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Assessment of Oral Epithelium Cytomorphological Changes among Water Pipe Smokers

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Abstract:

***Objective**: The study aimed to assess the cytomorphological changes in buccal mucosa among water pipe smokers by cytological methods. **Methodology**: One Hundred subjects were studied, 70 water pipe smokers (ascertained as cases) and 30 non water pipe smokers (ascertained as controls). **Results**: Of the 70 subjects who were accustomed to water pipe use, cytomorphological atypical changes appeared in 33/70(47.1%) of the cases and none of the controls. The risk of cytological atypia associated with water pipe smoking and the 95% confidence interval was Odd Ratio (OR) = 55.9863 (3.2922 - 952.0998), P = 0.0054. Moreover, inflammatory cells infiltrates were observed in 3/70(4.3%) of the cases and 1/30(3.3%) of the controls. The risk of inflammatory cells infiltrates associated with water pipe smoking and the 95% confidence interval was and OR = 1.2985 (0.1296 to 13.0134), P = 0.8242. **Conclusion:** Water pipe smoking is associated with significant oral epithelium changes among Sudanese users. Water pipe smokers should undergo interval screening programs, for detection of precancerous and cancerous cases.*

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Key words: Oral Epithelium, Atypia, Water pipe, Sudanese

INTRODUCTION

Tobacco consumption remains to be the leading preventable cause of disease and death worldwide because an estimated six million deaths each year are attributed to tobacco habit [1,2]. Despite the fact that cigarette smoking remains the main tobacco killer worldwide, for many people, especially youth, tobacco use and addiction is kept by means other than cigarettes smoking [3].

Water pipe tobacco smoking (WTS) (referred to in various regions as shisha, Goz, hookah, narghile, and hubble bubble) is an emerging tobacco product globally, particularly among adolescents and young adults who may see WTS as a safe alternative to cigarettes smoking [4]. Many users, believe that water pipe smoke is far less dangerous than cigarette smoke because the smoke passes through water, which they believe acts as a filter. Unluckily, the water only acts as a cooling agent, not as a filter for nicotine, tar, or carcinogens. Moreover, the cooling procedure forces the smoker to be inhaled much deeper, triggering the smoke to infiltrate deeper into the lungs [5,6]. According to water pipe smokers, the recent resurgence in waterpipe popularity is due to the introduction of *Maassel* (a specially prepared tobacco with sweetened fruit flavors and mild aromatic smoke), the media, and social trends [7].

The 2001 Sudan national Global Youth Tobacco Surveys (GYTS) presented a prevalence of 17.1% (male=25.6%, female=6.1%) for ever smokers, 6.1% (male=10.8%, female=1.9%) for current cigarette users, and 13.5% (male=17.2%, female=10.4%) for users of non-cigarette tobacco products [8]. There were significant gender differences in the

prevalence of ever cigarette users (21.8%; male=13.1%, female=6.5%, $p<0.05$) and current cigarette users (6.9%; male=4.9%, female = 1.3%, $p<0.05$) but not among users of non-cigarette tobacco products (14.7%; male=6.8%, female=6.1%). Adolescent tobacco use was significantly associated with availability of monthly income or allowance, exposure to tobacco industry promotions, and tobacco-use behavior of familial relations. Knowledge about the harmful effects of secondhand smoke was related with decreased likelihood of tobacco use [9].

In spite of the fact that cigarette-like adverse health effects associated with water pipe tobacco smoking and rise in its use among youth, it is a much underexplored research zone in various dimensions. Therefore, the aim of this study was to measure the effect of water pipe smoking in oral epithelium cytomorphological pattern.

MATERIALS AND METHODS

One hundred cytological materials were collected from volunteers in Al-Ubayyid city, 70 of the total volunteers were water pipe smokers (ascertained as cases), while the remaining 30 were non-smokers (considered as control group). Each participant in this study was told about the study before taking the specimen during the interview to obtain personal data.

Buccal smears were taken from the subject by using small size tooth brush to harvest the buccal mucosa cells by scraping technique. Wet smears were fixed immediately in 95% ethanol for 15 minutes, then allowed to dry and subsequently stained using Papanicolaou staining method. Quality control measures were adopted during sample collection and processing.

Atypia was assessed cytologically by using the criteria described Ahmed et al.[10] (2003).The presence of two or more

of the following features were consistent with atypia: nuclear enlargement associated with increased nuclear-cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately prominent nucleoli, irregular nuclear membranes and bi-or multi-nucleation, scanty cytoplasm, and variation in size and/or shape of the cells and nuclei.

