



Research Article

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Multiple Risk Factors of *Candida Albicans* Associated Denture Stomatitis

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Abstract

Background and Objectives: Opportunistic oral fungal infections have spread, especially in denture wearers. Denture stomatitis (DS) is a common inflammatory reaction with multifactorial etiology. It is usually associated with *Candida species*, particularly *Candida albicans*, due to its high virulence, ability to adhere and form biofilms on oral cavity tissues and denture surfaces. This study highlights the risk factors of *Candida albicans*-associated denture stomatitis. The study was carried out at Prosthodontics Departments at the Faculty of Dentistry- Sana'a University, Sana'a city - Yemen.

Study design: The study group consisted of 288 denture wearers suffering from DS. Data including demographic data of the patients, clinical information, and risk factors of DS recorded in predesigned questionnaire.

Result: There was a significance association between male (OR=2.33, $p<0.001$), older age patients (OR=6.8, $p<0.001$), denture poor fitness (OR=8.2, $p<0.001$), older age denture (OR=14.6, $p<0.001$), denture manufacturers under supervision of general dentist (OR=2.9, $p<0.001$), non-ideal denture quality (OR=4.8, $p<0.001$), irregular denture cleaning (OR=2.1, $p<0.001$), and often overnight denture wear (OR=1.7, $p=0.04$).

Conclusion: Management of predisposing risk factors is key in order to prevent recurrence of DS. Treatment methods may consist of: treatment of any underlying systemic risk factors, improvement in the fit of existing dentures, replacement of existing dentures, improved denture hygiene.

Keywords: *Candida albicans*-associated denture stomatitis; Denture; Ds risk factors; Sana'a city; Yemen

Introduction

Longer life expectancy has led to an increase in the ageing population worldwide. This growth in the number of elderly led to an increase in the number of people requiring removable dentures [1,2]. The current rates of edentulism have been estimated to be between 7 percent and 69 percent of the adult population internationally [3]. *Candida* is present in the oral cavity in two different forms, as floating planktonic cells (*blastospores*, *blastoconidia*) and/or in an organized biofilm. Poor oral hygiene, practices such as failure to remove the denture whilst sleeping and poor denture cleansing allows the accumulation of biofilm [4-6]. Biofilm is defined as structured microbial community that is attached to a surface, consisting of more than 10^{11} cells per gram

of dry weight [4,7] and bounded by a self-produced extracellular matrix. Biofilms are created adhering to living tissue such as mucosal surfaces or to abiotic surfaces such as oral prostheses, implanted medical devices, and intravascular catheters [8]. It has been recognized that unlike dental biofilm, the biofilm that forms on denture materials possesses a massive population of yeasts [9]. A spongy denture tissue surface, full of nutritive substances, is an idyllic incubator for species such as *Candida albicans* [10]. *Candida albicans* is a normal flora in limited number in the oral cavity of 45-65% of healthy individuals with a higher prevalence recovered in young adults and children [10]. In denture wearers, the prevalence of *Candida* raised up to 100% [7,11-17]. In addition *Candida* can be opportunistic, which can be explained by the fact that dentures

reduce the flow of oxygen and saliva to the underlying tissue creating a local acidic and anaerobic micro-environment that favours yeast overgrowth. Additionally, *Candida* has affinity for the acrylic surface of dentures and non-renewing surfaces such as teeth, dental fillings [17-22]. Surface characteristics of denture base acrylic resins, such as hydrophobicity, have generally been acknowledged to be one of the factors contributing to the adhesion, which is a crucial step in biofilm formation [23-25]. *Candida albicans* biofilms are frequently associated with the occurrence of denture stomatitis [20,24,26]. This study highlights the risk factors of *Candida albicans*-associated denture stomatitis.

