

ASSOCIATION OF PATHOLOGICAL ACTIVITY OF ORAL CANDIDA AND DENTURE WEARING HABIT IN DENTURE WEARERS

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ABSTRACT

Objective: To assess the effect of denture wearing on oral candidal activity through candida selective medium inoculated with standardized dilution salivary samples obtained from denture wearers.

Methodology: A cross sectional, comparative study during March-September 2007 was conducted at Khyber College of Dentistry Peshawar. Using a structured proforma, data were collected from 50 subjects (50 Denture wearers). Stimulated salivary samples were collected 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.

Results: Pathological candidal activity was seen in denture wearers. However, the association between the denture wearing habit and candidal activity was found significant, meaning that continuous denture wearing always showed a high pH change (*t*-value of 0.01) and mean pH decrease of 2.2 in continuous denture wearers as compare to 1.6 in only day time wearers. Beside this denture plaque score, type of dentures and denture hygiene maintenance was found to have insignificant association with the candidal activity.

Conclusion: Within the limits of this study, denture wearing significantly enhanced the pathogenic activity of oral candida. The importance of rest to denture supporting tissue is re-emphasized.

Key Words: *Candida albicans*, Biochemical activity, Denture hygiene maintenance, Denture plaque score

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INTRODUCTION

Candida albicans as a significant entity in the oral cavity has been recognized since antiquity¹. In 1885 Black first reported denture induced pathosis and identified acidogenic microorganisms from the intaglio surface of

dentures and corresponding mucosa as a causative factor for “Sore mouth under plates”. In 1936, Cahn first suggested that acidogenic microorganisms are responsible for causing denture sore mouth were *Monilia albicans*².

Candida albicans is the dimorphic fungus in a sense that in addition to yeasts and pseudo hyphae it can form true hyphae as well³. It basically exists as ovoid, blastopore and hyphal or mycelia phases⁴. The yeast form is commensal and relatively harmless, while the hyphal form is invasive, pathogenic and the cause of clinical candidiasis⁵. Therefore the ability to switch from yeast to filamentous form, together with the expression of hypha associated genes, is important for virulence of the opportunistic fungal pathogen *Candia albicans*^{6,7}. So morphologic transition from the yeast to hyphal state is one of the key factors in candida albicans pathogenesis. In the hyphal form, candida by producing acid proteinase has been considered more pathogenic than its yeast form^{6,8-11}. The acid proteinase secretion, a characteristic of candidal activity, has several

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implications including, value in evaluating the virulence of candida albicans^{8,12,13}.

Assessment of virulence of candida is best done by the technique determining the biochemical activity of acid production. It is considered a more relevant method in comparison to the counting of colony forming units (CFUs) on inoculated culture media plates in which candida species grow as oval, budding yeast cells (3-6 micro meter in size). The CFUs determination simply gives information of the presence and extent of candida that might not reflect its parasitic activities. The method of assessing, biochemical activity, by measuring acid production (pH change of the medium) is not only accurate and reliable but is also appropriate as it addresses the pathogenic form of candida that is productive of acid proteinase as one of its important virulent factor^{8,12,14}.

The aim of the present investigation is to compare the extent of the pathogenic activity of candida by biochemical activity in a group of subjects wearing maxillary palatal coverage acrylic dentures in our community. Because this kind of work about the determination of oral candidal activity in the oral cavity of the patients although has attracted the investigators abroad but so far none in ours¹⁶.

To address the issue, in the first place relevant literature on the related topic has been attempted to clarify the existing level of knowledge. Subsequently by following a well-designed research methodology, data have been collected from those wearing maxillary palatal coverage acrylic dentures. The data have been presented and discussed in light of the available information. Finally, recommendations for further work have been given.

METHODOLOGY

A cross sectional comparative study at Khyber College of Dentistry Peshawar was designed and conducted during the period from March to September 2007. The participants were 50 subjects, wearing palatal coverage acrylic maxillary dentures designated as DW's. In order to assess the effects of denture wearing over the oral candidal activity, denture wearing factors are divided into further subgroups like Denture type(DT) divided into Removable partial denture or Complete denture wearers, denture wearing habit(DWH) divided into day time wearers and day/night time wearers, Denture hygiene maintenance(DHM) divided into those using tape water with finger or paste brush / antiseptic cleansers and Denture plaque score(DPS)divided into those samples with no plaque on the dentures and those with 25-50% plaque accumulation over

the dentures. 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 the following 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 (1cm³) for five minutes and spit saliva into a sterile tube. All Saliva specimens were collected between 9am and 12pm. The collected saliva samples were quickly transported to the nearby located microbiology laboratory of the Zoology Department of Peshawar University.

