RESEARCH ARTICLE

Effects of Tobacco Smoking on the Dorsum of the Tongue and Buccal Epithelium

Abdullah Faraj Al Shammari[1], Ibrahim Khalil AL Ibrahim[1], Amjad Ibrahim Alaauldeen[2], Randa Fouad Merza[2], Hussain Gadelkarim Ahmed[3,4]*

Abstract

Objective: The aim of this study was to assess the effects of tobacco smoking on the dorsum of the tongue and buccal epithelium. **Methodology:** This case control cross-sectional study was conducted with 174 smoking and non-smoking volunteers living in the city of Hail, Northern KSA. Cytological Materials were obtained from buccal mucosa and dorsum of the tongue, and assessed using cytopathological methods. **Results:** In buccal smears, cytological atypia was observed in 17 out of 101 (16.8%) smoker cases but only 3/73(4.1%) of the controls. For cytological atypia in buccal and tongue smears, the adjusted odd ratio (OR) and the 95% confidence interval (CI) were found to be 4.7 (1.3-16.8), P < 0.016)) and 4.3 (0.93- 20.2), P <0.06)), respectively, in the two sites. **Conclusion:** Tobacco smoking is a major risk factor for occurrence of cytological atypia, which might subsequently develop into oral precancerous and cancerous lesions. Oral exfoliative cytology is an easy and cheap non-invasive procedure which appears highly suitable for screening populations at risk of developing oral cancer.

Keywords: Tobacco smoking- buccal mucosa- cytological atypia- tongue- oral

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Introduction

In 2015, the number of tobacco smokers in the world was estimated to be more than 1.1 billion, with males more than females. Although tobacco smoking is globally declining, but its prevalence appears to be increasing in the WHO Eastern Mediterranean Region and the African Region (WHO, 2016). Tobacco smoking is prevalent in the Saudi population among different age clusters. The prevalence of smoking in males ranges from 13-38% (median = 26.5%), whereas in females it ranges from 1-16% (median = 9%) (Bassiony, 2009).

It is well established that tobacco use plays a major role in the etiology of several cancers particularly oral cancer (Ahmed, 2013; Alshammari et al., 2015, Ahmed, 2013), lung cancer (Hecht, 2012) and cardiovascular disease (Marano et al., 2015). Tobacco smoking has several bad effects on the oral cavity, including staining of teeth and dental restorations, and the occurrence of oral diseases such as smoker's palate, smoker's melanosis, coated tongue, oral candidiasis and periodontal disease, and implant failure (Reibel, 2003). The occurrence and severity of periodontal diseases are higher among tobacco smokers than compared to non-smokers. Cessation of tobacco use has a beneficial effect on halting the development of periodontal diseases and on the outcome of periodontal treatment (Sham et al., 2003).

The dentists are encouraged to implement oral investigations that focus on oral pre-cancer and cancer detection, but other oral changes occur with tobacco use should also be considered. The oral mucosa is composed of stratified squamous epithelium and masticatory/ keratinized (hard palate, dorsum of the tongue, and keratinized gingival) and lining mucosa (floor of the mouth, ventrolateral surface of the tongue, soft palate complex, labial vestibule, and buccal mucosa). Tobacco use affects the surface epithelium, resulting in numerous changes ranging from an increase in pigmentation to thickening of the epithelium (hyperkeratosis). Tobacco use can affect the minor salivary glands on the hard palate and increase the risk for periodontal disease and oral cancer (Taybos, 2003; Juntanong et al., 2016).

Cigarette smoking is a major public health issue in the Kingdom of Saudi Arabia (KSA) in recent years, particularly among adolescents (Algorinees, et al., 2016). Tobacco import in Kingdom of Saudi Arabia (KSA) has increased from 1996 compared to 2012. This increment resulted almost doubled number of smokers especially in males from 21% in 1996 to 37% in 2012. Death attributable to tobacco usage was estimated to account for

*[1]College of Dentistry, [3]College of Medicine, [4]Molecular Diagnostic sand Personalized Therapeutics Unit, University of Hail, [2]Faculty of Dentistry, Umm Al-Qura University, Mekka, Kingdom of Saudi Arabia (KSA). *For Correspondence: hussaingad1972@yahoo.com*

280,000 premature deaths in KSA over the same period (Almutairi, 2015).

