

Original Article

Presence of pathogenic candidal hyphae in patients with palatal coverage appliances/prostheses

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Submitted: 26/10/2018. Accepted: 28/05/2019. Published online: 28/05/2019.

Abstract Oral candidiasis is an opportunistic infection of the oral cavity. This study aimed to determine prevalence of oral candidiasis in patients with palatal coverage orthodontic appliances and prostheses and its risk factors. Three groups of patients were recruited after an informed consent. The two tested groups were the denture and the upper removable orthodontic appliance (URA) groups while patients with no prosthesis/appliance acted as control. The assessments included demographic profile, denture/URA age, night-time wearing and duration of wearing. Signs and symptoms of candidiasis were examined intra-orally. Unstimulated salivary flow rate test was also performed. Smears samples were taken from palatal mucosa and impression surfaces of denture/URA and stained with Periodic Acid Schiff (PAS). The data were analysed using descriptive and chi-square tests. A total of 86 patients were recruited; denture (n=30), URA (n=22), control (n=34). Denture and URA groups had significantly more positive cases of candidal infection (56.7 and 72.7% respectively) as compared to control group (20.6%). Among the subjects with oral candidiasis, denture patients were detected to have more severe amount of candidal hyphae (20%) than URA (13.6%) group. The relationships between oral candidiasis and these risk factors i.e. age, night-time wearing, daily total hours of wearing and denture/URA hygiene were statistically significant ($p < 0.05$). Thus, patients who are wearing palatal coverage removable appliances or prostheses have higher prevalence of oral candidiasis. The risk is greater with confounding factors such as age, duration of wearing and oral hygiene.

Keywords: Candidal hyphae; denture-related stomatitis; oral candidiasis; orthodontic appliance.

Introduction

Oral candidiasis is a common opportunistic infection of the oral cavity. It is usually caused by overgrowth of endogenous *Candida albicans* (*C. albicans*). The spores of *Candida* are commensal, harmless form of dimorphic fungus that becomes invasive and pathogenic hyphae when there is a disturbance in the balance of flora or in debilitation of the host (Zunt, 2000). The incidence of *C. albicans* isolated from the oral cavity has been reported to be 45% in neonates, 45%-65% of healthy children, 30%-45% of healthy adults, 50%-65% of people who wear removable dentures (Akpan and Morgan, 2002). It has been shown that upper removable appliances and denture with palatal acrylic coverage contributes to colonization of *C. albicans* on

the palatal mucosa (Webb *et al.*, 1998; Mahmoudabadi and Drucker, 2003). The initial step in candidiasis is adherence of the *C. albicans* yeast to an epithelial cell surface (King *et al.*, 1980). It is an important prerequisite for the colonization of *C. albicans* especially in environments like oral cavity where there is plenty of factors for dislodgement, such as saliva-flushing action, masticatory movements, and continuous epithelial renewal.

C. albicans has the potential to switch between the yeast and the hyphal mode of growth and modify the surface antigen. After the development of a filamentous or pseudohyphal form, the organisms become invasive (Bilhan *et al.*, 2009). Hyphae have been observed to adhere to and invade host tissues more readily than the yeast form, suggesting that filamentous growth may

contribute to the virulence of this major human pathogen (Leberer *et al.*, 1997).

The aetiology for candidiasis could be derived from systemic and local factors. The systemic factor would include extremes of age, smoking, diabetes mellitus (Dorocka-Bobkowska *et al.*, 2010), Cushing's syndrome, malignancies, nutritional deficiencies such as Vitamin B12 deficiencies, use of broad-spectrum antibiotics (Dougall *et al.*, 1995), long-term steroid therapy (Kennedy *et al.*, 2000) and immunologically compromised patients due to AIDS/HIV infection (Sangeorzan *et al.*, 1994).

Local factors that can contribute to candidiasis include wearing orthodontic appliances or dentures (Mahmoudabadi and Drucker, 2003; Salerno *et al.*, 2011), poor denture/appliance hygiene (Salerno *et al.*, 2011), night-time wearing of denture, xerostomia due to Sjogren syndrome (Torres *et al.*, 2003) or post-radiotherapy of head and neck. However, there are scarce data on the amount of oral candidiasis between the two appliances.