Subjects and Laboratory Methods

Cross sectional study was carried out in the Prosthodontic department at the Faculty of Dentistry- Sana'a University, from 11/2016 to 2/2017 on 288 Yemeni subjects (males and females), aged more than 35 years old. Data were collected by predesigned questionnaire. Samples were collected by wiping premoistened sterile cotton wool swabs on upper and lower denture fitting surfaces. For mycological investigations each sample was inoculated into both media: Sabouraud's dextrose agar and a differential and selective culture media as *Candida* Chromogenic media. Plates were incubated aerobically for 48 to 72 hours at 37°C and were identified systematically by Colonial morphology, wet mount preparation, Gram's stain and germ tube.

Table 1: The relationship between sex and age (as risk factors) and *Candida albicans* stomatitis.

	<i>C. albicans</i> positive n=168		OR	CI	χ^2	p
	No	%				
Sex						
Male n= 171	114	66.7	2.33	1.4- 3.9	12.03	<0.001
Female n= 117	54	46.2	0.43	0.2- 0.17	12.03	<0.001
Age						
< mean age n=122	41	33.6	0.1	0.09-0.2	53	<0.001
≥ mean age n=166	127	76.5	6.4	3.8-10.8	53	<0.001
Total n=288	168	58.3				

Mean age=59.5years

Table 2: The connection between *Candidal* stomatitis and denture fitness and age of denture.

Factors	<i>C. albicans</i> positive n=168		OR	CI	χ^2	p
	No	%				
Denture fitness						
Good n= 52	22	42.3	0.4	0.2-0.8	6.7	0.009
Fair n= 99	46	46.5	0.5	0.3-0.7	8.7	0.003
Poor n= 137	100	73				<0.001
Denture age (mean in month= 20.2 months)						
< mean n=126	33	26.2	0.07	0.04-0.12	95	<0.001
≥ mean n=162	135	84.4	14.6	8.2-26	95	<0.001

When denture fitness was considered, there was significance association between the poor fitness and *Candida albicans* DS (OR=8.2, CI=8.2-26, and $p<0.001$) (Table 2). Also, there was a highly significant association between older age denture (mean ≥ 20.2 months) and contracting *Candida albicans* stomatitis DS (OR=14.6, CI= 8.2-26, with $p<0.001$) (Table 2).

Data Collection

Data including demographic data of the patients and risk factors of *Candida albicans* DS. The findings were recorded in a predesigned questionnaire with laboratory results.

Ethical Approval

We obtained written consent from all cases. Assent was taken from participants before collecting the specimens. The study protocol was reviewed and approved by the Ethics Committee of Sana'a University, Faculty of Medicine and Health Sciences.

Result

This study was conducted on a total of 288 patients contracting DS attending Prosthetic department at the Faculty of Dentistry-Sana'a University from 11/2016 to 2/2017. Male patients accounted for 59.4% while female patients accounted for 40.6% of the total cases. The patient's age ranged from 35-65 years, and most of the patients were at the age group of +65 years, (32.6%). There was a significant association between male and high risk of contracting *Candida albicans* DS (OR=2.33, CI=1.4 to 3.9, and $p<0.001$). There was a significant association between older age patients (≥ mean age=59.5 years) and contracting *Candida albicans* DS (OR=6.8 times, CI=3.8-10.8, with $p<0.001$) (Table 1).

Table 3 shows the association of *Candidal* stomatitis with multiple risk factors of denture stomatitis. There was risk of contracting DS with denture manufacturers supervision under general dentist (OR=2.9, CI= 1.6-5, with $p<0.001$), non-ideal denture quality (OR=4.8, CI=2.8 -8.8, with $p<0.001$), irregular denture cleaning (OR=2.1, CI= 1.3-3.5, with $p<0.001$), but no significant

effect with frequency of denture cleaning per day. Also, there was risk of contracting *Candida albicans* DS with often overnight denture wear (OR=1.7, CI= 1.0-31, with $p=0.04$), but no significant

association between overnight denture solutions or dental checkup per year and contracting *Candida albicans* DS.

Table 3: The association of *Candidal* stomatitis with multiple risk factors of denture stomatitis.