However, most of the tobacco related studies from KSA focused on the epidemiological factors rather than the exact effects on tissues. Studies focused on the early detection and prevention of these tobacco related consequences on the oral cavity are deemed important. Oral Exfoliative Cytology (OEC) is a cost effective methodology and may be the best method for the initial evaluation and diagnosis of oral precancerous and cancerous conditions (Ahmed et al., 2009). Therefore, the objective of the present work was to assess the effects of tobacco smoking on the dorsum of the tongue and buccal epithelium.

Materials and Methods

In this case control cross sectional study, a total of 174 Saudi volunteers living in the city of Hail, Northern KSA were investigated. Of the 174 study subjects, 101were deemed as current tobacco smokers (ascertained as cases) and 73 were non tobacco users (ascertained as controls). Cytological materials were obtained by scraping the surface of the buccal mucosa and dorsum of the tongue. The obtained materials were used for preparation of two direct smears, which were immediately fixed in 95% ethyl alcohol for 15 minutes then sent to the pathology laboratory at Molecular Diagnostic sand Personalized Therapeutics Unit, University of Hail for subsequent cytological processing.

Sample collection

Oral exfoliative cells were collected from buccal mucosa (covering both cheeks), dorsum of the tongue by a wooden tongue depressor and the obtained materials were directly smeared on clean glass slides and immediately fixed in 95% ethyl alcohol, while they were wet, and finally sent to the cytopathology laboratory for further processing.

Sample processing

Ethyl alcohol fixed smears were hydrated by using descending concentrations of 85% alcohol through 50% alcohol to distilled water for two minutes in each stage. Then smears were treated with Harris' Hematoxylin for five minutes, to stain the nuclei, rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for a few seconds to remove the excess stain. Then they were immediately blued in tap water for 5 minutes. For cytoplasmic staining they were treated with Eosin for two minutes, then washed in water. The smears were then dehydrated in ascending alcoholic concentrations from 70% through two changes of 95% and absolute ethyl alcohol for two minutes for each change. The smears were then cleared in Xylene and mounted in DPX (Distrene polystyrene Xylene) mounting medium. All reagents used were from Thermo Electron Corporation, UK.

Cytology Assessment

The smears were initially assessed for quality reliability by an experienced cytotechnologist. To increase the reliability and reproducibility, firm quality control measures were used. All obtained cytological finding were compared to standard illustrated web images.

Smears were examined under low (10X) power using a light microscope. All included smears showed satisfactory staining quality with blue nuclei, orange cytoplasm. To avoid the assessment bias, cytological smears were labeled in such a way that the examiner was blinded to the groups (case group or control group) of each subject. Cytological atypia was assessed cytologically by using the criteria described by Ahmed et al., (2003).

Data analysis

Statistical Package for Social Sciences (version 16) was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals (95% CI), as well as the Odd Ration (OR) were used. P value less than 0.05 was considered statistically significant.

Ethical consent

Each participant was asked to sign a written ethical consent during the questionnaire's interview, before the obtaining of the specimen. The informed ethical consent form was designed and approved by the ethical committee of the College of Medicine (University of Hail, KSA) Research Board.

Results

The present study investigated 174 volunteers, their ages ranging from 9 to 66 years with a mean age 30 years. Of the 174 study subjects, 122/174(70.1%) were males and 52/174(29.9%) were females, giving males' females ratio of 2.34: 1. Of the 122 males, 70/122(57.4%) were cases and 52/122 (42.6%) were controls. Of the 52.0 females 31/52(59.6%) were cases and 21/52(40.4%) were controls. With the regard to the age, most of the study subjects were found among the age range 26-35 years. The age distribution is relatively similar among cases and controls with the exception of age group < 18 years, most of them were controls as indicated in Table 1. In regard to the education, the great majority of cases were with secondary level of education representing 52/101(51.5%) followed by university's level constituting 36/101(35.6%), as indicated in Table 1. Figure 1. Figure 1, showing a comparative description of the study population by demographic characteristics. Each category was compared

