Assessment Of Candida Species Colonization And Denture-Related Stomatitis In Shisha (Waterpipe) Smokers Under Complete Denture In Some Upper Egyptian Patients

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Abstract

Background:

Increasing the prevalence of oral Candidal growth is associated with the habit of smoking and increased with denture wearing, and many of these carry a potential risk for the development of denture-related stomatitis. Shisha smoking in the rural areas in Egypt is more prevalent than cigarettes, especially in old males. There have been no studies that report the prevalence of candidal growth and denture related stomatitis under complete dentures in shisha smokers, in rural regions, of south Egypt.

Materials and Methods:

A total of 30 edentulous participants were selected and examined to assess any oral mucosal changes (15 were shisha smokers and 15 were non-shisha users). complete dentures were constructed, For every patient, oral examination and samples were collected at the following intervals: before denture insertion, one, three, and six months after denture adaptation.

Results:

Shisha smoking affect significantly the mean count at different time interval (1,3,and 6 months) while the non shisha users was observed to show significant increase later at 3 and 6 months. Confluent growth was not observed among non- shisha users while it appears in about 7% of the shisha smokers group. Moderate and severe inflammatory grades of palatal mucosa was noticed among shisha smokers compared to mild grade only seen in one case of non-shisha users after 6 months of insertion.

Conclusion:

shisha smoking increases the prevalence of candidal growth under complete dentures and increases the probability of denture-related stomatitis to occur.

Keywords: candida, denture stomatits, shisha smoking.

Introduction

Shisha is an instrument for smoking tobacco, which is popular in Upper Egypt. A number of synonyms have been used to describe shisha including narghile, sheesha, hookah, hubble bubble, argileh, , nargila, nargileh and goza [(1)]. In the present study, we shall refer only to the word shisha. To smoke a shisha, hot coals are kept in indirect contact with tobacco and the smoke is either to be inhaled or blown away. More recent variations on this theme include the use of tobacco flavoured with molasses. This mixture is called tobamel (tobacco plus 'mel' for 'honey' in Latin) or mu'essel ('honeyed', in Arabic) [(2)]. Interestingly, shisha smoking in the Upper Egypt is more prevalent than cigarettes in old males, especially in rural areas, and it is thought that it may be a substitute for cigarettes, because of cost savings. Many in the Upper Egypt think that shisha is harmless with no addiction, while it is considered as a good substitute for cigarettes, based on the belief that relative to cigarette smoking, the water filtration of the smoke in the pipe removes harmful toxicants. Hence, using shisha is common in many cafes and entertainment centers. However, some studies have reported high levels of toxic substances, such as, nicotine[(3)], carbon monoxide, heavy metals, and chemical carcinogenesis in shisha smoke[(4,5)]. A single narghile smoking session is equivalent to the formaldehyde released from 17 cigarettes and acetaldehyde from five cigarettes[(4)]. The smoking mechanism of the shisha (sharing behavior) creates many potential pathogen-transmission pathways. When shisha smokers contaminate or come into contact with contaminated shishas or smoking materials, a potential exposure-pathway to fungal, bacterial, and viral pathogens is created between shisha smokers. Due to poor access to modern sanitation, this population is particularly susceptible to transmission of infectious agents.

A variety of intra oral changes were found in denture wearers, one of which was inflammation. Inflammation of the denture –bearing mucosa can take several forms. Diffuse inflammation as a result of trauma and candidal infection was the most common type seen. The denture surface retains candidal organisms with subsequent release of irritating breakdown products. . Of the patients with candidal stomatitis, 28% were aware of a burning or tingling sensation under the denture while the remainder was asymptomatic[(6)]**.** It was suggested that the inflammatory process of stomatitis favors the colonization of Candida. These results could have new implications for diagnosis and management of the condition[(7)]**.**

Significantly few studies have reported the effects of narghile smoking in the oral cavity[(8)]. The habit is known to promote periodontal bone loss[(9)], and increase the possibility of acute osteitis (dry socket) after exodontias[(10,11)]. Interestingly, to our knowledge, no studies have reported an association between narghile smoking and oral candidiasis despite there being a positive correlation between tobacco smoking in

general and increased oral carriage of *Candida* species[12] . Accordingly, this study aims to investigate the effect of Narghile (Shisha) smoking on Candidal growth in complete denture wearers.