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 Performa. 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 4ml of distilled water in a sterile beaker. It was homogenously mixed in a standardized manner. Using a Petri dish 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 Petri dish. Similarly into 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 Petri dish separately, with the help of digital pH meter (Jenco Co-USA). After this, one ml of the diluted saliva was added to each of three sections of the Petri dish. 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 Petri dish. 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 Petri dish 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 secession and on its conclusion. All the readings were checked and recorded in the data collection sheet.

In order to objectively evaluate the candidal activity, a simple method of measuring candida biofilms activity using pH change in the liquid selective medium was used in the present study. As secretion of acid proteinase is a characteristic of candida pathological activity. Thus the test has a value in evaluating the virulence of candida albicans. To verify that the medium was selective only to candida we prepared the same medium to which agar was added for gelation purpose. In the first place to confirm that the liquid media was selective for candida albicans, we divided the media plate into two parts. On left side we inoculated the plate with bacteria (E.Coli) through streaking method. While on other half of the plate we made streaks of a drop of inoculum from a patient salivary sample. After 24 hours incubation we got creamy colonies of candida on the right side of the plate, while on the left side we got no growth even after 48 and 72 hrs. This showed that the media was specifically selective for isolation of the candida and that no bacteria could grow on it. This property was conferred by the presence of antibiotics (Genticin and Chloramphenicol). Secondly, to evaluate the results

further we cultured about 6 more samples. In these we also found creamy oval shaped colonies of candida albicans after further 24 hours incubation.

For the purpose of this study following operational definitions were used.

OPERATIONAL DEFINITIONS

Candidal Activity: For the purpose of this study, candida activity will be determined from the extent of acidic activity as seen by the change in the pH of medium, inoculated with a standardized portion of subject saliva to reflect the presence of candida in its virulent / active form.

Denture hygiene maintenance: In the study it is defined as the method in use by the participant for the maintenance of his/ her denture hygiene.

1. Simply cleaning dentures with tap water and finger.
2. Uses tooth paste/brush in addition to above.
3. Uses antiseptic denture cleansers in addition to 1 and 2 above.

Denture plaque level: In the study denture plaque level was defined by the plaque accumulation on the fitting surface of the dentures and categorized as follow.

1. No plaque noted.
2. Upto 25% denture surface is covered with plaque/debris.
3. Upto 50% denture surface is covered with plaque/debris.

For this study following hypothesis was derived: Pathogenic candidal activity is more pronounced in the oral cavity of persons wearing palatal coverage dentures having day and night denture wearing habits with poor denture hygiene maintenance and poor plaque control.

Using the statistical package for social sciences (SPSS) version 10, the data were statistically analyzed. Descriptive statistics in the form of mean, standard deviation, minimum, maximum (age and pH change in inoculated medium) and denture wearing habit were done.

T-test was applied to data in order to find out the significance among the full time denture wearers, part time denture wearers, different methods for denture hygiene maintenance and denture plaque score.

RESULTS

Mean pH change in the removable partial denture wearers was 1.8 ± 0.6 as compared to 2.0 ± 0.9 in the conventional complete denture wearers. The type of denture had insignificant

association with the observed candidal activity as seen from the average change in pH of the medium, with a p-value 0.49 (Table 2).

Among the denture wearers 48% were using maxillary dentures only during the daytime, while 52% were using dentures day and night. Mean pH change in the denture wearers with only daytime wearing habit was 1.6 ± 1.0 as compared to 2.2 ± 0.5 in those with continuous day/night denture wearing habit. The variable of denture wearing habit had a highly significant association with the candidal activity as seen from the levels of average change in pH of the medium, with p-value 0.01 (Table 2).

Among the denture wearers, 44% patients were using simple tap water with finger for cleaning of their dentures. While 56% patients were using toothbrush with paste or soap for cleaning their dentures. And only 2% patients were found using additional antiseptic cleansers for the

denture.

Mean pH change in the group of participants using tap water/ finger was 2.0 ± 0.8 as compared to 1.8 ± 0.9 in those using paste/brush/antiseptic cleansers. However the association between the variable denture hygiene maintenance and the candidal activity as seen from average change in pH of the medium was found insignificant (Table 2).