Thus, the objective of this research is to assess and compare the amount of *C. albicans* hyphae on palatal mucosa of patients with palatal coverage removable appliance. In addition to that, the risk factors associated with the presence of candidiasis were also investigated.

Materials and methods

This was a cross sectional study where patients undergoing orthodontic or prosthodontic treatment in the Dental Faculty, Universiti Kebangsaan Malaysia that fulfilled following inclusion criteria were recruited: (1) those with good general health, (2) wearing dental appliances or prostheses with palatal coverage for at least more than 3 months (in tested group), (3) who were not under immunosuppressive medications such as antibacterial, antifungal drugs, steroids for 3 months before study, (4) without medical conditions of type II diabetes mellitus, HIV/AIDS, asthma/COPD. Patients were divided into 3 groups: (1) upper removable orthodontic appliance, (2) upper removable acrylic denture and (3) patients who did not have any palatal coverage denture nor appliance acted as control.

Ethical approval from university's ethical committee and consent form were obtained prior to the start of this study (UKM 1.5.3.5/244/SPP/DD/2011/010(2)).

A questionnaire was formulated and validated by pretesting it to 10 patients. Amendment to the questions were made accordingly. The questionnaire was divided into two parts. Part 1 obtained the demographic profile i.e. gender and age. Age of the subjects were categorized according to the age group i.e. adolescent (10 to 19 years old), adult (20 to 49 years old) and elderly (> 50 years old) (Sabharwal *et al.*, 2015). Part 2 assessed the risk factors associated with oral candidiasis.

Clinical evaluation of intraoral soft tissue was performed by three examiners in a well-illuminated and dry environment, focusing mainly on the signs and symptoms related to oral candidiasis such as presence of redness, soreness and swelling at the corner of the mouth and palate. Inflammation on the palatal mucosa was recorded according Newton's classification (Altarawneh *et al.*, 2013).

The appliance/prosthesis cleanliness was evaluated by assessing the amount of dental plaque. The appliance/prosthesis hygiene index was used to score dental plaque at the impression surface; score of 1: Good (no or very little plaque), score of 2: Fair (less than half of the denture base covered by plaque), score of 3: Poor (more than half of the denture base covered by plaque) (Bilhan *et al.*, 2009).

Unstimulated salivary flow is measured by draining into a graduated test tube for 10 minutes. Subjects were instructed not to masticate, swallow and speak during the process (Fox and Ship, 2008). This is carried out for at least 1 hour after eating, before the saliva collection. Saliva flow rate is expressed in ml/min; whole unstimulated salivary flow of 0.2 ml/min or less is considered as abnormal (Naik and Pai, 2011).

The presence of candidal hyphae is highly diagnostic of the candidal infection. It was diagnosed by taking smear layers from the palatal mucosa and fitting surface of the appliance/prosthesis of the patient using standard wooden tongue spatula moistened with normal saline. A few gentle strokes of the spatula were done in order to scrap out

some of the desquamated epithelium. It was then transferred onto glass slide and stained with Schiff reagent (Merck KGaA, Germany) for the periodic acid Schiff (PAS) reaction. The diagnosis of oral candidiasis was based on the presence of hyphae of *Candida* species together with clustered keratinized cells.

The hyphae were demonstrated on a PAS smear within surface stratified squamous epithelium. The stained slides were initially scan under low magnification (4x objective magnification) microscope (Olympus binocular/ CHK2-213 light microscopy) to locate foci of desquamated keratinocytes together with candidal hyphae if any. Then, the higher magnification of 10x to 40x objectives were used to confirm the presence of candidal hyphae. The authors, consisting of one calibrated oral pathologist (NI) and three dental students (LYY, LML and MHL), had counted the presence and number of candidal hyphae which had morphology index (Mi) value of greater than 3 for true hyphal cells (Merson-Davies et al., 1991). The quantification of the hyphae from oral samples was defined as scanty, moderate or severe. These were labelled respectively as three classes: 1: 1-10 hyphae, 2: 11-20 hyphae and 3: > 20 hyphae.