Factors	<i>C. albicans</i> positive n=168		OR	CI	χ^2	p
	No	%				
Denture manufacturers under supervision of						
General dentist n=216	140	65	2.9	1.6-5	14.9	<0.001
Dental consultant n=72	28	38.9	0.34	0.19-0.5	14.9	<0.001
Denture quality						
Non-ideal n= 212	145	68.4	4.8	2.8-8.8	33.4	<0.001
Ideal n=76	23	30.3	0.2	0.11-0.03	33.4	<0.001
Denture hygiene						
Regular cleaning n=171	87	50.9	0.4	0.28-0.75	9.4	0.001
Irregular cleaning n=117	81	69.2	2.1	1.3-3.5	9.4	0.001
Freq of cleaning per day						
Once n=33	18	54.5	0.8	0.3-1.5	0.46	0.49
Twice n=43	25	55.5	0.9	0.5-1.9	0.008	0.97
Three n=67	31	46.3	0.52	0.3-0.9	5.2	0.02
More n=28	13	46.4	0.6	0.2-1.2	1.8	0.17
Often Overnight denture wear						
Yes n=75	51	68	1.7	1.0-31	3.9	0.04
No n=213	117	54.9	0.6	0.3-0.9	3.9	0.04
Overnight denture solution						
Water n=151	80	53	0.6	0.3-1.0	3.8	0.05
Saline n=13	6	46.1	0.6	0.19-1.8	0.8	0.36
None n=49	31	63.2	1.2	0.6-2.4	0.59	0.44
Dental checkup per year						
Yes n=25	11	44	0.5	0.2-1.2	2.3	0.12
No n=263	157	59.7	1.9	0.8-4.3	2.3	0.12

Discussion

In the current study the rate of *Candida albicans* DS was 58.3%. This high rate reflects the power of *Candida albicans* to form biofilm on the denture. There are many factors affect adhesion and biofilm formation of *Candida* on denture surfaces, such as surface roughness of the inner surface of the prosthesis, salivary pellicle, hydrophobic and electrostatic interactions, receptor-ligand binding [1,7,15,22,23,28,29]. Also, there are several reports to suggest the relationship between surface roughness and *Candida albicans* adherence to denture materials. Huh *et al.* and Hahnel *et al.* reported that significantly higher number of *Candida albicans* was observed on roughened than on smooth surfaces [22,28]. Denture base cracks may become to be one of the best sites for microorganism propagation and provides protection from shear forces, even during denture cleaning [29,30]. The second step in DS is adherence of *Candida albicans* to host epithelial cells is a critical first step in the infection process [6-8,23,29-33]. It is essential for both colonization and subsequent induction of mucosal disease [31]. These microorganisms can stick and proliferate through the hard and soft tissues of the oral cavity [29]. Also, the fact that both *Candida* and epithelial cell surface are negatively charged means that

there are disgusting forces retarding their adhesion. Nevertheless, there are other attractive forces such as Lifshitz-van der Waals forces, hydrophobic interactions, and Brownian movement forces. The sum of these nonspecific forces will determine whether the initial nonspecific adhesion between fungal and epithelial cells will be established [8,23].

When gender of patients in the current study was considered, there was significance association between the male patients and risk of increase *Candida albicans* DS (OR=2.33, CI=1.4-3.9, and $p<0.001$) (Table 1). Our result is in contrast with previous reports by Chopde *et al.*, Javed *et al.* and Naik & Pai in which they have been reported that oral *Candida* colonization is significantly higher in females compared to males; however, this relationship remains controversial [16,34,35]. The *Candida* colonization lower rate in females compared to males in our study might be explained by the findings of Li-Hui *et al.* in which salivary pH values were significantly lower in females than that in males (acidic salivary pH is unfavorable condition growth for *Candida albicans*) [36]. When age was taken into account in the current study, there was a highly significant association between older age group (\geq mean age=59.5 years) and contracting *Candida albicans* DS (OR=6.8 times, CI=3.8-

10.8, with $p < 0.001$) (Table 1). This result can be explained by the fact that older age people generally suffer from systemic illnesses, changes in nourishment and their salivary characteristics as low saliva production and contents [16]. Advancing age is also a risk factor for denture stomatitis in the elderly, because cell-mediated immunity, which provides protection against *Candida albicans* infection declines with age. According to Ryu et al., some oral environmental factors, such as un-stimulated salivary flow rate and age of subjects, are associated with higher numbers of microbes in the saliva of complete denture wearers. Those authors suggest that a reduction in the salivary flow rate with aging induces an increase in concentration of microbes in saliva [37].

