BIOCHEMICALLY ASSESSED PATHOLOGICAL ACTIVITY OF ORAL CANDIDA IN DENTURE AND NON DENTURE WEARERS

Fazal Ghani, Mohammad Ali Chughtai, Syed Akram Shah

Department of Prosthodontics and Zoology
Khyber College of Dentistry, Sardar Begum Dental College &
University of Peshawar, Peshawar - Pakistan

ABSTRACT

Objective: *To assess the candida activity through recording pH changes in candida selective medium inoculated with standardized dilution salivary samples obtained from dentures wearers and non denture wearers.*

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 DWs and 50 NDWs). Stimulated salivary samples were collected from each subject. One ml of standard dilution saliva was mixed with 4 ml sterile liquid candida-selective medium. The extent of candida activity was then determined, biochemically, through change in pH of the medium 24 hrs after inoculation and incubation at 37°C. The influence of age, gender, socioeconomic status, smoking habits, medication-use, oral-hygiene maintenance method, denture-plaque level, and level of salivary function was also investigated.*

Results: *Pathological candida activity was seen both in DWs and NDWs. However, it was significantly higher in DWs (p value = 0.00) with mean pH decrease of 1.9 as compared to 0.8 in NDWs. Except age, the effect of gender, socioeconomic status, medications, oral hygiene maintenance on the oral candida activity was insignificant both within and between groups.*

Conclusion: *Not-with-standing the varying effect of other variables, denture-wearing significantly enhanced pathogenic activity of oral candida. The importance of meticulous oral and denture hygiene maintenance and rest to denture-supporting tissues is re-emphasized.*

Key Words: *Candida albicans, Biochemical activity, denture related stomatitis, denture wearing.*

INTRODUCTION

Since antiquity, the dimorphic oral commensal yeast known as candida albicans is considered the most common cause of opportunistic fungal infection in oral cavity[1-3]. It has been shown that the extent of commensal existence of intraoral candida varies from 20-50% in a healthy dentate population and up to 75% in that wearing dentures[4-7]. Although, the factors influencing pathogenecity of oral candida in an individual have not been fully understood, consensus exists on the contributory role played by a number of local and general factors transforming these commensals into parasitic pathogens[7-9]. These factors are categorized as pre-disposing, initiating and perpetuating factors. Among the pre-disposing factors, important one include; age [2, 10-13] and poor oral and denture hygiene[14,15]. While immuno-suppressive states[16,17], prolonged use of broad spectrum antibiotics[18,19], low pH of saliva[10] and denture related trauma and infection[16,17] are considered as factors initiating oral candida infection. The factors perpetuating oral fungal infection include tobacco smoking, medications, systemic illnesses and physiologic conditions such as pregnancy[10, 14, 18-24].

Candida can be isolated and diagnosed by a number of conventional and advanced methods. Despite their individual specificity and sensitivity,

limitations and advantages and disadvantages, the available methods have been extensively used for isolation and identification of candida. Among these include; denture brushing technique[25,26], swabbing, replica culturing, smearing and scraping technique[27–30], isolation from saliva[6, 31, 32], fluorescent and optical brightening (di-amino-stilbenes) technique[32], automated blood culture[33,34], molecular methods like polymerase chain reaction (PCR)[35,36], nucleic acid sequence-based amplification (NASBA)[37,38], amplified fragment length polymorphism (AFLP)[39] and randomly amplified polymorphic DNA analysis(RAPDA)[40]. The advanced techniques have enabled the identification of candida up to the species level and hence the selection of appropriate therapeutic approaches.

Morphologic transition from the yeast to hyphal state is one of the key factors in the pathogenic activity of candida albicans. In the hyphal form, candida, by producing acid proteinase, has been considered more pathogenic than its yeast form[41-44]. The acid proteinase secretion, a characteristic of candida activity, has several implications including, value in evaluating the virulence of candida albicans. The proteinase secretion also determines the candida adhering and colonizing ability to the host tissue. It is also involved in tissue invasion and destruction[45-47]. 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. 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[45, 46, 48].

This kind of work about the determination of candida activity in the oral cavity of patients although has attracted the investigators' attention in other countries but so far none in ours[31]. Furthermore, the main objection to the use and applicability of findings of studies from abroad has been the use of population with un-matching characteristics in terms of socioeconomic, geographical and ethnic variables. All these make such work less relevant for local applicability unless confirmed otherwise.