Materials and method

Thirty completely edentulous patients were selected from the outpatient clinic. Fifteen of them were shisha smokers and fifteen were non-shisha users. Patients' age ranged between 40-60 years old with an average age of 50 years. All the participants were males. Non-shisha users were those who never had a history of smoking or using shisha. All participants signed a written informed consent form after the objective of the study was described to them by one of the researchers. The participants' history of systemic conditions was also recorded. Clinical oral examinations were performed. All the patients were having good general health, free from systemic diseases that may affect the oral condition e.g. diabetes mellitus, anemia and immuno-deficiency states as indicated by the medical history, with no previous denture experience, have a well formed residual ridge, free from sever bilateral undercuts, bony specules or sharp ridges, with healthy mucosal coverage free from any signs of inflammation, ulceration or hyperplasia. Patient with excessive salivation or stick ropy saliva was excluded. Those having dry mouth were not also considered. All patients had nearly the same social standard.

Patients selected for this study were divided into two groups, each composed of 15 patients. Group (I) are non-shisha users, Group (II) are shisha smokers. For both groups conventional complete dentures were constructed. The impression technique followed in this study was the same in all patients to avoid the possible effect of different impression techniques on the degree of plaque accumulation and hence on C. albicans colonization[6]**.** For every patient, upper and lower conventional heat cured acrylic dentures were constructed.

At time of denture delivery, patients were instructed to clean their dentures after each meal under tap water, no other mechanical or chemical means were used, which might affect plaque accumulation and its microbial flora. Patients were instructed to remove their dentures during sleep because wearing dentures day and night increases plaque accumulation and hence, increases the risk of developing denture stomatitis[13]**.**

For every patient, the samples were collected at the following intervals: before denture insertion, one, three and six months after denture adaptation.

A- Before denture insertion:

The palatal mucosa of all patients was sampled using the impression culture technique developed by *Bahn et al*[14] . An alginate upper impression was made in aluminum stock tray. Boxing of the upper impression was done by placing one strip of beading wax* just below the tray borders and sealing it. A strip of boxing wax ** was applied around the beading wax to create a mold used to confine the molten growth medium. Sabouraud`s dextrose agar medium was prepared (65gm of the powder was

suspended in one liter of distilled water and then brought to the boil to dissolve completely. Sterilization was made by autoclaving at 121°C for 15 min). After the medium was cooled to 40° C, sodium benzyl penicillin 1.5mg/ml and streptomycin sulphate 5mg/ml was added to suppress growth of bacteria. 30 to 40 ml of cooled liquid medium was poured into the boxed impression to approximately 1cm above the shallowest portion of the impression. This was done within a maximum of one hour after impression making.The agar replica was allowed to solidify for 15 min. and the beading and boxing wax were removed from the impression. The agar replica was gently freed from the impression and inverted into a sterile Petri-dish.The replica was incubated aerobically for 24-48 hr at 37° C. After 24 hr, the plates were examined visually for colony morphology characteristics of yeasts. Yeast colonies appeared as white, opaque, raised with a yeasty odor.If no colonies were visible, the plate was incubated for further 24 hr before a negative result was recorded. The colony forming units (CFU) of yeast-like organisms were quantitated according to a modification of the scale developed by Olsen, (1974)[15] and adopted by Bergendal et al., (1979)[16] as follows: No colonies = 0 (Fig, 1a), 1 - 100 colonies =1 (Fig,1b), greater than 100 colonies =2 (Fig,1c), confluent growth =3 (Fig, 1d). A minimum of three colonies were randomly taken from different locations on each agar replica, gram stained and examined under the light microscope, when the microscopic picture confirmed the presence of yeast cells (Fig 2), subcultures on sabouraud`s dextrose agar slopes were made to be subjected to the germ tube test for the identification of Candida albicans.

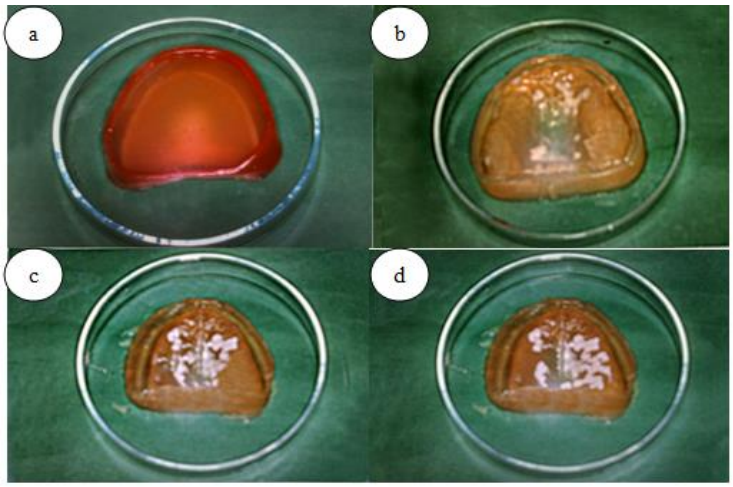

Fig (1): a) Sabouraud's dextrose agar medium shows no candida albicans colonies, b) Sabouraud's dextrose agar medium shows less than 100 colonies, c) Sabouraud's dextrose agar medium shows more than 100 colonies, d) Confluent growth .