All data collected were analysed using the Statistical Package for the Social Sciences (IBM SPSS) version 21. Chi-square test was used to analyse the significance of each risk factor. The chi-square tests were carried out to establish the statistical significance between candidiasis to other risk factor parameters. A *p*-value of less than 0.05 was considered statistically significant.

Results

Demographic profile of subjects

A total of 86 subjects were included in this study, aged from 13 to 74 years old, of whom 34 subjects were in non-appliance group (control), 30 subjects wore upper acrylic denture and 22 subjects wore upper removable orthodontic appliance (URA). In denture group, most of the patients are elderly (n=28), while orthodontics patients were of younger age with 8 adolescents and 14 adults when compared to the control

group in which majority were adults (n=26) (Table 1). There was an equal number of patients in each gender, female (n=17) and male (n=17) recruited in control group. Majority of the patients wearing upper removable denture were male (n=18) while in contrast more than 90% of the patients wearing upper removable appliance were female (n=20) as shown in Table 1.

Prevalence of candidiasis

In control group, majority of the subjects had no evidence of candidiasis (79.41%). However, in the denture and URA groups, more than half were positive cases as seen in Table 1. There was statistically significant difference in the prevalence of candidiasis in both denture and URA groups compared to control group ($p < 0.05$). Most of the affected subjects in all three groups presented in scanty amount of *Candida*. Severe cases were noted in the denture group (n=6).

Risk factors

a) Gender

There were 16 male and 23 female patients found to have candidiasis infection. In both genders, the positive result was found highest in scanty amount of candidiasis detected. When using the Pearson Chi-Square Test to compare the prevalence of candidiasis between gender group, there was no statistically significant difference ($p > 0.05$). (Table 2).

b) Age

Many positive cases noted with an increasing trend with age (Table 2). The highest positive cases were noted in elderly subjects (n=18, 45%). Most of the cases were scanty and were found in 18 adults and elderly. An association between the amount of *Candida* with the different age group was observed ($p < 0.05$) when analysed using Pearson chi-square test.

c) Medical problems

Hypertension was the most common medical problems and a wide range of other diseases were noted in the control group and denture patients. However, in URA group, all the patients are fit and healthy without any medical problems. In denture

group, hypertension (n=10) and other medical problems (n=12) were detected in one third of the patients respectively. Compared to the control group, only a small number of patients were medically compromised (n=5). Since hypertension is the most common disease in this study, cross tabulation of hypertension with presence of candidiasis is observed and it was found that more than half of the hypertensive patients presented with positive candidiasis result (Table 2). The significant test was done to compare prevalence of candidiasis with presence of hypertension. However, it was found not statistically significant ($p>0.05$).

d) Drugs

Medications taken by patients were anti-hypertensive drugs, aspirin and other drugs which correlate with their medical problems. All hypertensive patients were taking antihypertensive drugs (n=12). Eight positive smear results were found from 12 patients taking antihypertensive drugs (Table 2). No significant difference was found when tested between prevalence of candidiasis and antihypertensive drugs.

e) Smoking status

There was total of 15 smokers and 71 non-smokers recruited. The highest number of smokers was from denture group (n=10). From the cross tabulation of smoker with amount of candidiasis, 34 patients with positive smear result were non-smokers (85%), only 6 positive results were from smokers (Table 2). From Pearson Chi-Square test, no significant difference was found between smoking and prevalence of candidiasis ($p<0.05$).

f) Symptoms

When five symptoms of oral candidiasis from the questionnaire listed in Table 2 below were evaluated, only 23 patients experienced the symptoms and 63 patients had no symptoms at all. There were 6 patients' complaint about burning of the mouth or throat, 14 patients had bad breath, and 3 patients experienced bad taste when eating. Presence of redness, soreness and swelling at the corners of the mouth noted by 2 patients and pain when eating spicy food or drinking acidic beverage

experienced by 3 patients. In each cross tabulation of individual symptom for patients with positive symptom, nearly half of them presented with positive *Candida* infection except in symptom at angle of mouth. All patient that complaint on redness at the corner of mouth was detected with severe candidiasis infection. (Table 2).