The aim of the present investigation is to compare the extent of the pathogenic activity of candida in a group of subjects wearing maxillary palatal coverage acrylic dentures, with that of a group of individuals, who have never used such dentures. It is hypothesized that pathogenic candida activity is more pronounced in the oral cavity of persons wearing palatal coverage dentures, as compared to those not wearing such dentures In addition, the possible association of the observed candida activity with age, gender, socioeconomic status, smoking habits, medication-use, oral hygiene maintenance method, denture plaque level and level of salivary function of patients shall also be investigated.

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 subjects selected through convenience sampling technique and divided in two equal groups comprising of 50 subjects wearing palatal coverage acrylic maxillary dentures designated as DWs and 50 individuals not wearing such denture designated as NDWs. 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 a subjects' saliva containing candida. Stimulated salivary samples, from each participant, 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 ($1cm^2$) 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 Parasitology Unit, Zoology Department, University of Peshawar.

In the laboratory, the collected salivary sample was first vortexed for a minimum 30 seconds at 3000 revolutions per minute (rpm) in a centrifuge machine (Hettich Universal, Germany). This removed the entrapped air bubbles to precisely assess 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 specimen was opened. With the help of sterile pipette saliva sample was measured to determine the salivary function level as its flow rate during the saliva sample collection time. The volume of collected saliva was used for categorizing the subject salivary functional level. A person with saliva volume up to 0.5 ml/min or more saliva was included in the normal salivation group, while those having less than 0.5 ml/min saliva were placed in the hypo-salivation group.

Saboraud dextrose medium was prepared in bulk under aseptic condition in laminar flow (Fitted with Hepa filter-0.2 micro meter, France and supplied by Scientific Technical Corp, Lahore, Pakistan). Initially, 10 g of peptone and 20 g of glucose was added in 1000 ml 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 liquid medium to room temperature, 0.1g Gentamycin (Reckitt Benckiser Ltd) and 0.25g Chloramphenicol (Parke Davis Co; Ltd; Karachi, Pakistan) 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 a subject was then diluted with 5 ml of distilled water in a sterile beaker. It was homogenously mixed in a standard manner. Using a Petridish with three-compartments, a tube containing 4ml of liquid selective medium was taken from refrigerator and its content transferred to a compartment of the Petridish. Similarly, into each of the remaining two sections of the Petri dish, 4ml of the selective medium were poured. 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 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, UK) for 24 hrs 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 was calibrated before starting each session and on its conclusion. All the readings were 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, minimum, maximum for the age and the recoded pH changes, frequency tables and percentages for gender, socioeconomic status, salivation status of the patient, smoking habit, medication, level of oral hygiene maintenance, type of denture in use, denture wearing habit, denture hygiene maintenance and denture plaque level were done.

T-test was applied to data in order to find out the significance of differences between the data of DWs and NDWs and pathogenic activity of candida (pH change) as well as the influence of the confounders. To find out the association between DWs and NDWs and other variables (age, gender, socioeconomic status, salivation status of the patient, level of oral hygiene maintenance), Chi-square test was used. Chi-square test was also used to find association between age, gender, socioeconomic status, oral hygiene maintenance and salivation status of the patient and candida activity (average change in pH). As the variables of smoking and medication-use, had expected cell count less than 5 in more than one cell, Fisher's exact test was applied to these data so as to find out association of smoking and medication-use with candida activity (average change in pH) in DWs and NDWs.

RESULTS

The age range of 100 subjects was 40-60 years with the mean age 50.7$\pm$ 6.1(S.D). Of these 44 % were in the age range 40-50 years with mean age 44.5$\pm$2.9 years, while 56% were in the age group 51-60 years, having mean age 55.5$\pm$2.6 years. The DWs in the age group 40-50 years had mean age 43.8$\pm$2.4 as compared to the age group 51-60 years which had the mean age 55.7$\pm$2.8. The NDWs in the age group 40-50 years had mean age 45.1$\pm$3.1, as compared to 55.2$\pm$2.4 for NDWs belonging to the age group 51-60 years.

A mean pH change of 1.4$\pm$0.9 was noted in patients having their ages between 40 to 50 years as compared to1.3$\pm$1.1 with ages between 51 to 60 yrs (Tables 2 & 3). As seen in Table 3, the subjects' age had a significant association with the candida activity (p value 0.008), whereas it had an insignificant association with the case type (DW or NDW). Out of 100 participants, there were 47% male and 53% were females. Among the DWs, 48.9% were male and 50.9% females; whereas NDWs were 51% male and 49% females. Among

Table 1: Inclusion and exclusion criteria for the subjects

Inclusion Criteria:

For Denture Wearers (DWs):

1. Patients with age between 40-60 years.
2. Having no active infection in the mouth.
3. Have been presently wearing palatal coverage acrylic denture for a period of at least one month.

