THE EFFECT OF SALIVATION STATUS AND DENTURE WEARING ON ORAL CANDIDAL ACTIVITY OF THE PATIENT

MOHAMMAD ALI CHUGHTAI, BDS, FCPS, FFDP (PROSTHODONTICS)
AMJID NASEER, BDS, MSC
MUHAMMAD SARTAJ KHAN, BDS, FCPS

ABSTRACT

The objective of this study was to see the effect of salivation status on the oral candidal activity and also to see the effect of denture wearing / non-wearing on the salivation status of the patient.

In the methodology a cross sectional, comparative study during March-September 2007 was conducted at Khyber College of Dentistry Peshawar and Zoology Department, University of Peshawar (Pakistan). Using a structured proforma, data were collected from 100 subjects (50 Denture wearers and 50 non denture wearers). Stimulated salivary samples were collected and measured after vortex for 5 minutes from each subject. One ml of standard dilution saliva was mixed with 4ml sterile liquid candida –selective medium. The extent of candida activity was then determined, biochemically, through change in pH of the medium 24hrs after inoculation and incubation at 37 °C.

The results showed that 65 % patients had normal salivation rate, as compared to 35% belonging to the hyposalivatory group. Among the denture wearers 52.3% were having normal salivation status and 45.7% having hyposalivation status. While among non-denture wearers 47.7% participants were having normal and 54.3% with hyposalivation status. Mean pH change in the normal salivation group was 1.2 ±1.0 as compared to 1.5 ±1.0 in the hyposalivation group. Association between the variable salivation level and candidal activities (average change in pH of the medium) was insignificant with p-value 0.73. Also association found between the case type (denture / non denture wearers) and salivation status of the patients was insignificant, having p-value 0.52.

It was concluded that within the limitations of this study, the salivation status of the patients had an insignificant association with the oral candidal activity.

Key Words: Candida Albicans, Biochemical activity, Salivation Status.

INTRODUCTION

As mouth is an important pathway for many microorganisms entering the human body, so saliva also has an essential role in the first line defense against them in general. In addition to the barrier effect of saliva its mucous content, containing different proteins like lactoferrin, enzymes like Lysozyme and antibodies like IgA, has the capacity to clump or agglutinate microorganisms and also by the cleansing action of saliva. Candida albicans, dimorphic oral commensal yeast, is considered the most common cause of opportunistic fungal infection in oral cavity. A characteristic of candidal activity is its acid proteinase secretion which has several implications including a value in evaluating the virulence of...
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candida albicans. The proteinase secretion also determines the candida adhering and colonizing ability to host tissue. It is also involved in tissue invasion and destruction.

Lockhart et al\(^1\) demonstrated that the frequency and intensity of candidal colonization increased as a function of age, independent of denture use. This was attributed to a decrease in the immune status or salivary flow rate as expected in the elderly. Xerostomia is more prevalent in the elderly population, primarily due to increased use of drugs because of their susceptibility to disease\(^3\). Clinical experience suggests that complete denture patients with xerostomia have more intense sore spots than patients with normal salivary flow\(^3\). Decrease in salivary flow rate is either because of senile atrophy of the salivary gland or due to use of multiple medications having xerostomic side effects, or other conditions causing xerostomia such as, radiotherapy, autoimmune diseases (Sjogren’s syndrome) causing salivary gland hypo function (SGHF), which leads to the pre-disposition for fungal infections. Saliva has a major ecologic influence on the microorganisms that attempt to colonize oral tissues\(^1\).

In another study Kreher found up to 60% decrease in salivary flow rate of patients taking two or more hyposalivatory drugs\(^4\). It has been reported that symptoms of dry mouth are significantly associated with the flow of stimulated, unstimulated saliva and both stimulated and unstimulated saliva in healthy adult and elderly individuals\(^5\). Whereas elderly people with normal salivary flow rate had no special risks for the development of oral diseases. Actually lack of salivary flow affects defense mechanism, making oral cavity susceptible to microbial growth. As a result swallowing frequency decreases, which is the most effective way of reducing the number of microbes from oral cavity by the flushing action of saliva\(^6\). Samaranayke et al also claimed that saliva mechanically cleans the oral cavity and reduces the adhesion of candida albicans to acrylic\(^7\). That’s why candidal infection is the most frequent findings among people with hyposalivation\(^8\). This may appear in the form of acute or chronic pseudo membranous candidiasis or atrophic lesions on the oropharyngeal surfaces\(^8\).

