Original Research Article

DOI: http://dx.doi.org/10.18203/2394-6040.ijcmph20175053

Self-perceived oral health status and cytomorphological changes in individuals with addictive oral habits: assessed by WHO oral health questionnaire for adults

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Received: 08 October 2017 Accepted: 26 October 2017

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ABSTRACT

Background: Oral diseases related to tobacco use are relatively widespread and common in the Pakistani community but epidemiological data regarding maintenance of oral hygiene and suggested treatment is scarce. Therefore, this study was conducted in an under developed district (Muzaffargarh) of Punjab, Pakistan to evaluate oral health status and to determine frequency of cytomorphological changes in buccal mucosa of habitual tobacco users.

Methods: Oral examination of all the subjects was carried out using mouth mirrors and gauze under good illumination. Cytology samples were taken from buccal mucosa. The smears were later stained with Papanicolaou stain and microscopy was performed. All the cytological data was recorded in the respective proformas. Data was analysed using IBM SPSS software version 20. Descriptive statistical analysis was performed. A p value of ≤ 0.05 was considered significant.

Results: When cross relations were studied among the demographics, addictive habits and cytological variables, soft drink usage was associated with epithelial atypia (p=0.05), marijuana usage was associated with inflammatory infiltrate on cytology (p=0.047) and snuff/niswar usage was associated with inflammatory atypia (p=0.048). No associations were found among the other studied variables.

Conclusions: The results of cellular changes in the smears from subjects with addictive habits can be utilized as an educational and screening tool in cessation therapy. The results attained in this study proposed that tobacco addictive habits produce cellular modifications in clinically normal mucosa on exfoliation cytology.

Keywords: Oral addictive habits, Oral cytology, Smoking, Naswar, Paan, Chewing tobacco

INTRODUCTION

World Health Organization (WHO) has defined health as "A state of complete physical, mental and social wellbeing and not merely an absence of disease or infirmity". This means that merely absence of disease should not be regarded as health rather health is the

complete wellbeing of a person. Disease free oral cavity and good oral hygiene is as important as any other healthy part of the body. Therefore, individual with active disease in oral cavity cannot be regarded as healthy.¹

Multiple diseases occur in the oral cavity, including dental problems such as caries and periodontal problems

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such as gingivitis, periodontitits and yet others like mucositis, lichen planus, etc. These are painless and less destructive in the beginning, but as time passes they transform into more destructive chronic diseases and thus can affect different body organs. Cure and prevention of these diseases is necessary and its awareness among general population has become important so that the overall quality of life gets better.² It has been documented that many oral diseases lead to systemic diseases and various systemic diseases have oral presentation that reenforces the significance of good oral health in general well-being of an individual. In developing countries, basic facilities of life, such as food, clothing, shelter and medical facilities have overshadowed the need of oral health and thus it remains low on the priority list.³

Cigarette smoking (CS) and chewing tobacco (CT) is common in Pakistan. South East Asia consumes more than 90% of the world's total smokeless tobacco and around 100 million people of Indo-Pak subcontinent are habitual to smokeless tobacco use. 4,5 CT is available in and commercial both traditional preparations. Traditionally it is available as a betel leaf wrap which contains multiple ingredients including areca nut and slacked lime. Flavoring agents are added to enhance the taste. In commercial form it is available as gutka, zarda, khaini, mishri, etc. Commercial preparations contain tobacco coated areca nut along with different spices including saffron and cardamom. Flavoring agents and sweeteners are also added which make the preparation more palatable and addictive. 6-9

Tobacco use affects the oral cavity in multiple ways ranging from inert harmless lesions such as oral to highly malignant carcinomas. pigmentation Researchers have detected strong association between tobacco use and multiple oral lesions including mucositis, leukoplakia, submucous fibrosis and oral cancers. Oral cancers account for 3% of total malignancies in the Western world, whereas the situation in Pakistan is different. In Pakistan oral cancer is relatively more common and is ranked 3rd on the list of most common malignancies. This much variation in the distribution of cancer could be due to local habits of tobacco use. 10-13 Frequency of tobacco use and time duration for which active ingredients remain in contact with mucosal tissue play an important role in the pathologic transformation of mucosa. Therefore, chances of malignancy are many fold higher in chronic tobacco abusers. 14,3