For Non-Denture Wearers (NDWs):

1 Fulfilling the criteria 1, 2 as for group A but no denture wearing experience.

Exclusion Criteria:

1. Patients with age less than 40 and more than 60 years.
2. Patients with a history of major salivary glands pathology or extirpation.
3. Ill and medically compromised.
4. Patients with reduced intelligence and mental handicaps or having serious Illnesses.

Table 2: Association between age, gender, SES*, smoking and case type (DWs & NDWs)

Variable		No	DWs* No. (%)	NDWs* No. (%)	x^2Value	df	p-value
Age	40-50 yrs	44	19 (40.9)	25 (54.5)	1.4	1	0.22
	51-60 yrs	56	31 (57.1)	25 (46.4)			
Gender	Male	47	23 (48.9)	24 (51)	0.04	1	0.84
	Female	53	27 (50.9)	26 (49)			
SES*	Up to 2000/month	35	19 (54.3)	16 (45.7)	0.68	2	0.71
	Upto10000/month	42	19 (45.2)	23 (54.8)			
	> 10000/ month	23	12 (52.2)	11(47.8)			
Smoking	Yes	3	2 (66.7)	1 (33.3)	0.34	1	0.55
	No	97	48 (49.4)	49 (50.5)			

Total No=100 , *SES = Socioeconomic status, *DWs = Denture wearers, *NDWs = Non denture wearers

the male participants mean pH change was 1.2±1.0 and among females 1.4±0.9 (Table 3). Gender of the subjects had insignificant association (p-value 0.31) with the observed candida activity as determined from the change in pH of the medium for both the DWs and NDWs (Table 3).

Some 35% subjects had low income with average income less than 2000 Pakistani Rupees per month, with 42% patients having moderate income (10000 Pak Rupees per month) and 23% having high income (> 10000 Pak Rupees per month). The distribution of DWs was; 54.3% having low income, 45.2% having moderate income and 52.2% high income. While NDWs were; 45.7% had low income, 54.8% moderate income and 47.8% high income groups. The mean pH change among the poor income group was 1.7±1.1; moderate income group was 1.2±0.9 and in the high income group was 1.0±0.8 (Table 3). The variable socioeconomic status had an insignificant association (p-value 0.15) with the observed average change in pH of the medium (Table 3). Even insignificant association (p-value 0.71) was found between the socioeconomic status and the case type (DW / NDW).

Only 3% subjects had a history of smoking tobacco. The distribution of these among DWS was 66.7% as compared to 33.3% and among NDWs. In contrast 97% of the non-smoking patients, 48 (49.4%) were DWs and 49 (50.5%) were NDWs. Mean pH change among the smokers was found to be 0.3±0.2, while in non-smokers 1.4±1.0 (Table 3). Insignificant association (p-value 0.55) was found between smoking habit and case type (DW / NDW). Also insignificant association was found between the smoking habit and the candida activity (Table 3).

Only 9% subjects used medicines having xerostomic side effects while 91% were not using any such medications. The distribution of the total 9 subjects using xerostomic drugs were 5 (55.5%) DWS and 4 (44.4%) NDWs. In contrast the distribution of 91 subjects not using any medicines (having xerostomic effect) were 45 (49.5%) DWs and 46 (50.5%) NDWs. Mean pH change in the medication using group was 1.1±0.7 as compared to 1.4±1.0 not using such medications.

Among the medication-using group 33.3% participants had normal salivation as compared to

Table 3: Association between age, gender, socioeconomic status, smoking and candida activity as seen from pH change* in the medium

Variable		No	PH-change* $\overline{X} \pm S.D$ (Min-Max)	x^2value	df	p-value
Age	40-50 yrs	44	1.4 ±0.91 (0.06-2.9)	11.7	3	0.008
	51-60 yrs	56	1.3 ±1.1 (.0-3.3)			
Gender	Male	47	1.2 ±1.06 (.0-3.3)	3.5	3	0.31
	Female	53	1.4 ±0.98 (0.06-3.3)			
SES*	Upto2000/month	3	1.7 ±1.12 (.0-3.3)	9.3	6	0.15
	Upto10000/month	42	1.2 ±0.97 (0.06-3.3)			
	>10000/ month	23	1.0 ±0.81 (0.1-2.8)			
Smoking	Yes	3	0.3 ±0.23 (0.2-0.6)	5.04	3	0.16
	No	97	1.4 ±1.02 (.0-3.3)			

Total No=100, *SES = Socioeconomic status, *pH change = Average change in pH of the medium.