Saliva or oral fluid as it preferably should be called is a mixture of pure glandular secretions, gingival crevicular fluid, oral microbes and epithelial cells\(^10\). Saliva performs some important functions in the oral cavity. Saliva acts as a lubricant in the oral cavity and provides a buffer system that protects the oral cavity. Change in pH of saliva affects oral flora. The pH of saliva from resting salivary glands is slightly less than 7.0, but during active secretion, it approaches 8.0\(^11\). According to the study of Mac-Farlane and Samaranayake\(^12\), low pH of saliva can cause a reduction in the number of Veillonella, commensal Neisseria and Micrococcus species. While the growth of candida species, streptococcus mutans and lactobacillus was favoured. It has been suggested that the colonization of candida enhances in the acidic environment\(^13\).

Saliva also maintains the tooth integrity. Saliva is saturated with calcium and phosphate ions. Interaction with saliva results in post eruption maturation through diffusion of such ions as calcium, phosphorus and chloride into the surface apatite crystals. Such maturation increases surface hardness, decreases permeability and heightens the resistance of enamel to caries\(^14\). Unconditioned reflexes can also stimulate salivary flow\(^15\). In addition mastication also affects the salivary flow rate via periodontal mechanoreceptors and mechanical stimulation of the oral mucosa and tongue\(^3\). According to Hibino et al\(^16\) saliva can play a role in modulating oral candidal populations in oral cavity.

The aim is to compare the effect of salivation rate on the extent of candidal pathogenic activity in a group of subjects wearing maxillary palatal coverage acrylic dentures, with that of a group of individuals, who have never used such dentures.

A well designed research methodology was followed by collecting the data and saliva samples from those not wearing dentures as well as those wearing maxillary palatal coverage acrylic resin dentures. In the light of available information all the data have been presented and discussed.

**OBJECTIVE**

The objectives of this study were:

— To see the effect of salivation status and denture wearing/ non-wearing on the oral candidal activity of the patient.
OPERATIONAL DEFINITION

Normal Salivation and Hypo salivation

Salivation status was considered normal, if the stimulated salivary flow rate was equal or more than 0.5ml/min, whereas less than 0.5ml/min salivation will be considered as hypo- salivation.

HYPOTHESIS

Pathogenic candidal activity will be more pronounced in persons having hyposalivation with or without denture wearing.

METHODOLOGY

A cross sectional comparative study at Department of Prosthodontics, Khyber College of Dentistry Peshawar and Parasitology Unit at the Department of Zoology, University of Peshawar was designed and conducted during the period from March to September 2007. The participants were 100 out of those 50 were non denture wearers (NDW) while 50 subjects were wearing palatal coverage acrylic maxillary dentures (DW). Criteria for inclusion and exclusion of subjects in the study are given in Table 1.

The study was approved by the Institutional Research Evaluation Unit. After informed consent and willingness for participation, data collection from each participant were obtained using pre-structured data collection sheets.

For the purpose of this study, candida activity reflecting the presence of candida in its virulent/active form was determined from the extent of acidic activity as assessed from the decrease in the pH of medium that was inoculated with a standardized portion of subject saliva containing the candida.

Stimulated salivary samples from each of the participants were collected in a standardized manner. To start saliva collection subjects were first asked to swallow all the saliva in the mouth, then to chew a piece of modeling wax (1cm²) for five minutes and spit saliva into a sterile tube. All Saliva specimens were collected between 9 am and 12 pm. The collected saliva samples were quickly transported to the nearly located microbiology laboratory of the Zoology Department, University of Peshawar.

