Original Article

Evaluation of Candidal Carriage Among Smokers and Non-Smokers

Umar Irfan, Salik Rasool, Perveen Memon, Shazia Irum, Bushra Jabeen, Faraz Khan

ABSTRACT

Objectives: To determine the *Candidal* carriage among smokers and non-smokers and with different intra-oral sites including examination of various biotypes of *Candida*.

Study design and setting: Cross-sectional based study conducted at Dr. Ishrat ul Ebad Khan Institute of Oral Health Sciences and Dow International Dental College, Karachi, from May 2017 till April 2018.

Methodology: Comprised 100 patients (50 smokers and 50 nonsmokers) between 20 and 60 years of age. The collection was performed through sterile cotton swab to evaluate oral *Candidal* carriage and the colonizing *Candida* species using Sabouraud Dextrose Agar (SDA) and API20C AUX (BIOMERIEUX). Data was analyzed Spss version 20.

Results: A total of 100 participants (50 smokers and 50 non-smokers) were evaluated for *candidal* carriage. The common age group was 20-30 years in both the groups, without significant difference (p-value 0.79). Frequency of *candidal* carriage was comparable among smokers 14 (28.0%) to non-smokers 10 (20.0%), with a statistically insignificant p-value 0.35. Based on various biotypes among smokers and non-smokers, *Candida albicans* was 9(18%) and 7(14%), *Candida glabrata* was 4(8%) and 2(4%); and *Candida tropicallis* was 1(2%) each for both smokers and non smokers. Dorsum of tongue harbored all prevalent biotypes i.e. *Candida albicans*, *Candida glabrata and Candida tropicalis* as statistically significant among smokers (p-value 0.04).

Conclusion: Candidal carriage was comparable among smokers and non-smokers. *Candida albicans* and *Candida glabrata* were the common biotypes predominantly among smokers.

Key Words: *Candida albicans*, Oral cavity, Tobacco smoking.

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INTRODUCTION:

Fungi are aerobic micro-organisms.[1]The major human fungal pathogens belong to genus *Candida*, mainly *Candida albicans*, which causes different types of infections in humans. Infections caused by *Candida albicans* frequently affect the immunocompromised patients.[1]

Umar Irfan
Lecturer, Department of Oral Pathology
Dow International Dental College.

Salik Rasool
Associate Professor, Head of Department Of Oral Pathology
Dow International Dental College, Karachi.
Email: salik.rasool@duhs.edu.pk

Perveen Memon
Associate Professor, Department Of Oral Biology
Liaquat University of Health Sciences, Hyderabad

Shazia Irum
Assistant Professor, Department Of Pathology
Dow Dental College, Karachi.

Bushra Jabeen
Associate Professor, Department of Prosthodontics
Dow International Dental College, Karachi

Faraz Khan
Lecturer, Department of Pathology
Dow International Dental College, Karachi.

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Oral *Candida* species, mainly *Candida albicans* are frequently isolated from the oral mucosa of humans, with oral carriage prevalence varying between 17-75% in all healthy individuals[2], mainly the children and younger adults[3]. The increased risk factors for oral candidal carriage in humans documents age, female gender, pregnancy, wearing of dentures, immune suppression, hypo-vitaminosis, iron deficiency, steroid treatment, poor oral hygiene[2-4], xerostomia, salivary pH[5] and systemic diseases, such as chronic hyperglycemia.[2-4]

During the recent years, there has been a dramatic increase in fungal infections, mainly due to increase in number of immunocompromised patients, such as patients infected with HIV and patients undergoing chemotherapy due to cancer[3]. It has been noted that bio-films associated with denture stomatitis is not only caused by *Candida Albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis* and *Candida dubliniensis* are the additional risk factors for the disease.[3] The use of tobacco has been considered as the most common risk factor for development of oral candidal infections.[2-3] According to World Health Organization (W.H.O), it is estimated that the use of tobacco will turn out to be single most common health leading problem by the year 2020.[6-7] It accounts for six million deaths yearly[8], which is expected to cause more than 8 million deaths annually by the year 2030.[8-9]