Table 4: Association between case type (DW & NDW) and candida activity

Case Type	No	pH change* $\overline{X} \pm S.D$ (Min-Max)	t-value	df	P-value
DWs*	50	1.9 ±0.84 (0.2-3.3)	6.2	98	0.000
NDWs*	50	0.8 ±0.89 (.0-3.3)			

* DWs = Denture wearers, * NDWs = Non denture wearers, * pH change = Average change in pH.

66.6% having hypo-salivating state. While in the group not using any medication 68.1% were normally salivating as compared to 31.9% as hypo-salivating. There was a significant association between the intake of medicines and the salivation status (p–value 0.03). The association was insignificant between the variable of medication use and the DW and NDW status of the subject(p-value 0.72). Similarly, the association between the medication use and the candida activity as seen from average change in pH of the medium was insignificant (p-value 0.25).

Some 64% subjects were using simple tap-water and finger for cleaning mouth as compared to 32% using tooth paste/brush and only 4% used mouth-wash in addition to tooth paste/brush for the maintenance of their oral hygiene. To determine level of significance for the oral hygiene habits, the data for using antiseptic mouth-wash using tooth paste/brush was pooled. Among DWs, 53.1% were using tap water with the finger for mouth cleaning and 44.4% were using tooth paste/brush/ mouth-wash. While among NDWs, 46.9% were using tap-water with the finger for mouth cleaning and 55.6% were using tooth paste/brush/mouth-wash. Mean pH change in the group using tap water with the finger for mouth cleaning was 1.5±1.0 and in the group using tooth paste/brush/ mouth-wash was 1.0±0.8. The variable "method of oral hygiene maintenance" had insignificant association with the "DW and NDW status of subjects". Also an insignificant association was found between the methods for oral hygiene maintenance and candida activity as determined from the average change in pH of the medium (p-value 0.09).

Mean pH change for the DWs (n = 50) was 1.9 0.8, while in NDWs (n = 50) it was 0.8±0.8. Among the NDWs about 62% showed an average change in pH of the medium in the minimal pH range i.e. 0 - 0.7 as compared to DWs where only14% exhibited this pH range. Similarly, among DWs, 20% showed maximum pH change i.e. 2.8-3.3 as compared to NDWs, where only 6% showed this pH range. Thus the change in pH had a highly significant association with the variable "DW or NDW status of the subject and "candida activity" as seen from the average change in pH of the medium (p-value 0.00).

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

Among the DWs, 44% patients used simple tap-water with finger for cleaning of their dentures as compared to 58% using tooth-brush with paste or soap and/or antiseptic denture cleanser for maintaining the hygiene of their dentures. 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 candida activity as seen from average change in pH of the medium was found insignificant (Table 4).

The dentures of only 4 % participants had no plaque or debris on the fitting surfaces as compared to 80% patients in whom 25 % of the denture fitting surface was covered with plaque or 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. Mean pH change in the DWs with their dentures having no plaque was 1.9±1.01 as compared to1.9±0.8 in case of DWs having denture surfaces covered with plaque. The association between the denture plaque levels and candida activities as seen from the average change in pH of the medium was found insignificant.

As many as 65 % patients had normal salivation rate, as compared to 35% who belonged to the hypo-salivating group. Among the DWs 52.3% were having normal salivation status and 45.7% having hypo-salivating status. While among NDWs 47.7% had normal and 54.3% with hypo-salivating status. Mean age of normally salivating subject was 50.9 6.2 and 50.2±5.8 years of hypo-salivating subjects. Mean pH change in the normally salivating subjects was 1.2±1.0 as compared to 1.5±1.0 in the hypo-salivating subjects. Association between the variable salivating status and candida activity as seen from the average change in pH of the medium was insignificant (p-value 0.73). Also association found between the case type (DW/NDW) salivating statuses of the subjects was insignificant (p-value 0.52).

DISCUSSION

For the virulence of candida, the biochemical technique of acid production determination is considered a more relevant method in comparison to the counting of CFUs on inoculated culture media plates. Because the conventional method of CFUs counting has several shortcomings, particularly in respect to its precision[31], in a way that CFUs' determination simply gives information of presence and extent of candida that might not reflect its parasitic activities. Thus in order to objectively evaluate the candida 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, this method 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 hrs 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.