In the laboratory, the collected salivary sample was first vortexed for at least 30 seconds at 3000 revolutions per minute (rpm) in centrifuge machine (Hettich Universal-Germany) in order to remove entrapped air bubbles for assessing the volume of saliva collected. The tube was then transferred to the Laminar flow. Under aseptic conditions in the laminar flow, the centrifuged tube containing the salivary specimens was opened. With the help of sterile pipette saliva sample was measured first and the salivary flow rate thus determined was recorded on the Proforma. The volume of collected saliva was used for categorizing the subject salivary functional level.

Saboraud dextrose medium was prepared in bulk under aseptic condition with in laminar flow (Fitted with Hepa filter-0.2 micrometer, France and supplied by Scientific Technical Corp, Lahore-Pakistan). Initially 10 g of peptone and 20 g of glucose was added in 1000ml of distilled water and then heated till boiling to dissolve the medium completely. Then this medium was autoclaved at 121°C for 15 min. It was then allowed to bench cool. On bench cooling this medium to room temperature, 0.1g Gentamycin (Reckitt Benckiser Ltd) and 0.25g Chloramphenicol (Parke Davis Co; Ltd; Karachi, Pak) was added to the solution to make it selective for candida. The initial pH of the prepared medium was 6.9, with a shelf life of one month after preparation. During this period its pH remained constant. Sterile closed test tubes filled with 4ml of medium were stored in the refrigerator at 4°C.
Subsequently one ml of collected saliva of each subject was then diluted with 5 ml of distilled water in a sterile beaker. It was homogenously mixed in a standardized manner. Using a Petridish with three compartments, 4ml of liquid selective medium was taken from the stored bulk of liquid selective medium and was poured in a compartment of the petridish. Similarly in the remaining two compartments of the petri dish 4ml of the medium were added. The pH of the liquid medium was first confirmed for the stability of medium in each section of the Petridish separately, with the help of digital pH meter (Jenco Co-USA). After this, one ml of the diluted saliva was added to each of the three sections of the petridish. This facilitated 3 samples for each subject. The pH of the inoculated medium was then determined using the pH meter in each of the three sections of the petridish. The average value of pH was thus calculated. After this, inoculated medium was incubated (Gallenkamp Company-U.K) for 24 hours at 37°C. The pH of the three sections of the petridish was again measured and average value obtained. The change in pH of the medium was then determined by subtracting the average pH value of the incubated medium from the average value recorded immediately after inoculation with the subject salivary sample. The pH meter (Jenco Co-USA) was calibrated on each session before starting each session and on its conclusion. All the readings were checked and recorded in the data collection sheet.

Using the statistical package for social sciences (SPSS) version 10.0, the data were statistically analyzed. Descriptive statistics in the form of mean, standard deviation and minimum, maximum (age, salivation status and pH change in inoculated medium) were done. While chi square test was applied to get p value related to salivation status, case type (denture wearer/ non-denture wearer) and candidal activity.

RESULTS

The age range for the 100 participants was 40-60 years with the mean age 50.7 ± 6.1(SD). Of these 44% were in the age range 40-50 years with mean age 44.5 ± 2.9 years, while 56% were in the age group 51-60 years, having mean age 55.5 ± 2.6 years.

Among the denture wearers 48% were using maxillary dentures only during the daytime, while 52% were using dentures day and night.

As many as 65% patients had normal salivation rate, as compared to 35% belonging to the hyposalivatory group. Among the denture wearers 52.3% were having normal salivation status and 45.7% having hyposalivation status. While among non-denture wearers 47.7% participants were having normal and 54.3% with hyposalivation status. Mean age of normal salivation group was 50.9 ± 6.2 and 50.2 ± 5.8 among hyposalivary group subjects. Mean pH change in the normal saliva-

<table>
<thead>
<tr>
<th>Salivation status</th>
<th>No</th>
<th>DW* No. (%)</th>
<th>NDW* No. (%)</th>
<th>x²value</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSG*</td>
<td>65</td>
<td>34(52.3)</td>
<td>31(47.7)</td>
<td>0.39</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>HSG*</td>
<td>35</td>
<td>16(45.7)</td>
<td>19(54.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*NSG: Normal salivary group  
* DW: Denture Wearer  
*NDW: Non-Denture Wearer