g) Age of the appliance/prosthesis

The duration of wearing an appliance/prosthesis is classified into 3 groups: 3 months to 6 months, 6 months to 1 year, and more than 1 year. For patient wearing upper removable denture, more than half of the group received and wore it for more than 1 year (n=17, 56.67%), 11 patients received for 6 months to 1 year (36.67%), and only 2 patients receive for 3 to 6 months (6.67%). For URA group, in contrast with denture group, more than half of them were wearing newly received URA for 3 months to 6 months (n=15, 54.55%), 23% received for 6 months to 1 year and more than 1 year respectively (n=5). From the cross tabulation (Table 2), it can be seen that the number of patients with different amount of candidiasis within each duration groups were distributed with very small differences, from minimum 0 patient to maximum 5 patients in denture group and maximum 4 patients in URA group with candidiasis.

h) Time of wearing appliance/prosthesis

Patients wear their appliances or prostheses in daytime only, night-time only or both day and night. Around two third of denture patients wore their prostheses during the daytime (n=19). Unlike the denture patients, almost half of URA patients wore their appliance at night-time only (n=10). Similar amount of denture and URA patients wore both in the day and night-time (n=11). The number of total positive *Candida* smear result in daytime wearer, night-time wearer and all-day wearer were 10 patients, 8 patients and 11 patients respectively. 80% of night-time wearer and 75% all-day wearer had *Candida* infection, when compared to daytime wearer, lower percentage showed positive *Candida* infection (50%) (Table 2). Statistically, time of wearing had significance in the prevalence of candidiasis ($p<0.05$).

i) Duration of wearing appliance/prosthesis

Duration of wearing an appliance/prosthesis was categorized into 2 groups: less than 12 hours and more than 12 hours. For both denture and URA group, most of the patients wore for more than 12 hours, 25 denture patients and 14 upper removable appliance patients. From the thirty-nine wearers of more than 12 hours, twenty-six of them had presented with candidiasis infection (67%). Seven patients from less than 12 hours group had positive candidiasis result (54%). The amount of *Candida* was significantly associated with the hours of wearing of the appliance/prosthesis ($p < 0.05$) (Table 2).

j) Cleaning of the appliance/prosthesis

Only one denture patient did not have habit of cleaning denture daily, other patients cleaned their denture and appliance daily either with tap water or soap. However, there is still large number of patients had *Candida* infection with total of 34 patients from both denture and ortho group. The only patient with no cleaning habit had scanty *Candida* infection (Table 2). The cleaning habit was significantly associated with the amount of *Candida* present ($p < 0.05$).

k) Saliva flow rate

The normal unstimulated saliva flow rate was recorded as 0.2 ml/min and above. There were 57 with normal and 29 with abnormal saliva flow rate patients found. All test groups had higher number of patients with normal saliva flow activity. From all the abnormal saliva flow patients, less than half

of patients have positive *Candida* result ($n=11$). In contrast, more than half of normal saliva flow patients presented with positive *Candida* result ($n=19$) (Table 2).

Intraoral lesions

In this study, the relation of wearing an appliance/prosthesis with intraoral and circumoral soft tissue including angle of mouth, tongue and hard palate were observed by inspection for any abnormalities. All results are shown in Table 2. The condition at the angle of mouth in control group was normal; only one patient from each of the tested group presented with fissuring. The denture patient who had fissuring angle of the mouth had severe candidiasis infection while the URA patient with fissuring did not have any *Candida* infection. For tongue lesion, the abnormalities observed were fissure tongue. Only one control patient and three denture patients have fissure tongue. Among four fissure tongue cases, two of them presented with *Candida* infection, with one scanty and one severe amount of candidiasis. Therefore, similar to angle of mouth, high prevalence of candidiasis infection was detected in normal tongue patients. On the hard palate, most of the abnormalities were found in denture patient. Twelve denture patients had red spot lesion, nine of them presented with Newton's type 1 and three patients with Newton's type 2. In URA group, only one patient had Newton's type 1. There is no abnormality found on the hard palate from control group. Nine from thirteen red spot lesions cases were found to have positive infection.