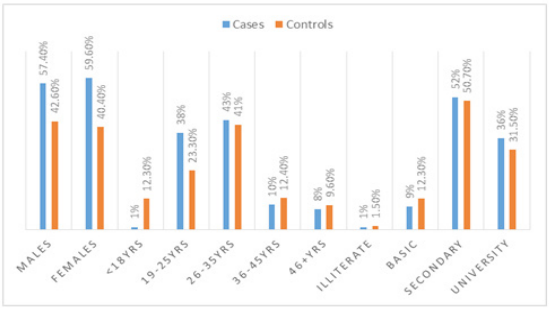


Figure 1. Description of the Study Population by Demographic Characteristics

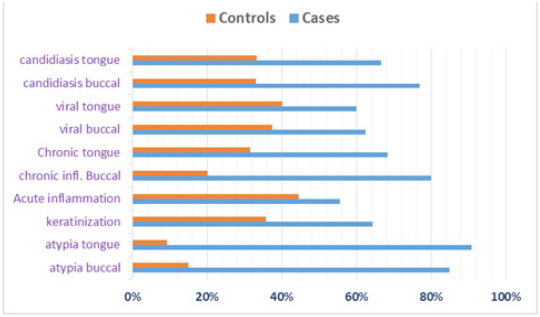


Figure 2. Comparison of Sample Site of Different Variables among Cases and Controls

in its entire group with cases and controls.

In regard to education level, the great majority of the cases were categorized among those with higher level of education as indicated in Table 1.

Table 2, summarizing the distribution of the study population by cytological findings. In buccal smears, cytological atypia was found among 17/101 (16.8%) of the cases and 3/73(4.1%) of the controls. Of the 17 cases, 13/17(76.5%) were identified with mild cytological atypia and 4/17(23.5%) with moderate atypia, hence, all 4 subjects with cytological atypia among controls were identified with mild category. In tongue smears, cytological atypia was found among 11/101 (10.8%) of the cases and 2/73(2.7%) of the controls. Of the 11 cases, 9/11(81.8%) were identified with mild cytological atypia and 2/11(18.2%), hence, all 2 subjects with cytological atypia among controls were identified as mild category (See Microphotograph 1and 2). For the cytological atypia among buccal and tongue smears, the adjusted Odd ratio (OR) and the 95% Confidence Interval (CI) were found to be 4.7 (1.3-16.8), P < 0.016)) and 4.3 (0.9- 20.2), P

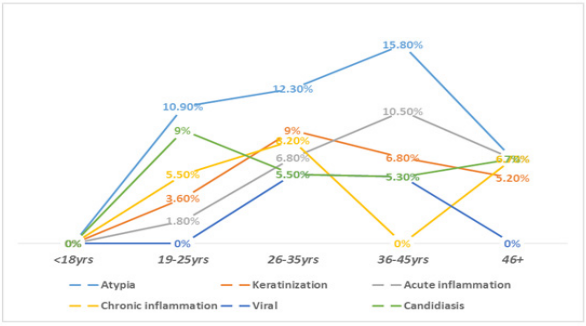


Figure 3. Comparison of the Percentages of the Cytological Findings within Each Age Group

Table 1. Distribution of the Study Population by Demographical Characteristics

Variable	Category	Cases	Controls	Total
Sex	Males	70	52	122
	Females	31	21	52
	Total	101	73	174
Age in years				
	<18.0	1	9	10
	19-25	38	17	55
	26-35	43	30	73
	36-45	10	9	19
	46+	8	7	15
Level of Education				
	illiterate	1	1	2
	Basic	9	9	18
	Secondary	52	37	89
	University	36	23	59

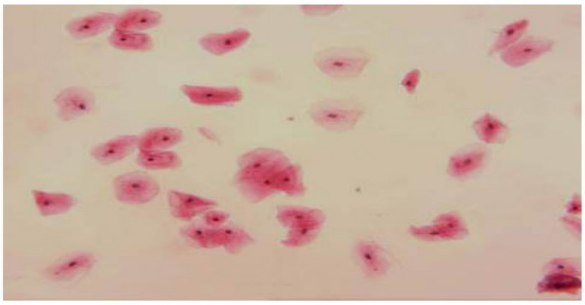
<0.06)), respectively.

Keratinization in the buccal mucosa was demonstrated among 9/101(8.8%) of the cases and 5/73(6.8%) of the controls. For the keratinization, the adjusted OR and the 95% CI were found to be 1.3 (0.43- 4.14), P < 0.622).