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*Beading wax sticks (KERR manufacturing Company, USA).

** Extra-tough pink base plate wax. The hygienic corporation/Akron, OHIO, USA

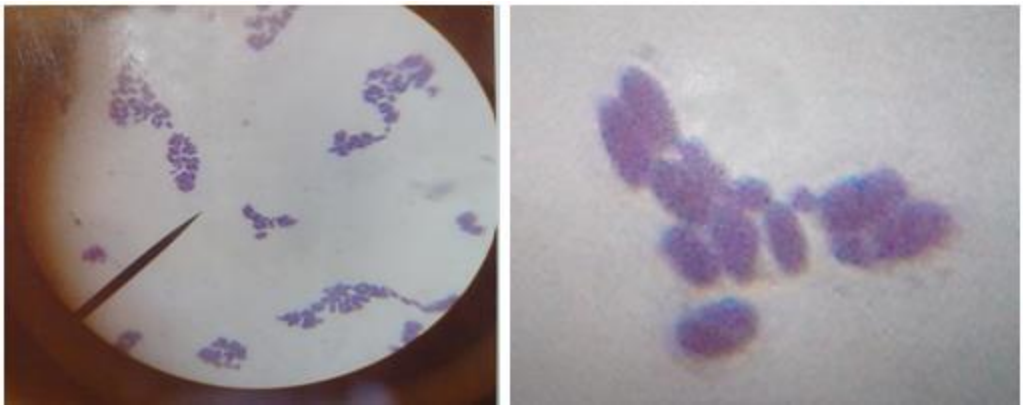


Fig. (2): microscopic picture confirmed the presence of yeast cells

B) After denture adaptation:

Each time the patients were recalled during the follow-up period, the following procedures were carried out:

1. **Sampling of the palatal mucosa**:

The palatal mucosa was sampled following the same impression culture technique used before. A subject was considered to be a carrier of Candida albicans if one or more colonies were found on any plate.

2. **Clinical evaluation of the palatal mucosa**:

The palatal mucosa was visually inspected for any sings of inflammation or ulceration. Patients were also directly questioned if they were satisfied with their dentures or complaining of pain, soreness or stinging sensation. Qualitative differentiation of the inflammatory intensity was made; distinguishing between slightly inflamed, moderately inflamed and severely inflamed mucosa.

The clinical inflammatory index used was adopted from *Budtz-Jorgensen and Bertram*[17] as follows:

- Slightly inflamed: slight erythema, scraping with a spatula does not produce any reaction of pain .
- Moderately inflamed: distinct erythema, scraping with a spatula produces a reaction of pain.
- Severely inflamed: the mucosa is firey red, scraping with a spatula produces a reaction of pain and bleeding of the mucosa involved.

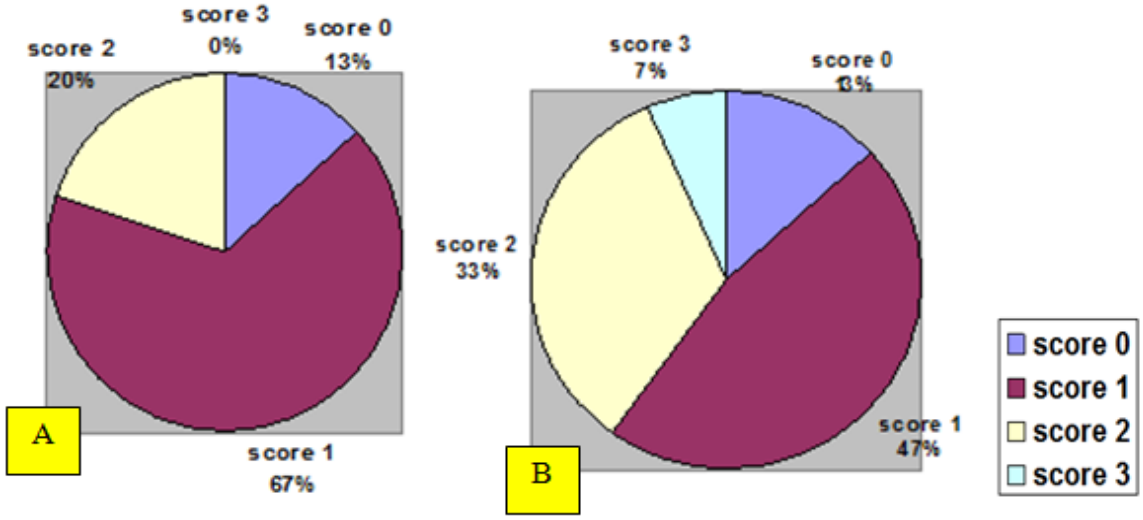
- Data were collected and statistically analyzed by Spss version 17 Software used for analysis according to *Trapp*[18].