The effects of cigarette smoke on the oral mucosa are both chemical and thermal. Use of tobacco is a primary cause of many oral diseases and adverse oral health conditions. Studies conducted in some industrialized countries have shown that smoking alone is responsible for more than half of the periodontitis cases in adults.[5,6,8,9,10]

Some studies showed that cigarette smoke cause increased *Candida albicans* adhesion and growth as well as biofilm formation in association with increased secretion of proteolytic enzymes, particularly aspartyl proteinases[2,4,10]. Additionally, other studies have reported that *Candida* increases epithelial atypia and leads to epithelial hyperplasia and malignant conditions.[2]The significance of identifying *Candida* species is important for understanding the epidemiology, pathogenicity as well as treatment of oral *Candidiasis.*

To date, there is insufficient data regarding the candidal carriage in local population among the smokers and non-smokers.

The objectives of this study was to determine the *Candidal* carriage among smokers and non-smokers and with different intra-oral sites including examination of various biotypes of *Candida.*

METHODOLOGY:

This cross sectional study was conducted at the department of Oral diagnosis outpatient department at Dr. Ishrat Ul Ebad Khan Institute of Oral Health Sciences, and Dow International Dental College, DUHS Karachi. The study duration was from May 2017 to April 2018. This research was conducted under ethical consideration. The internal board review of D.U.H.S approved the consent form and research protocol. The participation was voluntary and informed consent was obtained before being included in the study. Using PAS v11, two groups with a sample of 50 each with 95% power to identify the difference between the group proportions. Under the null hypothesis and alternate hypothesis the proportions in the groups are 0.325 and 0.675 respectively at the level of significance 0.05.[5]

The participants between the age of 20-60 years were included comprising of 50 smokers and 50 non-smokers in each group. Samples of participants were taken using the criteria of Canadian Tobacco Use Monitoring Survey-2015 (CTUMS). Convenient sample technique was used. The participants who were smokers were inducted in the study group and every non-smoker was inducted in control group. Exclusion criteria were immunocompromised patients, patients on antibiotics corticosteroids, antiglycemic agents, blood pressure medicines (known to alter candida microbiota), xerostomia any other white lesion other than candidiasis, denture wearers and orthodontic treatment cases.

The study parameters were based on the following factors; age, gender, smoking, candidal carriage, oral site and biotypes.

The participants were advised not to eat or drink for at least 2 hours. A sterile cotton-tipped swab was used. The samples were then collected from dorsal surface of tongue, commissural and buccal mucosae. (The reason for collecting the sample from these sites is due to the fact that the anatomy of the tongue favors the accumulation of carbohydrates which allows a favorable environment for candida growth as compared to the other intra-oral sites, e.g., buccal mucosae and commissural mucosae.) The swab samples were then placed in a glass tube, transported to the Department of Pathology, Dow Diagnostic Reference and Research Laboratory, Ojha Campus, Dow University of Health Sciences, Karachi and inoculated directly onto Sabouraud Dextrose Agar plates (SDA).The samples were then incubated for 24-48 hours at 37^{o}C. The cultured plates were then visually examined for detection of whitish creamy growth of yeast like colonies of *Candidal* biotypes. Gram staining of colonies was done with gram-positive and gram- negative controls. The identification of gram-positive yeast like colonies was further processed for species level identification by inoculation on API 20C AUX (BIOMERIEUX) with standard McFarland. Sabouraud dextrose agar plates (SDA) and I20C AUX (BIOMERIEUX) kits for the evaluation of Candidal carriage were used as this is most the relevant technique and widely accepted.

Sabouraud Dextrose Agar (SDA) was prepared by the following method:

- Suspend 65g of Sabouraud in 1L of distilled water, add polysorbate (tween-80), and boil to dissolve completely.
- Sterilize by autoclaving at 121^{0}C (15lb pressure) for 15mins.
- Dispense 15ml amount in Petri dish.
- Allow it to solidify at room temperature. Final pH should be between 5.6-6.2 at 25^{0}C (room temperature).

Strip preparation:

- To obtain a humid atmosphere, an incubation box was prepared with lid and tray. It was filled with approximately 5ml of distilled water into the honeycombed wells of the tray.
- On the elongated flap of the tray, recording of the strain reference was performed.
- The strip was placed in the incubation tray after its removal from individual packing.