Table 1 Comparison of subjects' demographic profile, amount of candidiasis risk factors and intraoral lesions between control, denture and removable orthodontic appliance (URA) groups

		Group (n)		
		Control	Denture	URA
Demographic profile:				
Gender	Female	17	12	20
	Male	17	18	2
Age	Adolescent	4	0	8
	Adult	26	2	14
	Elderly	4	28	0
Amount of candidiasis:	Negative	27	13	6
	Scanty	5	8	8
	Moderate	2	3	5
	Severe	0	6	3
Risk Factors:				
Medical problems	Hypertension	2	10	0
	Others	3	12	0
	None	30	11	22
Drugs	Anti-hypertension	2	10	0
	Aspirin	1	0	0
	Others	3	12	0
	None	29	11	22
Smoking status	Smoker	4	10	1
	Non-smoker	30	20	21
Symptoms	Burning of the mouth and throat	2	3	1
	Bad breath	6	5	3
	Bad taste	2	1	0
	Redness, soreness and swelling at the corners of the mouth	0	2	0
	Pain when eating spicy food or drinking acidic beverage	2	0	1
	None	24	22	17
Appliance/prosthesis age	3 months to 6 months	-	2	12
	6 months to 1 year	-	11	5
	More than 1 year	-	12	5
Time of wearing	Day only	-	19	1
	Night only	-	0	10
	Both	-	11	11
Daily total hours of wearing	Less than 12 hours	-	5	8
	More than 12 hours	-	25	14
Cleaning appliance/prosthesis	Yes	-	29	22
	No	-	1	0
Appliance/prosthesis hygiene	Good	-	10	8
	Moderate	-	11	9
	Poor	-	9	5
Saliva flow rate	Normal	20	19	18
	Abnormal	14	11	14
Intraoral Lesions:				
Angle of mouth	Fissuring	0	1	1
	No abnormality detected	34	29	21
Tongue	Fissured tongue	1	3	0
	No abnormality detected	31	27	21
Hard palate	Newton's Type I	0	9	0
	Newton's Type II	0	3	0
	No abnormality detected	34	17	21

Table 2 Prevalence of candidiasis in different risk factor parameters

Parameters		Prevalence of candidiasis				χ^2
		Negative	Positive			
			Scanty	Moderate	Severe	
<u>Risk Factors</u>						
Gender	Male	21	9	2	5	NS
	Female	26	12	8	3	
Age	Adolescent	4	3	2	3	0.047
	Adult	28	9	5	0	
	Elderly	14	9	3	6	
Medical problems	Hypertension	4	3	2	3	NS
	Others	3	4	2	0	
	None	127	55	26	22	
Drugs	Anti-hypertension	5	3	2	3	NS
	Aspirin	0	1	0	0	
	Others	3	5	1	1	
	None	129	51	57	22	
Smoking status	Smoker	9	3	1	2	0.578
	Non-smoker	37	18	9	7	
Symptoms	Burning of the mouth and throat	3	2	0	1	NS
	Bad breath	7	5	0	2	
	Bad taste	1	2	0	0	
	Redness, soreness and swelling at the corners of the mouth	0	0	0	2	
	Pain when eating spicy food or drinking acidic beverage	1	1	1	0	
	None	126	53	29	22	
Appliance/ prosthesis age	3 months to 6 months	5	4	4	1	NS
	6 months to 1 year	5	5	2	4	
	More than 1 year	9	7	2	4	
Time of wearing	Day only	10	4	1	5	0.000
	Night only	2	2	5	1	
	Both	7	10	2	3	
Duration of wearing	Less than 12 hours	6	3	4	0	0.000
	More than 12 hours	13	13	4	9	
Cleaning appliance/prosthesis	Yes	19	15	8	9	NS
	No	0	1	0	0	
Appliance/ prosthesis hygiene	Good	15	3	0	0	0.000
	Moderate	4	12	2	2	
	Poor	0	1	6	7	
Saliva flow rate	Normal	28	13	10	6	0.468
	Abnormal	18	8	0	3	
<u>Intraoral Lesions:</u>						
Angle of mouth	Fissuring	1	0	0	1	NS
	No abnormality detected	45	21	10	8	
Tongue	Fissured tongue	2	1	0	1	NS
	No abnormality detected	42	19	10	8	
Hard palate	Newton's Type I	2	1	4	3	NS
	Newton's Type II	2	1	0	0	
	No abnormality detected	17	8	5	42	