Results

Mycological results

Table (1) shows The percentage of the colony forming unit scores in the non-shisha users and shisha smokers group.

Non- shisha users									Shisha smokers							
CFU Score	Zero Time		One Month		Three Months		Six months		Zero Time		One month		Three Months		Six months	
	n=	%	n =	%	n =	%	n =	%	n =	%	n =	%	n =	%	n =	%
Score 0	9	60.0	8	53.33	5	33.33	2	13.33	7	46.66	4	26.66	2	13.33	2	13.33
Score 1	6	40.0	7	46.66	8	53.33	10	66.66	8	53.33	10	66.66	7	46.66	7	46.66
Score 2	0	0.0	0	0.0	2	13.33	3	20.0	0	0.0	1	6.66	6	40.0	5	33.33
Score 3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	6.66



Score zero = no growth.

Score one = 1<CFU <100.

Score two = CFU>100.

Score three = confluent growth.

Fig. (3): The percentage of the colony forming unit scores six month after denture adjustment. A) In the non- shisha users group. B) In the shisha smokers group.

The means of colony forming unit (CFU) scores in the non- shisha users and shisha smokers groups:

For the non shisha users group the calculated mean of the CFU score was 0.4000 before denture insertion that showed an increase to be 0.4667, 0.800, and 1.0667 after one, three and six months after denture adjustment. Statistical analysis revealed a significant increase ($P<0.05$).

For the shisha smokers group the mean of CFU scores was 0.5333 before denture insertion, that showed steady increase to be 0.8000, 1.2667 and 1.3333 after one, three and six months respectively. This increase was found to be statistically significant ($P<0.05$) except at the period from three to six months ($P>0.05$).

Table (2) shows the means of colony forming unit (CFU) scores in the non- shisha users and shisha smokers groups respectively.

Non- shisha users group					Shisha smoker group			
	Mean	N	Std. Deviation	Significance	Mean	N	Std. Deviation	Significance
Before	.4000	15	.50709	t_{paired} 0.435 P >0.05 NS	.5333	15	.51640	t_{paired} 2.256 P <0.05 S
One month	.4667	15	.51640		.8000	15	.56061	
Before	.4000	15	.50709	t_{paired} 2.449 P < 0.05 S	.5333	15	.51640	t_{paired} 4.036 P <0.05 S
Three months	.8000	15	.67612		1.2667	15	.70373	
Before	.4000	15	.50709	t_{paired} 4.183 P <0.05 S	.5333	15	.51640	t_{paired} 4.000 P <0.05 S
Six months	1.0667	15	.59362		1.3333	15	.81650	
One month	.4667	15	.51640	t_{paired} 2.092 P <0.05 S	.8000	15	.56061	t_{paired} 3.500 P <0.05 S

Three months	.8000	15	.67612		1.2667	15	.70373	
One month	.4667	15	.51640	t_{paired} 3.154 P <0.05 S	.8000	15	.56061	t_{paired}4.000P <0.05 S
Six months	1.0667	15	.59362		1.3333	15	.81650	
Three months	.8000	15	.67612	t_{paired} 2.256 P <0.05 S	1.2667	15	.70373	t_{paired}1.000P >0.05 NS
Six months	1.0667	15	.59362		1.3333	15	.81650	