RESULTS

This study assessed cytological atypia in 100 study subjects their ages ranged from 19 to 50 years with the mean age of 25 years. The great majority of the cases were at age range 21 to 30 years. Of the 100 study subjects 70 are water pipe users, and 30 are non-smokers. Of the 70 cases, 32/70(42.9%) were found to use water pipe only, 27/70 (38.6%) were found to use cigar beside water Pipe , 2/70(2.9%) were toombak users(2.9%) and 2/70(2.9%) were alcoholic, as shown in Fig1.

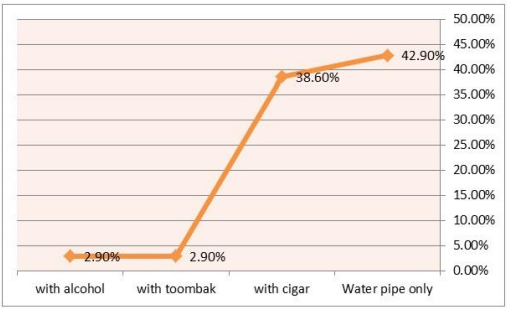

Figure 1. Description of the cases by exposure

Of the 70 subjects who were accustomed to water pipe use, cytomorphological atypical changes appeared in 33/70(47.1%) of the cases and none of the controls. The risk of cytological atypia associated with water pipe smoking and the 95% confidence interval was Odd Ratio (OR) = 55.9863 (3.2922 -952.0998), P = 0.0054. Of the 33 cases with cytological atypia, 25/33 (75.8%)

were found with mild cytological changes and the remaining 8/33(24.2%) were found with moderate changes. None was categorized with severe cytological atypia, as indicated in Fig 2.

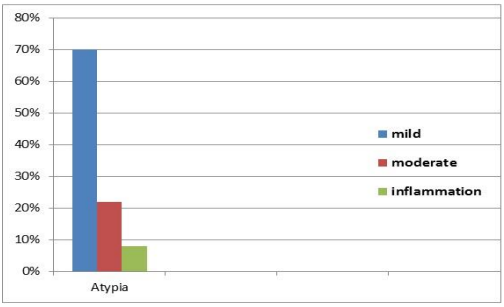

Figure 2. Description of cases by cytomorphological changes

In regard to the duration of exposure, 11/70 (15.7%) were found to have smoked for one year, 21/70(30%) smoked for a duration of 2-4 years and 38/70 (54.3%) smoked for more than 5 years, as indicated in Fig3. The cytological atypia found to increase with the increase of the duration of smoking water pipe, most of the moderate changes were detected among those who smokes for more than 5 years, followed by those form 2-4 years, as indicated in Table1, Fig 4.

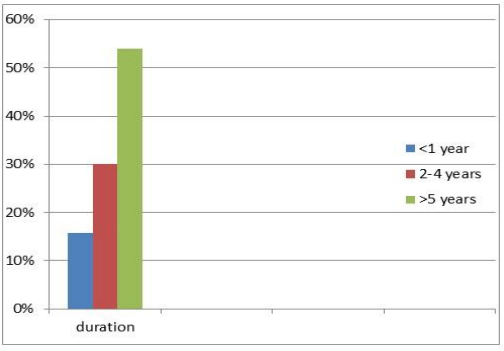

Figure 3. Description of cases by duration of water pipe smoking

Table1: Description of atypia by duration of water pipe smoking

	Atypia			Total
	Mild	Moderate	Inflammation	
Less than 1 year %	5 13.9%	2 6.5%	1 2.8%	8 22.2%
2-4 years	6 16.7%	1 2.8%	1 2.8%	8 22.2%
More than 5 years	14 38.9%	5 13.9	1 2.8%	20 55.6%
Total	25 69.4%	8 22.8%	3 8.3%	36 100%

Inflammatory cells infiltrates were observed in 3/70(4.3%) of the cases and 1/30(3.3%) of the controls. The risk of inflammatory cells infiltrates associated with water pipe smoking and the 95% confidence interval was and OR = 1.2985 (0.1296 to 13.0134), P = 0.8242. Notably inflammatory cells infiltrates were also appear to increase with duration of exposure, as indicated in Table.1