Discussion

This is a cross sectional clinical study where smear technique was used to detect the presence of *Candida spp.* in patients with palatal coverage prosthesis or appliance. The conventional diagnostic methods for recovery of yeasts in oral cavity are swabs and smears (Olsen and Stenderup, 1990). These methods are non-invasive, safe and convenient which examined for blastospores, hyphae, and inflammatory cells to ensure rapid presumptive diagnosis. While oral smear is a fast and inexpensive method, swab takes much longer time for the result and more expensive. In addition, swab also offers good sensitivity and specificity in determining the species of *Candida* (Coronado-Castellote and Jiménez-Soriano, 2013). However, in the present study, we only wanted to determine the presence of candidal hyphae which indicates pathogenicity. *Candida* is a normal commensal of the oral cavity and if it is not in pathogenic state, it is presented as blastospores.

The result from this study suggested that patients who were wearing palatal coverage removable appliances or prostheses had higher prevalence of oral candidiasis. The presence of mild, moderate or severe colonies of candidal hyphae may represent the severity stages of infection. For example, the mild type of patients may not have developed the sign or symptom yet. The result from this study can be used to educate the clinicians and patients who is wearing palatal coverage appliances/ prostheses that they may be predisposed to the risk of candidiasis.

Both denture and URA groups have more than half of subjects presented with *Candida* colonization on the palatal mucosa and fitting surface of prosthesis or appliance. Placement of palatal coverage prosthesis/appliance in the oral cavity results in alteration of microflora. It produces a microenvironment conducive to the growth of *Candida* with low oxygen, low pH, and an anaerobic environment. These enhanced adherences of *Candida* to acrylic together with reduced saliva flow under the surfaces of the denture fittings thus increase risk of candidiasis (Akpan and Morgan, 2002). In the present study, the

amount of candidal hyphae was more on the fitting surface of prosthesis or appliances when compared to the palatal mucosa. This finding can be correlated to a study where they found that *C. albicans* are more readily adhere to the porous acrylic resin rather than the mucosa (Mahmoudabadi and Drucker, 2003). Furthermore, the roughness of the fitting surface of an appliance/prosthesis as a result from the impression of normal morphology of one's palate e.g. rugae can contribute to the *Candida* colonization as compared to the polished surface of the appliance/prosthesis.

Prosthesis- or appliance-related stomatitis is resulted by interaction of various local and systemic factors and is a multifactorial disease (Webb et al., 1998; Naik and Pai, 2011). The present study evaluated many predisposing factors at once, which include age, medical problems, drug taken, whole unstimulated salivary flow rate, smoking, oral hygiene, wearing of appliance or denture that cover the palate (susceptible tissue), denture/URA hygiene, night-time wear of denture or appliance, daily total hours of wearing denture/URA. The present study showed that some predisposing factors such as age, denture/URA hygiene, night-time wearing, daily total hours of wearing (more than 12 hours per day) have more significant role in the development of oral candidiasis.

Mean age for URA and dentures wearers is 22.3 and 60.2 years old respectively. The large differences of age between the tested groups were inevitable. This is a normal clinical scenario where URA wearers were usually adolescent to young adult who needs either interceptive orthodontic treatment or being treated for a mild case of malocclusion. However, denture wearers were usually adult and elderly who has loss their teeth due to caries or periodontal problems. The present study showed an increase in the prevalence of oral candidiasis with increasing age. Elderly can develop numerous physiological and/or pathological conditions that will influence their oral microbial balance or maintenance of their oral health (Bianchi et al., 2016). Another study also showed that individual with increase age will have reduced immunity, which make elderly

become predisposed to infection (Akpan and Morgan, 2002). However, this finding should be interpreted with caution because of the large age differences between the tested groups.

The major medical problem among the present subjects was hypertension. Until now, hypertension or hypertensive drugs do not have any proven relation to oral candidiasis. The present study also did not reveal any significance of oral candidiasis in relation to either hypertension or any medical and drug history of a patient.

Abnormal saliva flow was detected in 33.7% of subjects in the present study. A study had showed that presence of *Candida spp.* was inversely proportional to salivary flow rate (Torres et al., 2003). However, the present study showed no increase in the oral candidiasis risk among those with low salivary flow. The difference in findings may due to the difference in the technique used to collect whole unstimulated saliva.