The present sample of subjects seems too small for drawing definitive conclusions specifically it was too small for the effect of some of the confounding variables. For example, there were only 3 smokers as compared to 97 non-smokers. Also few subjects belonged to the high-income group as compared to moderate and low-income group, therefore relative influence of each could not be easily determined. Similarly, in case of denture hygiene/ oral hygiene habits, conditions were not too different and thus conclusion regarding the relative influence of each could not be precisely ascertained.

A significant association was found between the age and candida activity as seen from average change in pH of the medium (Table 3). The present results as showing significant association between the candida activity and the age in the relatively healthy old people are in agreement to those of Lockhart's et al findings who demonstrated that, the frequency and intensity of candida colonization increased as a function of age, independent of denture use[2]. Also our study results are in partial agreement to those of Zaremba et al,[13] who isolated yeasts of genus candida (by culturing swabs on Sabourad agar media) at a comparable rate from the adults with and without dentures, as well as in middle aged (35-44yrs) and elderly subjects (56-92 yrs). However significant difference was observed between elderly subgroups aged 56-70years (35%) and advanced age subgroups 71-92 years (74%). Whereas, our results are in contradiction to those

of Ikebe et al[31]. Reason for this may be that our study is based on the age groups 40-60 years and there are no advanced age group participants in the study as compared to the study done by et al[31]. Our results are also in contradiction to those of Figueiral et al findings showing decrease in the prevalence of DRS and yeasts in older subjects[49]. Also in another study Kulak and Arikan found insignificant association between the denture stomatitis and age[50]. Reason for contradiction might be that our study is based on healthy normal middle aged individuals as compared to these studies which included DRS patients in addition to symptom-less participants.

The mean pH change observed in females (1.4±0.9) was higher than that in males (1.2±1.0), and that the differences seen were insignificant. This finding is in agreement to those of Ikebe et al, showing insignificant association between the gender and candida activity[31]. In another study Kulak and Arikan also found insignificant association between gender and denture stomatitis[50]. But on the other hand our results are in contradiction to those of Figueiral[49] who showed higher prevalence of DRS in the females as compared to males. Reason may be that majority of women in their study were in the postmenopausal age group (mean age-59.75 years), which may have some influence on higher prevalence of DRS[49]. In our study females were in the age range 40-60 years. This might be a possible reason that insignificant effect of the gender on candida activity was observed.

The change in pH due to candida activity was more in the subject belonging to the poor socioeconomic status in comparison to those having moderate and high income. However, the observed effect of the socioeconomic status on the candida activity was not significant. In general economic status of the patient has some role in the maintenance of oral hygiene. It is quite natural that a person who cannot afford the normal status of living, he would not be using tooth paste or antiseptic mouth-wash for the maintenance of oral hygiene. But the contradiction to the general statement might be that our study is comprised of only 35% poor participants whereas 65% participants were belonging to moderate and high-income group. We did not include the recording of the educational level of the patients. Had this been done, the effect of this as well as the socioeconomic status of patients on the candida activity would have been clearer, especially in the DW group.

Generally it is a belief, that smoking would cause increase in the candida colonization. Our study results are in contradiction to those of Alkumru and Beydemir[22] who claimed that candida carrier rate for complete and partial denture wearers were greater in smokers than non-smokers. Also our study results are in contradiction to those of Barbeau et al[21], who found smoking to be associated with most extensive inflammation and declared it as a risk factor for DRS. Contradiction is also found to the local study conducted by Rasool et al, who found a higher prevalence of candida albicans in the smokers as compared to non smokers[9]. Similarly, in another study Kulak and Arikan also found statistically significant relationship between denture stomatitis and smoking habit as well as between candida colonization[50]. But reason for this contradiction might be explained on the basis that in our study the number of smokers were too few as compared to those not smoking tobacco.

Findings of this work are in agreement to those of Narhi et al[11] and Pajukoski et al[12] showing that medicine was the main factor causing salivary gland hypofunction (SGH) in the elderly. Specifically multiple medications being clearly associated with SGH in the elderly. This effect will be seen as reduced rate of both stimulated and non-stimulated whole saliva flow with the increasing number of medications[11]. Our study results are also in agreement to Kreher et al who found up to 60% decrease in salivary flow rate of patients taking two or more hypo-salivatory drugs[51]. But according to his study use of drugs with hypo-salivating side effects was related to more increase in candida glabrata as compared to candida albicans in saliva, DRS and denture sore sites[51].