The dentures of only 4 % participants had no plaque or debris on the fitting surfaces as compared to 80% patients denture surfaces were covered with 25% plaque and debris and 6% patient's denture had plaque and debris on more than 50% surfaces. To find out the level of significance data was pooled into two groups. Those whose dentures had no plaque and the other having denture surfaces covered with plaque/debris (Table 2). Mean pH change in the denture wearers with their dentures having no plaque was 1.9 ± 1.01

Table 1: Inclusion and exclusion criteria for selected subjects

Inclusion Criteria	
1.	Patients with age between 40-60 years
2.	Having no active infection in the mouth
3.	Patients presently wearing palatal coverage acrylic denture either complete or partial coverage but having minimum of six teeth replaced for at least one month
Exclusion Criteria	
1.	Patients with a history of major salivary glands pathology or extirpation
2.	Ill and medically compromised patients
3.	Patients with reduce intelligence, mental handicaps or having serious illness

Table 2: Association between denture wearing habit, denture hygiene maintenance, and denture plaque score with oral candidal activity {Average change in ph of the medium} (n=50)

	Variable	No. (%)	pH Change * $\bar{X} \pm S.D$ (Min-Max)	p-value
DT	RPD	22 (44)	1.8 ± 0.63 (0.5-2.8)	0.49
	CD	28 (56)	2.0 ± 0.98 (0.2-3.3)	
DH	Day time	24 (48)	1.6 ± 1.03 (0.2-3.3)	0.01
	Day/night	26 (52)	2.2 ± 0.50 (1.2-3)	
DHM	Using Tape water/fingers	22 (44)	2.07 ± 0.80 (0.2-3.3)	0.26
	Using paste/ brush/Antiseptic cleansers	28 (56)	1.8 ± 0.87 (0.2-3.1)	
DPS	No plaque noted	4 (8)	1.9 ± 1.01 (0.7-2.9)	0.99
	25-50% plaque noted	46 (92)	1.9 ± 0.84 (0.2-3.3)	

* pH change: Average change in pH of the medium

Abbreviations: DT: Denture type; RPD: Removable partial denture; CD: Complete denture
 DH: Denture wearing habit; DHM: Denture hygiene maintenance; DPS: Denture plaque score

as compared to 1.9 0.8 in case of denture wearers having denture surfaces covered with plaque (Table 2). The association between the denture plaque levels and candidal activities as seen from the average change in pH of the medium was found insignificant (Table 2).

DISCUSSION

In our study among the denture wearers, 44% participants were having acrylic removable partial dentures with substantial amount of palatal coverage, while 56% participants were using conventional complete dentures. Mean pH change in the removable partial denture wearers was 1.8 0.6 as compared to 2.0 0.9 in the conventional complete denture wearers. Acrylic removable partial dentures, having palatal coverage had a similar effect on candidal activity as the conventional complete dentures have on this (Table 2).

Our study findings were in contradiction to those of Ikebe et al, who found increase candidal activity in complete denture wearers as compared to the partial denture wearers¹⁶. Reason might be that we have selected only those participants as denture wearers, who had a substantial palatal coverage, also the age group difference between the two studies i.e. our study age group was between 40-60 yrs as compared to Ikebe's study whose study age limit included participants above 60years, criteria of patient selection for this study as compared to Ikebe et al study. Increase in age has substantial effect on the factors like salivary flow rate as well as immunity of the person¹⁶.

In addition denture induced stomatitis was detected only in maxillary denture wearers, which supports the theory that the prevalence of denture induced stomatitis is associated with the amount of tissue covered by a denture¹⁶. Whereas Jainkittiwong et al also found insignificant difference in denture related mucosal lesions among complete and partial denture wearers and concluded that rather than the amount of tissue coverage other predisposing factors may participate in the aetiology¹⁷.

Among the denture wearers 48% participants were using maxillary dentures only daytime, while 52% participants were using dentures day and night. Mean pH change in the denture wearers with only daytime wearing habit was 1.6 1.0 and 2.2 0.5 with continuous day/night denture wearing habit. This shows increase in the candidal activity with continuous denture wearing as compared to those who wear dentures only in the daytime. The candidal activity was significantly higher in patients having the habit of

continuous day and night denture wearing, as compared to daytime only with having p-value 0.01 (Table 2). This is in agreement to those of Khasawneh and al-Wahadni who found strong association between continuous denture wearing habit and higher candidal activity¹⁸. Even relatively more extensive inflammatory status of the denture supporting tissues has been noted by others in such patients¹⁹. As already established, the persistent and continuous denture pressure interrupting blood supply to supporting tissues might be involved as a reason. To avoid this to remain constantly present, routine removal of dentures at night or for some extended period has been recommended for maintaining the health of the tissues²⁰.