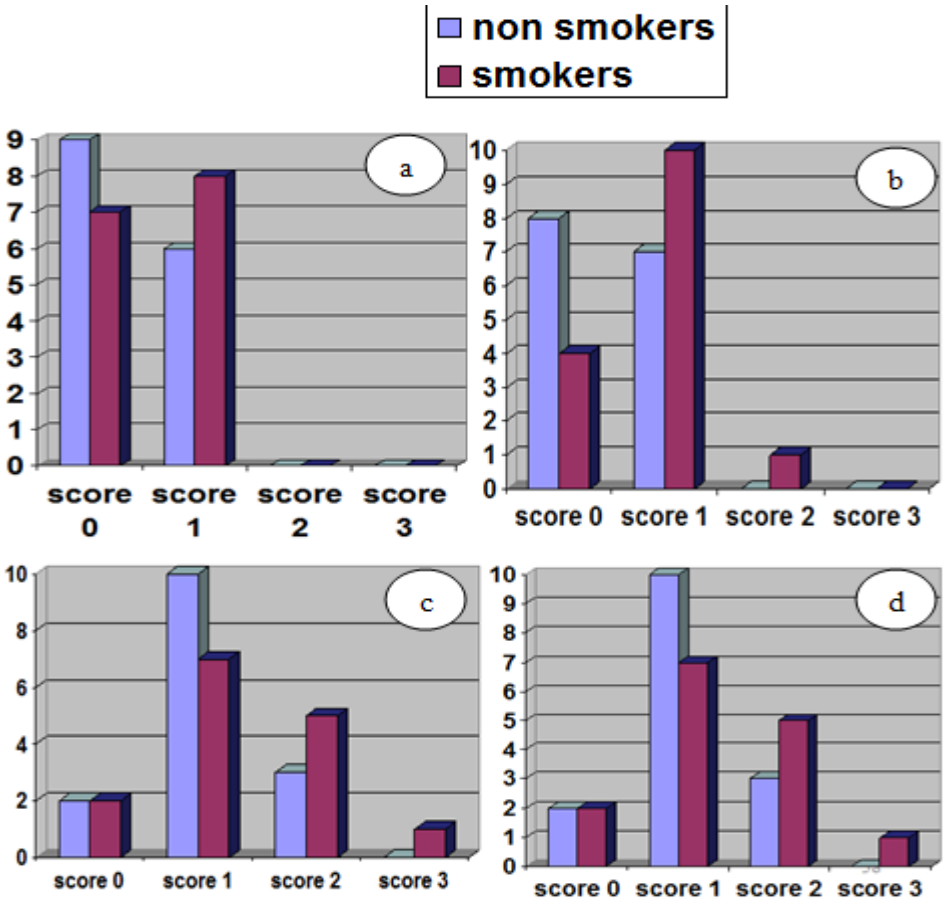


Fig. (4): The prevalence of the colony forming unit scores in the studied groups. a) Before denture insertion. b) One month after denture adjustment. c) Three months after denture adjustment. d) Six months after denture adjustment.

Effect of shisha smoking on colony forming units count (CFUC):

Table (3) shows the means of colony forming unit (CFU) scores between the non- shisha users and shisha smokers groups.

		N	Mean	Std. Deviation	Std. Error Mean	Significance
CFUC before	Non- shisha users	15	0.4000	0.50709	0.13093	t 0.714 $P > 0.05$ NS
	shisha Smokers	15	0.5333	0.51640	0.13333	
CFUC One month	Non- shisha users	15	0.4667	0.51640	0.13333	t 1.694 $P > 0.05$ NS
	shisha Smokers	15	0.8000	0.56061	0.14475	
CFUC Three month	Non- shisha users	15	0.8000	0.67612	0.17457	T 1.852 $P > 0.05$ NS
	shisha Smokers	15	1.2667	0.70373	0.18170	
CFUC Six month	Non- shisha users	15	1.0667	0.59362	0.15327	t 1.023 $P > 0.05$ NS
	shisha Smokers	15	1.3333	0.81650	0.21082	

Statistical analysis revealed a no significance difference (P>0.05) between the non-shisha users & shisha smokers before denture insertion , one month after denture adjustment, three months after denture adjustment and six months after denture adjustment.

II- Clinical results

One month after denture adaptation: Signs of mild inflammatory reactions were observed in two cases in shisha smokers group and non- shisha users group. Three months after denture adaptation: Signs of mild inflammation were observed in two cases and signs of moderate inflammation were observed in one case in shisha smoking group. However in the non- shisha users group there is only one case showed mild inflammation of the palatal mucosa. Six months after denture adaptation: Signs of mild inflammation were observed in two cases, Signs of moderate inflammation were observed in another one case, and one case also showed sever inflammation in shisha smoking group. However, in non- shisha users only one case showed mild inflammation of the palatal mucosa.