Preparation of inoculum:

- An ampule of NaCl 0.85% was used.
- A portion of yeast colony was obtained using a pipette by suction and a turbidity equal to 2 McFarland of a suspension was achieved.
- Finally, 2-4 drops of previous suspension was added into a newly opened ampule of C. medium.

Strip inoculation:

- The cupules are then filled with the obtained suspension in the ampule of C. medium.
- The lid is incubated at 30^{0}C for 48-72 hours after placing it on the tray.

Strip recoding:

- Compared the growth in each capules after the incubation period of 48 hours. It is a negative control. When control is less turbid than the cupules it indicates a positive reaction.

Identification:

- On the result sheet, using the profile index the reaction pattern was coded into a numerical profile. 3 groups were made to separate the tests and a number 1, 2 or 4 was marked for each group. A 7-digit number was obtained by adding numbers corresponding to positive reactions within each group. A 7-digit number created a numeric profile.

When a positive result with a value of 4 was obtained, the 21st test was done by the presence of hyphae (mycelium) or pseudohyphae (pseudo mycelium).

The data was analyzed on SPSS version 20. Frequency, Mean and Standard deviation were used as descriptive statistics. Chi-square test was implied for assessing the association of *Candidal* carriage and comparing the amount of *Candidal* carriage between smokers and non-smokers.

RESULTS:

A total of 100 participants that included 50 smokers and 50 non-smokers were investigated for possible Candidal carriage. Mean age of smokers was 30.10+10.20 years and non-smokers were 32.82+10.26 years. The most common age group was 20-30 years among both groups. Table 1. Regarding Candidal carriage distribution in terms of different intra-oral site, dorsal commissural buccal was the most common 6(12.0%) among smokers and 5(10.0%) among non-smokers, followed by dorsal commissural, buccal commissural, buccal dorsal and dorsal with percentage of 4.0%, 2.0%, 8.0% and 2.0% respectively among smokers and dorsal commissural, buccal commissural, commissural and buccal with percentage of 4.0%, 2.0%, 2.0% and 2.0% respectively among non- smokers.

According to the various biotypes among smokers and non-smokers, *Candida albicans* had a comparatively higher prevalence in smokers than non-smokers. Further details are given in Table 2. Regarding distribution of various biotypes according to buccal mucosa, *Candida albicans* was found among 6(12.0%) smokers and 5(10.0%) of non-smokers. *Candida glabrata* found among 3(06.0%) smokers and 2(04.0%) non-smokers. *Candida tropicalis* was only in 1(02.0%) smokers and 1(02.0%) non-smokers respectively, while there was no growth on buccal mucosa among 40(80.0%) smokers and 42(84.0%) non-smokers.

In terms of distribution of various biotypes according to commissural mucosa, *Candida albicans* was found among 5(10.0%) smokers and 6(12.0%) of non-smokers. *Candida glabrata* found among 3(06.0%) smokers and 1(02.0%) non-smokers. *Candida Tropicalis* was only in 1(02.0%) smokers and 1(02.0%) non-smokers respectively, while there was no growth on commissure mucosa among 41(82.0%) smokers and 42(84.0%) non-smokers.

Considering the distribution of various biotypes according to dorsum of tongue, *Candida Albicans* was higher in smokers than non-smokers. Further details are given in **Table 3**.

DISCUSSION:

Oral *Candida albicans*, is the most frequently isolated biotype from the oral cavities. W.H.O estimates that around 22% of the people over 15 years age worldwide consume smokeless tobacco which is a public health concern[21]. Our study results showed that frequency of *Candidal* carriage was high among smokers 14(28%), in contrast to non-smokers 10(20%), with a statistically insignificant p-value of 0.349. Similarly, in a study conducted by Darwazeh et al.[5,12,15] showed that the rate of *Candida* carriage was 84% in smokers and 74% in the non-smokers. In another study conducted by Keten et al, stated that *Candidal* infection was present in 58.3% of smokers (P = 0.018)[22]. Some studies have revealed a signi?cantly higher rate of *Candidal* carriage in the smokers compared with non- smokers[15]. The significance of identifying *Candida* species is important for understanding the epidemiology, pathogenicity and treatment of oral *Candidiasis*[16]. Several studies have, on the other hand reported that tobacco smoking either alone or in combination with other factors, is associated with increased