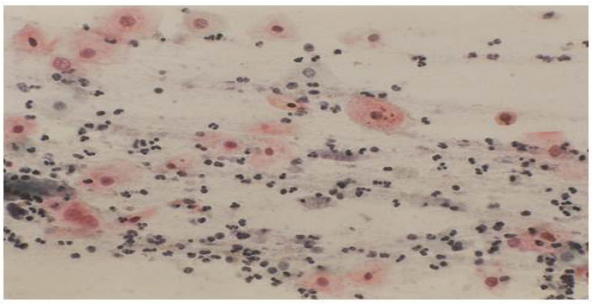
Acute inflammatory cells infiltrates were found in 5/101(%4.8) of the cases and 4/73(5.5%) of the controls. Chronic inflammatory cells infiltrates in buccal mucosa were revealed among 8/101(7.8%) of the cases and 2/73(2.73%) of controls, hence, in the tongue, the cells were identified among 13/101(12.8%) of the cases and 6/73(8.2%). For Chronic inflammatory cells infiltrates among buccal and tongue smears, the adjusted OR and the 95% CI were found to be (3.0(0.6-14.8), P < 0.16)) and (1.6(0.6- 4.6), P <0.33)), respectively, as shown in Microphotograph 2.

Cytological evidence of viral infection (presence of koilocytosis) was identified in 5 and 3 of the buccal smears of the cases and controls respectively. Candidiasis was identified in the buccal smears of 10/101(9.8%) of the cases and 3/73(4%) of controls, whereas, in tongue smears, it was identified among 4/101(3.8%) and 2/73(2.7%). For Candidiasis in buccal and tongue smears, the adjusted OR and the 95% CI were found to be 2.6 (0.9-9.7), P < 0.164)) and 1.5 (0.3- 8.2), P <0.66)), respectively.

Furthermore, variations between buccal smears and tongue smears for the different cytological findings were



Micrphotograph 1. Buccal Smear Obtained from Non-Tobacco Users Showing Normal Oral Exfoliative Epithelium Cells



Micrphotograph 2. Buccal Smear Obtained from Cigarette Smokers Showing Dense Inflammatory Cells Infiltrate with Some Features of Mild Cytological Atypia

Table 2. Distribution of the Study Population by Cytological Findings

Variable	Category	Cases		Controls	
		buccal	tongue	buccal	tongue
Cytological atypia					
	Normal	84.0	90.0	70.0	71.0
	Mild	13.0	9.0	3.0	2.0
	Moderate	4.0	2.0	0.0	0.0
	Severe	0.0	0.0	0.0	0.0
	Total	101.0	101.0	73.0	73.0
Keratinization					
	Low	6.0	1.0	3.0	3.0
	High	3.0	2.0	2.0	0.0
Inflammatory cells infiltrate					
	Acute	5.0	0.0	4.0	0.0
	Chronic	8.0	13.0	2.0	6.0
Evidence of Viral infection					
	yes	5.0	3.0	3.0	2.0
	No	96.0	98.0	70.0	71.0
Candidiasis					
	yes	10.0	4.0	3.0	2.0
	No	91.0	97.0	70.0	71.0

shown in Figure 2.

For males and females, cytological atypia, keratinization, acute inflammatory cells infiltrate, viral infection, and candidiasis were demonstrated in (18.0 and 2.0), (9.0 and 6.0), (9.0 and 0.0), (5.0 and 1.0) and (6.0 and 0.0), in this order, as indicated in Table 3.

In regard to the relationship between age and cytological findings and when comparing the percentages of these variables within each age group, the highest percentage of cytological atypia was found in age group 36.0-45.0 years followed by age ranges 26.0-35.0, 19.0-25.0 and 46.0+ representing 15.8%, 12.3%, 10.9% and 6.7%, respectively. For keratinization the highest percentage was observed in age group 26.0-35.0 followed by age ranges 36.0-45.0, 46.0+ and 19.0-25.0 constituting 9.0%, 6.8%, 5.2% and 3.6%, respectively. The highest percentage of acute inflammatory cells infiltrate was seen in age group 36.0-45.0 years, followed by 26-35.0, 46+

Table 3. Distribution of the Sex by Cytological Findings

Variable	Category	Males		Females	
		buccal	tongue	buccal	tongue
Cytological atypia					
	Normal	104.0	110.0	50.0	51.0
	Mild	14.0	10.0	2.0	1.0
	Moderate	4.0	2.0	0.0	0.0
	Severe	0.0	0.0	0.0	0.0
	Total	122.0	122.0	52.0	52.0
Keratinization					
	Low	5.0	4.0	4.0	4.0
	High	4.0	2.0	1.0	2.0
Inflammatory cells infiltrate					
	Acute	9.0		0.0	0.0
	Chronic	6.0	15.0	4.0	4.0
Evidence of Viral infection					
	yes	5.0	7.0	0.0	1.0
	No	117.0	115.0	52.0	51.0
Candidiasis					
	yes	6.0	5.0	0.0	0.0
	No	116.0	117.0	52.0	52.0