Discussion:

To investigate the effect of shisha smoking on the prevalence of candidal growth under complete denture, this study was conducted. The candidal growth under complete denture was measured, before denture insertion, One month, three months and six months after denture adaptations.

Results of this study revealed that one month after denture insertion, there is no increase in mean colonies count than before adaptation in non-shisha users group while there is significant increase in this count in shisha smoker`s group.

Furthermore, the mean count is much increased in shisha smokers than non-shisha users at 1, 3 and 6 month after denture adaptation, although it did not reach statistical significance yet.

Based on the results of this study, shisha smoking not only increases the mean count but also there is more than 6% prevalence of confluent growth in shisha smokers opposite to 0% in non- shisha users.

While most studies on the effect of smoking on candidal growth under complete dentures have only studied the effect of tobacco smoking, our results confirmed the increased prevalence of Candidal growth under complete denture and denture related stomatitis in shisha smokers.

Interestingly, no studies have reported an association between shisha smoking and oral candidiasis under complete dentures, despite there being a positive correlation between tobacco smoking in general and increased oral carriage of *Candida* species[19,20]. Opportunistic pathogens like candida was reported to be higher in smokers and shisha users compared to the control group[21]. Furthermore, clinical expertise suggests that some candidal infection invariably disappear following smoking cessation alone[22].

The presence of denture wear greatly affect the candidal carrier state, since candidal growth increases in a humid and closed area which is well established by the

fitting surface of the denture base. It has been shown that Candida albicans can easily adhere to and sometimes penetrate the acrylic surfaces[23]. When a denture is inserted, plaque on the fitting surface helps Candida to adhere and become established[24]**.** Tobacco smoking associated with denture friction on the oral mucosa also alters the mucosal surface, which may facilitate candidal colonization[25].

Alkumru and Beydemir[26], found more candidal carriers amongst complete or partial denture wearer smokers. *Daniluk et al* **[27],** found that oral cavity revealed only growth of candida albicans species, more frequently in patients with dentures (38\57, 66.7%) than in those without dentures (11\38, 28.9%). *Zomorodian[28]* demonstrated a significant association between the duration of denture wear and oral candidiasis. Furthermore, they noted a high prevalence of C. dubliniensis in complete denture wearers. Another study by *Shruti Nayak, [29]***,** stated that *Candida*l colonization was higher in denture wearers compared to non-denture wearers. Also, among risk factors evaluated, wearing dentures at night and smoking were associated with the most extensive inflammation **[7].** The finding of the study done by *Eun et al.*[30] had led them to suggest that smoking dictates oral *Candida* carriage in healthy individuals. A study done by *Shulman* [31] found that smoking increased candidal colonization in maxillary complete/partial and mandibular complete denture wearers but not in mandibular partial denture wearers. The relationship between candidal growth and smoking was put forward by *Sitheeque et al.*, [32] who found that tobacco smoking favors *Candida* colonization due to induction of increased epithelial keratin, reduced IgA and, possible, depression of PMNL (polymorphonuclear leukocytes) function. *Scully et al.*[33] reviewed that cigarette smoking provides nutrition for *C. albicans*, the fungus replicating by using polycyclic aromatic hydrocarbons as a source of carbon and energy[34].

Denture stomatitis is a common inflammatory lesion in the palatal mucosa of denture wearers who presenting a erythema of variable intensity and extension. Our results revealed that shisha smoking increases the probability of denture-related stomatitis to occur. This was in accordance with previous prevalence studies which varying between 10% and more than 65% [35]. An association between denture stomatitis and Candida spp., specially Candida albicans, has been reported[35,36,28].

Further studies are required in order to understand the role of shisha smoking in Candidal growth and mucosal inflammation in complete denture wearers. The limitation of this study is the small number of participants, and short time of follow up period, which we suggested to be increased for future studies .

As a conclusion, in this study, shisha smoking affect significantly the mean count at different time interval (1,3,and 6 months) while the non shisha users was observed to show significant increase later at 3 and 6 months. Confluent growth was not observed among non- shisha users while it appears in about 7% of the shisha smokers group. Moderate and severe inflammatory grades of palatal mucosa was noticed among shisha smokers compared to mild grade only seen in one case of non-shisha users after 6 months of insertion. Role of shisha smoking in candidal growth in complete denture

wearers, needs further studies, taking into account the frequency and depth of inhalation, and length of smoking session.

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