<table>
<thead>
<tr>
<th>Salivation status</th>
<th>No</th>
<th>x²value</th>
<th>df</th>
<th>pH change* ( X \pm S.D ) (Min-Max)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSG*</td>
<td>65</td>
<td>1.25</td>
<td>3</td>
<td>1.2 ±1.01 (.0-3.3)</td>
<td>0.73</td>
</tr>
<tr>
<td>HSG*</td>
<td>35</td>
<td></td>
<td></td>
<td>1.5 ±1.02 (0.1-3.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
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*NSG: Normal salivary group  
*pH change: Average change in pH of the medium  
*HSG: Hypo salivary group

<table>
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<tr>
<th>TABLE 2: ASSOCIATION BETWEEN SALIVATION STATUS AND CASE TYPE (DENTURE/ NON-DENTURE WEARERS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivation status</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>NSG*</td>
</tr>
<tr>
<td>HSG*</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*NSG: Normal salivary group  
* DW: Denture Wearer  
*NDW: Non-Denture Wearer

<table>
<thead>
<tr>
<th>TABLE 3: ASSOCIATION BETWEEN THE SALIVATION STATUS AND CANDIDAL ACTIVITY (AVERAGE CHANGE IN PH OF THE MEDIUM)</th>
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</thead>
<tbody>
<tr>
<td>Salivation status</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>NSG*</td>
</tr>
<tr>
<td>HSG*</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*NSG: Normal salivary group  
*pH change: Average change in pH of the medium  
*HSG: Hypo salivary group
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tion group was 1.2 ±1.0 as compared to 1.5 ±1.0 in the hyposalivation group. Association between the variable salivation level and candidal activities (average change in pH of the medium) was insignificant with p-value 0.73 (Table 3). Also association found between the case type (denture / non denture wearers) and salivation status of the patients was insignificant, having p-value 0.52 (Table 2).

DISCUSSION

In this study 65% patients had normal salivation rate, as compared to 35% with salivary hypo function (Table 2). Mean pH change in the normal salivation group was 1.2 ±1.0 as compared to 1.5 ±1.0 in the hyposalivation group. This shows that candidal activity in the hyposalivatory participants was more than the normal salivatory participants. But association between the salivation level and the candidal activities as seen through the average change in pH of the medium was insignificant (Table 3). Also insignificant association found between the case type (denture / non denture wearers) and salivation status of the patients, having p-value 0.52 (Table 2). Our results are in agreement to those of Figueiral et.al who also found insignificant association between the DRS and saliva characteristics. However he found a significant relationship between the increased presence of yeasts and hyposalivation.

On the other hand our results are in contradiction to those of Narhi et al18 who demonstrated that subjects having low stimulated salivary flow rate had significantly higher yeast counts than did those having normal stimulated salivary flow rates in a group of 368 Finns aged 76, 81 and 86 years18. Also Navazesh et al19 demonstrated that decrease in whole salivary flow rate was correlated with an increase in Candida albicans count19. Reason might be that, lack of saliva decreases the swallowing frequency, which is the most effective routine to remove microorganisms from the oral cavity6. Consequently Candidal infections are the frequent findings among people with hypo salivation20. Whereas elderly people with normal salivary flow rate possess no special risk group for the development of oral diseases21. Reason for the contradiction might be that, we evaluated candidal activity through bio chemical testing method, which is supposed to show the virulence of candida albicans. Because change in pH occurs due to secretion of acid proteinases, which is related to the capacity to adhere and colonize host tissues and is involved in tissue invasion and destruction22,23,24. Also the differences in age group among the sampling population can be a reason for this contradiction, as compared to others, who included elderly people in their studies. Also differences in the duration of hypo salivation status or the cause of hypo salivation (age related) that may be more persistent than that seen in our study patients (young) can be a reason for this contradiction with other studies.

CONCLUSION

Within limitations of the study stated earlier, the following conclusion could be drawn: Salivation status of the patients had an insignificant association with the oral candidal activity and the denture wearing/ non-wearing.

REFERENCES


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