Positive *Candida* cases were not significantly related to patient's smoking status. Even though smoking is considered as a risk factor of oral candidiasis in many studies (Akpan and Morgan, 2002; Tarçin, 2011) however the exact mechanism of action has not yet been established (Tarçin, 2011).

Dental plaque can become an ideal feeding ground for fungi and other microorganisms, leading to the development of infections and inflammation of the mucosa (Evren et al., 2011). In this study, denture or appliance hygiene status was found to be a significant risk factor for candidiasis as the plaque accumulates at the palatal surface.

Night-time wear of denture or appliance can increase risk of oral candidiasis. Presence of a denture on the oral mucosa can alter the local environment. It causes inaccessibility of the saliva and lack of mechanical cleaning by the tongue. During the night, the whole volume of the saliva decreases. Hence, prostheses/appliances act as reservoirs that harbours *Candida* (Sahebamee et al., 2011).

Subjects that wear denture or appliance more than 12 hours per day showed more prevalence of oral candidiasis. Continuous wearing of denture is proven to increase the likelihood of injury

to palatal mucosa and density of *C. albicans* on fitting surface of maxillary denture (Zissis et al., 2006). This also explains why subjects that wear their URA for more than 12 hours per day had increased risk of having oral candidiasis.

The majority of the present subjects did not present with any signs or symptoms of candidiasis. This is an expected result, as oral candidiasis is usually asymptomatic (Tarçin, 2011). However, when signs and symptoms present as seen in cases such as erythematous candidiasis, they may include mucosal bleeding, swelling, burning sensations, altered taste and bad breath (Webb et al., 1998). Those red lesions can be differentiated from erythroplakia by prescribing antifungal agent to patients and if the lesion healed, erythroplakia can be rule out. In order to get a definitive diagnosis, a biopsy can be done.

Therefore, the wearers of an orthodontic appliance or a prosthesis should take these precautions; correction of predisposing factors, maintenance of proper oral hygiene and oral prostheses and the use of antifungal agent based on severity of infection (Patil et al., 2015) in order to maintain a healthy intraoral environment.

Conclusion

Patients who are wearing palatal coverage removable appliances or prostheses have higher prevalence of oral candidiasis with severe cases noted in the denture wearer. Age, time & duration of wearing and oral hygiene are contributing factors in presentation of oral candidiasis.

Acknowledgement

We thank Ms. Zetty Saiful Bahry, laboratory assistant from Oral Pathology and Oral Medicine Department, for her laboratory assistance.

References

- Akpan A, Morgan R (2002). Oral candidiasis. *Postgrad Med J*, 78(922): 455-459.
- Altarawneh S, Bencharit S, Mendoza L, Curran A, Barrow D, Barros S et al. (2013). Clinical and histological findings of denture stomatitis as related to intraoral colonization patterns of *Candida albicans*, salivary flow, and dry mouth. *J Prosthodont*, 22(1): 13-22.