As denture fitness was considered, there was significance association between the poor fitness and *Candida albicans* DS (OR=8.2, CI=8.2-26, and $p < 0.001$) (Table 2). An ill-fitting denture may cause frictional irritation of the palatal mucosa and this facilitates invasion of *Candida* into the superficial layers of the epithelium. On occasions, a denture soft liner may be used to cushion the hard-acrylic material of the denture against the mucosa. Unfortunately, silicone rubber (the most frequently employed material for soft liners) is also a surface that *Candida* can readily colonize and actually invade [5]. The fact that the oral epithelium is continually replenished means that, in order to colonize the oral mucosa, *Candida* must be present in the mouth in sufficient numbers and with a high enough growth rate to allow their continued persistence [5].

Once age of the denture was considered in the present study, there was a highly significant association between older age denture (\geq mean denture age=20.2 months) and contracting *Candida albicans* DS (OR=14.6 times, CI=8.2-26, with $p < 0.001$) (Table 2). This result can be explained by that, denture age is shown to be an important factor as a result of poor fit, roughness, inadequate hygiene, and accumulation of plaque due to aging of denture [20,25,35]. It was reported, that aging of the denture and release of residual monomer with time results in poorer fit which affects the contamination of the denture. Moreover, denture age was proportional to *Candida* colonization and not to degree of inflammation [11,35]. Only 25% of individuals using dentures for <1 year were diagnosed with denture stomatitis, whereas >84% of those using dentures for >5 years had the disease [33].

As soon as oral/denture hygiene was determine, there was significance association between the negative response of cleaning and *Candida albicans* DS (OR=2.1, CI=1.-3.5, and $p = 0.001$) (Table 3). The present study result is similar to that reported previously in which the negative response of cleaning lead to accumulation of microbial plaque on the surface of the denture in contact with the mucosa [7]. Both the plaque accumulated on the denture and the poor oral hygiene contribute to the virulence of *Candida*, offering the clinical picture of *Candida*-associated denture stomatitis [7,12,16,17,20,25,35-40].

In the current study denture wear at night was taken into account as one of predisposing factor of DS (OR=1.7, $p = 0.04$) (Table 3). Our result is similar to that reported by Chopde et al. and Brondani et al. in which denture wear at night was considered as one of predisposing factor of DS [16,39]. When denture manufacturers supervision was measured, there was significance association

between denture prepared under supervision of general dentist and *Candida albicans* DS (OR=2.9, CI=1.6-5, and $p < 0.001$) while this risk is absent with denture prepared under supervision of dental consultant (Table 3). Furthermore, there was significance association between non-ideal denture and *Candida albicans* DS (OR=4.8, $p < 0.001$). Whether denture manufacturers and denture quality should be considered as predisposing factors are still a matter of debate. Several previous studies have reported that bad denture manufacture and non-ideal denture quality, either alone or in combination with other systemic or local factors, is associated with changes in the oral cavity, increased oral *Candida* colonization or with the development of oral candidosis.

Conclusion

Candida albicans DS is a condition that usually affects denture wearers and should be treated in anticipation of asymptomatic. DS needs a shared treatment method from both patient and clinician, and the role of the patient must be stressed. Management of predisposing risk factors is key in order to prevent recurrence. Treatment methods may consist of: treatment of any underlying systemic risk factors, improvement in the fit of existing dentures, replacement of existing dentures, improved denture hygiene. Either methods are in work, the main aim of treatment is to eradicate the biofilm from the patient's dentures. Habitual examination of patients suffering from DS is essential in order to guarantee long-term successful treatment of the situation.

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Conflict of Interest

No conflict of interest associated with this work.

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