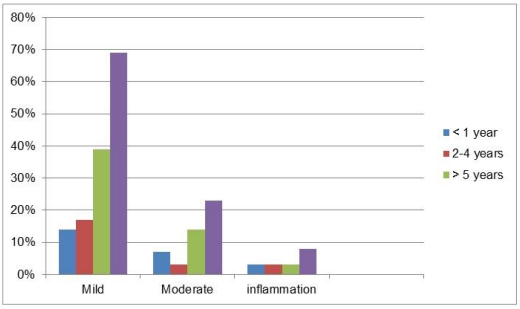

Figure 4. Description of atypia by duration of smoking

DISCUSSION

Oral exfoliative cytology has been used for the assessment of epithelial atypical changes [11,10] and screening for early diagnosis of the oral premalignant and malignant lesions[11,13]. It is known that the exfoliated cells are from the

superficial layer, while most of the atypical cells are located in the deeper layers. The degree of cytological atypia varied in many studies based on qualitative evaluation [14,15]. However, the current study represent results on the relationship between the occurrence of oral epithelial changes water pipe use. These findings indicated the strong association between water pipe use and risk of oral epithelial atypical changes which may develop into oral precancerous or cancerous changes.

Previous studies have suggested that oral mucosal epithelia of smokers and water pipe users are more susceptible to malignant alterations. In a cross sectional study, cytologic smear samples from the following three different areas: buccal mucosa, lateral surface of the tongue, and floor of the mouth (right) were taken from 40 smokers, 40 water pipe users, and 40 normal individuals. They found that smoking and using water pipe are effective in creating some quantitative cytometric alterations in oral mucosa; however, smoking shows greater effect in the cytometric alterations than using water pipe [16]. Also in another research study was conducted in Jeddah, Saudi Arabia, the most common form of tobacco used was cigarette smoking (65.6), followed by water pipe (38.1). By clinical conventional examination a high prevalence (88.8%) of soft tissue lesions was found among the tobacco users examined, and a wide range of lesions present in about 50% had hairy tongue, (36%) smoker's melanosis, (28.9%) stomatitis nicotina, (27%) frictional keratosis, fissured tongue (26.7%), gingival or periodontal inflammation (26%) and finally leukodema (20%), Suspicious (premalignant) lesions affected only 0.5% of the subjects, most prevalent was smokeless keratosis (6.3%), leukoplakia (2.3%), erytheroplakia (0.7%), oral submucous fibrosis (0.5%), lichenoid lesions (0.4%) The high level of oral mucosal soft tissue lesions (88.8%) may be attributed to the irritational effect of tobacco on oral structures [17].

Nevertheless, the frequency of cytological changes in this study were greater than what was reported in the previous, this might be due to non-exclusion of cigar smokers and toombak dippers as both can lead oral epithelial atypical changes. In a study to assess cellular proliferative activity of clinically healthy oral mucosal epithelial cells of toombak dippers and smokers by means of silver Nucleolar Organizer Regions (AgNOR) counts and nuclear areas via nuclear morphometry. Cytological atypia was ascertained in 6 tobacco users and could not be ascertained in non-tobacco users. Statistically mean AgNOR numbers per nucleus in the non-tobacco users (2.45±0.30) was lower than the toombak dippers (3.081±0.39, $p<0.004$), and the smokers (2.715±0.39, $p<0.02$), and mean nuclear areas of epithelial cells of toombak dippers (6.081±0.39, $p<0.009$) and smokers (5.68±10.08, $p<0.01$) was also significantly higher than non-smokers (5.39±9.4). The mean number of nuclei having more than 3 AgNORs was 28%, 19% and 7% in toombak dippers, smokers and non-tobacco users, respectively. These findings support the view that toombak dipping and smoking are severe risk factors for oral mucosal proliferative lesions and exfoliative cytology is valid for screening of oral mucosal lesions [18,19].

Furthermore, the deteriorative effects were found to increase with the increase of duration of exposure. Such findings were previously reported with cigarette smoking and toombak dipping [10]. And because the tobacco is a peripheral vasoconstrictor, it influence the rate at which the oral wound heals. Carbon monoxide and other chemicals produced during the combustion of tobacco can reduce the capillary blood flow. A clinical study has shown that a single cigarette can reduce the peripheral blood velocity by 40% in one hour [20].

This study also found that water pipe smoking is more common among younger individuals (21-30 years old) which may indicate the future hazard to important section of

population. Therefore, implementation of strategies to reduce the burden of tobacco use as well as, assigning screening programs are deemed important for future control of the diverse effect of tobacco.

Furthermore, this study has major limitations including the exclusion criteria, as well as, its cross sectional setting.

In conclusion: Water pipe smoking is associated with significant oral epithelium changes among Sudanese users. Water pipe smokers should undergo interval screening programs, for detection of precancerous and cancerous cases.

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