- Bianchi CM, Bianchi HA, Tadano T, Paula CR, Hoffmann-Santos HD, Leite DP Jr et al. (2016). Factors related to oral candidiasis in elderly users and non-users of removable dental prostheses. *Rev Inst Med Trop Sao Paulo*, **58**:17.
- Bilhan H, Sulun T, Erkose G, Kurt H, Erturan Z, Kutay O et al. (2009). The role of *Candida albicans* hyphae and *Lactobacillus* in denture-related stomatitis. *Clin Oral Investig*, **13**(4): 363-368.
- Coronado-Castellote L, Jiménez-Soriano Y (2013). Clinical and microbiological diagnosis of oral candidiasis. *J Clin Exp Dent*, **5**(5): e279-e286.
- Dorocka-Bobkowska B, Zozulinska-Ziolkiewicz D, Wierusz-Wysocka B, Hedzelek W, Szumala-Kakol A, Budtz-Jørgensen E (2010). *Candida*-associated denture stomatitis in type 2 diabetes mellitus. *Diabetes Res Clin Pract*, **90**(1): 81-86.
- Dougall HT, Smith L, Duncan C, Benjamin N (1995). The effect of amoxycillin on salivary nitrite concentrations: An important mechanism of adverse reactions? *Br J Clin Pharmacol*, **39**(4): 460-462.
- Evren BA, Uludamar A, Işeri U, Ozkan YK (2011). The association between socioeconomic status, oral hygiene practice, denture stomatitis and oral status in elderly people living different residential homes. *Arch Gerontol Geriatr*, **53**(3): 252-257.
- Fox PC, Ship JA (2008). Salivary gland diseases. In: Greenberg MS, Glick M, Ship JA (eds.), *Burket's Oral Medicine*, 11th edn. Hamilton: BC Decker Inc., pp. 191-194.
- Kennedy WA, Laurier C, Gautrin D, Ghezzi H, Paré M, Malo JL et al. (2000). Occurrence and risk factors of oral candidiasis treated with oral antifungals in seniors using inhaled steroids. *J Clin Epidemiol*, **53**(7): 696-701.
- King RD, Lee JC, Morris AL (1980). Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infect Immun*, **27**(2): 667-674.
- Leberer E, Ziegelbauer K, Schmidt A, Harcus D, Dignard D, Ash J et al. (1997). Virulence and hyphal formation of *Candida albicans* require the Ste20p-like protein kinase CaCl4p. *Curr Biol*, **7**(8): 539-546.
- Mahmoudabadi AZ, Drucker DB (2003). Comparison of mycelial production by *Candida albicans* isolated from different sources. *Iran Biomed J*, **7**(4): 187-189.
- Merson-Davies LA, Odds FC, Malet R, Young S, Riley V, Schober P et al. (1991). Quantification of *Candida albicans* morphology in vaginal smears. *Eur J Obstet Gynecol Reprod Biol*, **42**(1): 49-52.
- Naik AV, Pai RC (2011). A study of factors contributing to denture stomatitis in a north Indian community. *Int J Dent*, **2011**:589064.
- Olsen I, Stenderup A (1990). Clinical-mycologic diagnosis of oral yeast infections. *Acta Odontol Scand*, **48**(1): 11-18.
- Patil S, Rao RS, Majumdar B, Anil S (2015). Clinical appearance of oral *Candida* infection and therapeutic strategies. *Front Microbiol*, **6**:1391.
- Sabharwal S, Wilson H, Reilly P, Gupte CM (2015). Heterogeneity of the definition of elderly age in current orthopaedic research. *Springerplus*, **4**:516.
- Sahebjamie M, Shabestari SB, Asadi G, Neishabouri K (2011). Predisposing factors associated with denture induced stomatitis in complete denture wearers. *J Dent (Shiraz)*, **11**: 35-39.
- Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L et al. (2011). *Candida*-associated denture stomatitis. *Med Oral Patol Oral Cir Bucal*, **16**(2): e139-e143.
- Sangeorzan JA, Bradley SF, He X, Zarins LT, Ridenour GL, Tiballi RN et al. (1994). Epidemiology of oral candidiasis in HIV-infected patients: Colonization, infection, treatment, and emergence of fluconazole resistance. *Am J Med*, **97**(4): 339-346.
- Tarçın BG (2011). Oral candidosis: Aetiology, clinical manifestations, diagnosis and management. *MÜSBED*, **1**(2): 140-148.
- Torres SR, Peixoto CB, Caldas DM, Silva EB, Magalhães FA, Uzeda M et al. (2003). Clinical aspects of *Candida* species carriage in saliva of xerostomic subjects. *Med Mycol*, **41**(5): 411-415.
- Webb BC, Thomas CJ, Willcox MDP, Harty DWS, Knox KW (1998). *Candida*-associated denture stomatitis. Aetiology and management: A review. Part 2. Oral diseases caused by *Candida* species. *Aust Dent J*, **43**(3): 160-166.
- Zissis A, Yannikakis S, Harrison A (2006). Comparison of denture stomatitis prevalence in 2 population groups. *Int J Prosthodont*, **19**(6): 621-625.
- Zunt SL (2000) Oral candidiasis: Diagnosis and treatment. *J Practical Hyg*, **9**(5): 31-36.