A significantly higher level of candida activity in the DWs (1.9±0.8) was observed as compared to NDWs (0.8±0.8). Among the NDWs 62% subjects had average change in pH of the medium in the minimal pH range i.e. 0-0.7 as compared to DWs, where only14% exhibited this pH range. Minimal or no pH change in the medium could be explained on the basis that oral cavity is the principal candida reservoir in the body with 60% of subjects colonized asymptomatically in the general population[52]. These findings are in agreement to those of Ikebe et al who also found significant association between candida activity and maxillary denture wearing[31]. Our study results are also in agreement to many others showing that commensal existence of intraoral candida species could vary from 20-50% in a healthy dentulous population and up to 75% in a denture wearing population[53].

Candida activity was significantly higher in subjects wearing dentures day and night as compared to those wearing them during the

daytime. This is in agreement to those of Khasawneh and al-Wahadni who found strong association between continuous denture wearing habit and higher candida activity[54]. Even relatively more extensive inflammatory status of the denture supporting tissues has been noted by others in such patients[55]. As already established, the persistent and continuous denture pressure by 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 tissues[56]. But 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 wearing[57]. Such a finding may be explained by the differences in the study population used by Kulak and Arikan[50] 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 candida activity. It also highlight that denture wearing habit alone may not be all to explain candida 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.

Candida activities had an insignificant association with the methods of denture hygiene maintenance. This is in agreement to those of Kulak and Arikan who found insignificant association between the frequency of denture brushing and denture related stomatitis (DRS)[50]. Also results are comparable to those of Zisis et.al who also found insignificant association between the DRS and method for denture cleaning[58]. But they found significant association between the dentures with unsatisfactory cleanliness and DRS prevalence[58]. Our results are 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 maintenance[59]. Also Schou et.al found a significant relationship between plaque formations, denture immersion in alkaline peroxide cleanser and the presence of DRS[60]. 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.

The association between plaque level deposited on the fitting surface of the denture and candida activities was found insignificant. This is in agreement to that of Figueiral et. al who by studying 140 persons wearing removable maxillary acrylic prosthesis also found insignificant association between prosthesis hygiene and DRS[49]. 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 organisms[4]. However, it is the plaque quantity rather than its composition that is more important for the development of DRS[61]. Also our results are in contradiction to those of Abelson who related unclean removable dentures to DRS[62]. Koopmans also in a case control study found 10 times more yeasts on denture plaque in DRS than in control group[63]. Kulak and Arikan, also found pronounced association between poor denture cleanliness and DRS[50]. Differences in results could be in terms of level of denture hygiene, general health status and different subject population studied.

We found increased, though insignificantly different candida activity in the hypo-salivating subjects in comparison to that seen in normally salivating subjects. This was in agreement to those of Figueiral et.al who also found insignificant association between the DRS and saliva characteristics[49]. However they found a significant relationship between the increased presence of yeasts and hypo-salivation[49]. On the other hand our results are in contradiction to those of Narhi et al[64], and Nawazesh et al[65] who demonstrated that subjects having low stimulated salivary flow rate had significantly higher yeast counts than those having normal stimulated salivary flow rates. Reason might be that, lack of saliva decreases the swallowing frequency, which is the most effective routine to remove microorganisms from the oral cavity[66]. Consequently, candida infections are the frequent findings among hypo-salivating subjects[67]. On the other side, it has been shown that elderly people with normal salivary flow rate possess no special risk group for the development of oral diseases[68]. Reason for the contradiction might be that, we evaluated candida activity through biochemical testing method, which is supposed to show the virulence of candida albicans. Because change in pH occurs due to secretion of acid protienases, which is related to the capacity to adhere and colonize host tissues and is involved in tissue invasion and destruction[45-47]. Differences in age group among the sampling population can be another 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 subjects (young) can be a reason for this contradiction with other studies.

CONCLUSION

Within the limitations of this study, it is concluded that denture wearing itself is the main cause for significant increase in the pathological activity of oral candida. Furthermore, continuous denture wearing was noted as an aggravating factor for this activity. The association between oral candida activity and several co-variables including; subjects' age, gender, and socioeconomic status, use of xerostomic medicines, method for oral and denture hygiene maintenance, extent of plaque deposition on denture and subjects' salivation status was insignificant.

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Address for Correspondence:
Dr. Fazal Ghani
Department of Prosthodontics
Khyber College of Dentistry
University Campus, Peshawar - Pakistan
E-mail: fazalg55@hotmail.com