminated, the health of the alveolar and surrounding mucosa as well as the mucosa of the tongue will be affected. Further, these complete denture prosthesis with colonies of microorganisms depositing on them may be source of infection to the oropharynx and alimentary tract\(^\text{12,13}\).

It is now well established that there is a specific ecological niche beneath the fitting denture surface and the mucosa and saliva as a barrier. It contains a large quantity of proteins and glycoproteins which are readily adhered to the denture fitting surface. The process of protein adsorption and bacterial adhesion seem to be more important than mechanical retention for the colonization by microorganisms of the fitting denture surface and the underlying mucosa\(^\text{2,5,14}\). It was found that Streptococcus spp. predominate in early denture plaque formation. The association between streptococcal activity and denture stomatitis was previously established\(^\text{7-10}\).

It was found that bacteria colonization and subsequent biofilm formation on the denture fitting surface may lead to accumulation of denture plaque and stomatitis\(^\text{15}\). Adherence of microorganisms to the acrylic surface may be described as initial non specific interactions (electrostatic interaction, van der Waals forces) and specific interactions (receptor—ligand binding)\(^\text{6}\). Surface roughness and surface free energy acrylic resin as well as surface characteristics of adhering bacteria also contribute to the process of microbial colonization and denture plaque maturation\(^\text{16}\).

Sardin et al\(^\text{2,6}\) evaluated Streptococcal adherence (Streptococcus mitis, Steptococcus
parasanguinis, Streptococcus oralis and Streptococcus sanguis) to currently used prosthetic materials, after a salivary coating. They observed a relationship between the number of adherent bacteria and the physicochemical surface properties of bacteria. It has been demonstrated that in vitro adherence of Candida albicans to an acrylic surface increases when it is covered by a layer of Streptococcus sanguis or Streptococcus salivarius. Moreover, it was shown that strains of Streptococcus sanguis, Salivarius, mutans and Mitis coagglutinate with Candida albicans, may also affect oral mucosal adherence and colonization by yeasts. In the present study, there was no significant difference in the occurrence rate of Streptococcus species in patients with and without complete denture. But the denture wearing per se was shown to lead to a higher prevalence of Candida species in the oral cavity.

The second objective of the study was to analyze the salivary pH in complete denture wearers. This analysis showed the decrease in pH due to the presence of complete denture. The pH is an important parameter in oral microbial ecology. The saliva contributes to maintenance of the pH by two mechanisms. First, the flow of saliva eliminates carbohydrates that could be metabolized by bacteria and removes acids produced by bacteria. Second, acidity from drinks and foods, as well as from bacterial activity, is neutralized by the buffering activity of Saliva (molecular bio). Bicarbonate is the major salivary buffering system of saliva, but peptides, proteins, and phosphates are also involved. Bicarbonate concentration is directly related to flow rate of saliva. pH leads to increase colonization of Candida and hence increase in the prevalence of Candida isolation. The mean value of pH was lower in complete denture wearers (6.9) than in nondenture wearers (7.1). The mean value of pH 6.9 is regarded as suitable for growing Streptococcus species. In support of this, changes of salivary pH were found after the insertion of complete denture and dentures supported by implants. In conclusion, while various factors may influence the ecology of the mouth in the elderly, our data suggest that the oral micro flora is influenced by wearing of complete dentures.

**CONCLUSION**

In the present study following conclusions may be drawn, the proportions of Streptococcus species in saliva showed no change on wearing of complete dentures. The percentage of Candida colonies was greater in complete denture wearers. There is a decrease in the salivary pH in complete denture wearers and it approaches neutral in non-denture wearers.

**CONFLICTS OF INTEREST**

None declared

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**REFERENCES**


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