Table 1: Demographic characteristics of smokers and non-smokers n=100

Variables	Groups		P-value*
	Smokers	Non–smokers	
Age groups			0.786
20-30 years	22(44.0%)	25(50.0%)	
31-40 years	17(34.0%)	14(28.0%)	
40-60 years	11(22.0%)	11(22.0%)	
Total	50(100.0%)	50(100.0%)	
Intra-oral sites			0.398
DCB	6(12.0%)	5(10.0%)	
DC	2(4.0%)	2(4.0%)	
CB	1(2.0%)	1(2.0%)	
BD	4(8.0%)	00	
D	1(2.0%)	00	
C	00	1(2.0%)	
B	00	1(2.0%)	
Not found	36(72.0%)	40(80.0%)	
Total	50(100.0%)	50(100.0%)	
Candidal carriage			0.349
Yes	14(28.0%)	10(20.0%)	
No	36(72.0%)	40(80.0%)	
Total	50(100.0%)	50(100.0%)	

Mean age = 30.10+10.20 years of smokers and 32.82+10.26 years of non-smokers *chi-square

Table 2: Various biotypes among smokers and non-smokers n=100

Various biotypes	Groups		P-value*
	Smokers	Non–smokers	
No growth	36(72%)	40(80%)	0.711
Albicans	9(18%)	7(14%)	
Glabrata	4(8%)	2(4%)	
Tropicalis	1(2%)	1(2%)	
Total	50(100%)	50(100%)	

*chi-square

Table 3: Various biotypes in dorsum of tongue in smokers and non-smokers n=100

Biotypes of Candida	Dorsum of tongue		*P*-value*
	Smokers	Non–smokers	
No growth	37 (74%)	47 (94%)	0.044
Albicans	09 (18%)	01 (2%)	
Glabrata	03 (6%)	01 (2%)	
Tropicalis	01 (2%)	01 (2%)	
Total	50 (100%)	50 (100%)	
Biotypes of Candida	**Commissural mucosa**		***p*-value**
	Smokers	**Non–smokers**	
No growth	41 (82%)	42 (84%)	0.776
Albicans	5(10%)	6 (12%)	
Glabrata	3 (6%)	01 (2%)	
Tropicalis	01 (2%)	01 (2%)	
Total	50 (100%)	50 (100%)	
Biotypes of Candida	**Buccal Mucosa**		***p*-value**
	Smokers	**Non–smokers**	
No growth	40 (80%)	42 (84%)	0.952
Albicans	6(12%)	5 (10%)	
Glabrata	3 (6%)	2 (4%)	
Tropicalis	1 (2%)	1 (2%)	
Total	50 (100%)	50 (100%)	

*chi-square

Figure 1: **(a)** *Candida albicans* on dorsal surface, B= *Candida albicans* on buccal surface, C= No growth on commissural surface **(b)** *Candida tropicalis* on dorsal surface, B= *C. tropicalis* on buccal surface, C= *Candida tropicalis* on commissural surface **(c)** C. *albicans* on buccal surface, B= no growth on buccal surface, C= *C. glabrata* on commissural surface