and 19-25, representing 10.5%, 6.8%, 6.7% and 1.8%, in this order. The highest percentage of chronic inflammatory cells infiltrate was seen in age group 26-35 years, followed by 46+ and 19-25, representing 8.2%, 6.7% and 5.5%, respectively. Evidence of viral infection was found in age ranges 26-35 and 36-45 years, representing 5.5% and 5.3%, in this order. The highest percentage of candidiasis was observed in age group 19-25 years followed by age ranges 46+, 26-35 and 36-45 constituting 9%, 6.7%, 5.5% and 5.3%, respectively, as shown in Figure 3.

Discussion

This study explored the possibility of occurrence of oral proliferative activity associated with tobacco smoking; which might subsequently develop into oral precancerous and cancerous lesion among Saudi civilian. Moreover, this study confirmed the visibility of applying oral exfoliative cytological methods for early detection cytological atypical changes before developing into immoral cell line resulting in oral cancer.

Prevalence of oral cancer shows varied geographical distribution (Warnakulasuriya, 2009). Morbidity and mortality are higher in developing countries compared to developed countries (Ferlay et al., 2010). Prevalence of oral cancer is relatively high in most Arab countries including Saudi Arabia (Al-Jaber et al., 2016).The main etiologic factors in the carcinogenesis of oral cancer are tobacco usage and alcohol consumption (Notani, 2000; Ahmed et al., 2010). Tobacco usage, particularly, cigarette smoking is very common in Saudi Arabia especially among younger population. Most of studies from Saudi Arabia dealt with epidemiological settings and cognitive preventive measures. To the best of our knowledge, this is a pioneer study from Saudi Arabia aiming at implementing oral cancer early detection settings. Therefore, establishing of simple, non-invasive, cost-effective procedure is important for effective preventive strategies.

In the present study we examined oral epithelium cells both from buccal mucosa and dorsum of the tongue and both showed significant atypical proliferative changes. May studies have reported that tobacco consumption can lead to oral precancerous lesions, which can progressively develop into advanced oral cancer, even in the absence of clinical manifestations. In the course of malignant progression, alterations happen at the cellular level before clinical changes become evident. Thus, detection of high-risk precancerous oral lesions and intervention is a major key toward reducing the incidence, prevalence, death and cost of treatment associated with oral cancer. Early alterations in the oral mucosa can be identified OEC through screening of population at risk (Ahmed et al., 2003; Ahmed et al., 2009; Khot et al. 2015). OEC is well accepted by patients and it is a reasonable diagnostic and screening tool for diagnosis and early detection of oral mucosal lesions (Buch et al., 2014). The method has a well-known sensitivity of 94.0%, and specificity of 100% (Rajesh et al., 2012).

In the present study, we have tried to investigate samples from buccal mucosa as well as, the tongue to test the hypothesis that tobacco smoking more effect on the dorsum of the tongue than buccal mucosa. However, the effect on both sites was found to be statistically significant.

Furthermore, inflammatory cells infiltrates were more common among tobacco smokers than amongst non-tobacco users, particularly, chronic inflammatory cells, but the frequencies were statistically insignificant. Cytological evidence of viral infections was documented by presence of koilocytosis and were also identified in a number of study subjects, but it was statistically insignificant. Candidiasis was also evident in a number of cases in this study, but it was statistically insignificant.

When comparing tobacco related abnormalities with sex, all parameters were higher among males compared to females. This might be attributed to the fact that, males have higher frequencies of consumption than females, particularly in conservative Saudi community, since female's tobacco use is considered as social stigma.

In regard to age, although the overall effects were seemingly increased with the increase of age, which might be due to prolonged exposure, but still there considerable effect on the relatively younger population.

In conclusion: Tobacco smoking is a major risk factor for occurrence of cytological atypia, which might subsequently develop into oral precancerous and cancerous lesions. Tobacco smoking causes cellular proliferative atypical changes both in the buccal mucosa and dorsum of the tongue. These cytological atypical changes were found to increase tremendously among younger tobacco users. OEC is a non-invasive, easy and cheap method, therefore, it is highly suitable for screening population at risk. In our opinion tobacco smokers should undergo annual screening to detect early changes for suitable interventions.

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