Our results are contradictory to those of Kulak and Arikan, who did not found any significant relationship between the prevalence of DRS and overnight denture wearing²¹. Such a finding may be explained by the differences in the study population used by Kulak and Arikan²² and our study population. The differences could be in terms of level of oral hygiene, denture hygiene, and general health status and factors that influence candidal activity. It also highlight that simple denture wearing habit may not be all to explain candidal activity. The findings of Kulak and Arikan should not be taken as a premise for recommending the continuous use of prosthesis nor do our findings recommend discouraging patients against continuous denture wearing use.

The data in Table 2 shows mean pH change in the group of participants using tap water/ finger was 2.0 0.8 and 1.8 0.8 in the group using paste/brush/antiseptic cleansers. These findings show more candidal activity in those who used simple tap water /finger for hygiene maintenance as compared to those who used paste/brush/antiseptic cleansers. However, the observed differences had an insignificant association with the methods of denture hygiene maintenance and the candidal activity as seen through average change in pH of the medium (Table 2).

Our results are in agreement to those of Kulak, who found insignificant association between the frequency of denture brushing and denture stomatitis²¹. Also results are comparable to those of Zisis et al who also found insignificant association between the denture stomatitis and method for denture cleaning²². But he found significant association between the dentures with unsatisfactory cleanliness and denture stomatitis prevalence²³.

Denture hygiene maintenance is also important from the candidal colonization point of view. Our results are thus in contradiction to those

of Pranhos et al, who studied about the effects of different denture cleansing methods and concluded that best results were obtained by a combination of mechanical and chemical methods for the denture hygiene maintenance²⁴. Also Schou et.al found a significant relationship between plaque formations, soaking habits with alkaline peroxide and the presence of DRS²⁵. Reason for contradiction might be that only 2% of our patients used any antiseptic cleansers for the maintenance of denture hygiene, which do have a very important role in the maintenance of denture cleanliness. Secondly we have used biochemical testing approach for the evaluation of candidal activity, which is supposed to reflect the virulent nature of the candida albicans. While in other studies, a different method was used that considered isolation of candida In comparison to conventional colonies and the counting on the culture media plates that reflect the extent of candidal colonization and not their virulence.

The data in Table 20 show mean pH change in the denture wearers having no plaque found was 1.9 1.01 as compared to 1.9 0.8 in denture wearers having 25-50% denture surfaces covered with plaque. The denture plaque levels found insignificant association with the candidal activities as seen through the average change in pH of the medium (Table 2).

However our study results are in agreement to those of Markovic et al, who found only 30% denture surfaces clean in their²⁶. But interestingly he found that only 18% denture wearers were given instructions on denture cleaning by their dentists²⁶. Cardash and Rosenberg also claimed that many patients are not informed on how to care for their removable dentures²⁷. Older patients may have reduced manual dexterity for cleaning dentures and less than acute eyesight to detect the almost invisible layer of plaque on the acrylic resin²⁷. These might be the reasons for increased number of dentures having fitting surfaces covered with plaque in this study as well. Our results are in agreement to those of Figueiral et al who by studying 140 persons wearing removable maxillary poly-methyl-methacrylate prosthesis also found insignificant association between prosthesis hygiene and denture related stomatitis²⁸. But on the other hand our results are contradictory to Radford et al who reported the importance of the sequential development of denture plaque and its colonization by Candida organisms²⁹, but the plaque quantity rather than composition is more important for the development of DRS³⁰. Also our results are in contradiction to those of Abelson who related unclean removable dentures to denture stomatitis³¹. Koopmans also in a case control study found 10 times more yeasts on

denture plaque in DRS than in control group³². Kulak and Arikan, also found pronounced significance between poor denture cleanliness and denture stomatitis²². Differences in results could be in terms of level of denture hygiene, general health status and different subject population studied.

CONCLUSION

Within the limitations of the study stated earlier, it is concluded that denture wearing itself is the main cause for significant increase in candida pathological activity. The association between the denture wearing habit and candidal activity was also found significant, meaning continuous denture wearing always showed high pH change. The effect of denture plaque score level of the patients had also an insignificant association with the oral candidal activity.

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CONTRIBUTORS

MAC conceived the idea and planned the study. AN & MSK did the data collection and analyzed the study. FG supervised the study. All the authors contributed significantly to the research that resulted in the submitted manuscript.