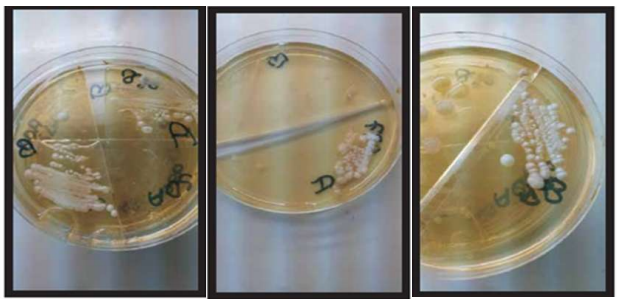
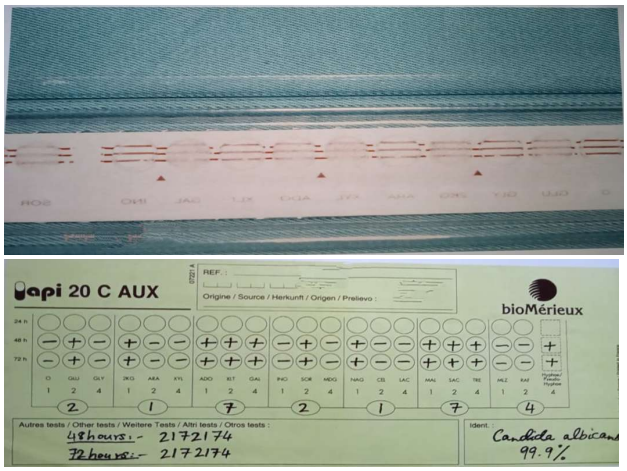


Figure 2**: (a)** Turbid and non turbid honey comb wells give a 7digit value. (**b)** 7 digit API Aux coding sheet positive for *candida albicans*



incidence of oral *Candida* colonization[14,17,19] and the relationship between frequency of smoking and the *Candidal* carriage is proportional[18,21,23]. In our study, a total 100 participants 50 smokers and 50 non-smokers were investigated according to *Candidal* carriage. Mean age of smokers was 30.10+10.20 years and non-smokers were 32.82+10.26 years, showed no significance (p-0.786). On other hand Keten et al, also reported that the mean age of the participants was 40.49 ± 12.89 years[21].

Regarding our study on distribution of various biotypes according to buccal mucosa, *Candida albicans* was found among 6(12%) smokers and 5(10%) of non-smokers. *Candida glabrata* found among 3(6%) smokers and 2(4%) non-smokers. *Candida tropicalis* was only in 1(2%) smokers and 1(2%) non-smokers respectively, while there was no growth on buccal mucosa among 40(80%) smokers and 42(84%) non-smokers, p-value 0.952. Keten et al12,16,19, reported that the most frequently isolatedÊ*Candida*Êspecies in all groups wereÊ*C. albicans,* followed byÊ*C. tropicalis*, in the present study. Consistently, it has been reported in the literature that the most frequently isolated oralÊ*Candida*Êspecies wasÊ*C. albicans*Êfollowed byÊ*C. tropicalis*Éboth in smokers and the normal population[5-10]. Frequency among commissural mucosa, *Candida albicans* was found among 5(10%) smokers and 6(12%) of non-smokers. *Candida glabrata* found among 3(6%) smokers and 1(2%) non-smokers. *Candida tropicalis* was only in 1(2%) smokers and 1(2%) non-smokers respectively, while there was no growth on commissure mucosa among 41(82%) smokers and 42(84%) non-smokers. Rodrigues et al, reported *Candida albicans* was the most common species (80.9%) frequently isolated from the tongue and buccal surface, followed by *C. tropicalis* (7.2%) frequently isolated from the tongue and palate[12,16,19]. Darwazeh et al, reported *Candida albicans* as (65%) frequently isolated from the tongue and commisure, followed by *C. tropicalis* (11%) frequently

isolated from the tongue[17,19,23,24].

Considering the distribution of various biotypes according to dorsum of tongue, *Candida albicans* was higher in smokers than non-smokers[14,20,25].

The research project had some limitations that have been addressed. Firstly, the participants in the study were only males, females were not included in the study. Secondly, quantification of oral candidal species was not done. Thirdly, the study was conducted on a limited population of Karachi and only two public sector hospitals were selected due to limitation of resources and budget. Fourthly, no ethnicity was taken into account, as candidal carriage may vary between various ethnic groups.

CONCLUSION:

It was evident that the candidal carriage was significantly high among smokers, compared to non-smokers. *Candida albicans* and *Candida glabrata* were the most common biotypes and found mainly among the smokers. Commissural mucosa and buccal mucosa were the most commont intraoral sites.

Author Contribution:
Umar Irfan: Introduction and Methodology
Salik Rasool: Discussion
Perveen Memon: Lab work
Shazia Irum: Lab work
Bushra Jabeen: Statistics
Faraz Khan: Results

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