Oral diseases related to tobacco use are relatively widespread and common in the Pakistani community but epidemiological data regarding maintenance of oral hygiene and suggested treatment is scarce. Further, no data is available on general population's awareness towards oral health and their oral habits. The limited data that is available is from the developed cities of Pakistan. Therefore, this study was conducted in an under developed district (Muzaffargarh) of Punjab, Pakistan to evaluate oral health status and to determine frequency of

cytomorphological changes in buccal mucosa of habitual tobacco users.

METHODS

This was a cross sectional study conducted between January 2016 to February 2016 in the Muzaffar Garh district, which is situated in the south of Punjab, Pakistan. The study was approved by the Ethical Review Committee of University of Health Sciences, Lahore, Pakistan. Non probability convenient sampling technique was used to collect samples from 45 subjects with addictive tobacco habits such as cigarette smoking, betel quid chewing, gutka, etc. An inclusion criterion was adults above the age of 18 years with any addictive tobacco habit for more than 6 months. Details of the study were explained to the subjects and after taking written informed consent each subject was interviewed. Demographics and other related data such as type of habit, duration, frequency, site of placement, the period of contact with the mucosa, and history of discontinuation of the habit was recorded on WHO oral health assessment questionnaire.

Oral examination of all the subjects was carried out using mouth mirrors and gauze under good illumination. Cytology samples were taken from buccal mucosa. Before sampling all subjects were made to rinse their mouth with water. Site of the smear was cleaned with a moist gauze piece. Wooden spatulas were used to collect cells for cytology from the site and these were transferred to frosted glass slides (already numbered) using circular motion. Glass slides were immediately sprayed with the fixative and kept in a container. These smears were later stained with Papanicolaou stain and microscopy was performed. All the cytological data was recorded in the respective proformas.

Data was analysed using IBM SPSS software version 20. Descriptive statistical analysis was performed. The significance of the study parameters was assessed by applying Chi-square test, keeping confidence interval and level of significance at 95% and 5% respectively. A p value of \leq 0.05 was considered significant.

RESULTS

All the participants in the above mentioned research were males with a mean age of 28.88±9.50 years and a range of 15 to 58 years. The samples were collected from a backward area of Punjab called Khangarh (District Muzaffargarh). The majority of the participants n=43 (95.6%) belonged to peri-urban area while n=2 (4.4%) belonged to a rural area. When the educational status of the participants was assessed, the majority had only completed their primary level of education as seen in Table 1.

When an oral examination was carried out, all the subjects had more than 20 teeth in their oral cavity and

only n=2 (4.4%) were using a partial denture. The majority of the participants, 80% (n=36) never visited a dentist in their lifetime as shown in Table 2. According to the survey the participants were happy with their oral health (Table 3).

Table 1: Educational background of the study subjects.

Educational Status:	Results (%)
No formal schooling	8 (17.8)
Less than primary	10 (22.2)
Primary school completed	17 (37.8)
Secondary school completed	04 (8.9)
High school completed	01 (2.2)
College/university completed	05 (11.1)

Table 2: Duration since last visit to dentist.

Duration	Results (%)		
Last visit to the dentist			
<6 months	6 (13.3)		
>1 year	1 (2.2)		
>2 years	2 (4.4)		
Never	36 (80)		

Table 3: Self oral assessment of the study subjects.

Own oral assessment	Results (%)		
Excellent	1 (2.2)		
Very good	5 (11.1)		
Good	21 (46.7)		
Average	12 (26.7)		
Poor	06 (13.3)		

Table 4: Frequency of teeth cleaning habits.

Teeth cleaning habits	Results (%)		
Never	1 (2.2)		
2-3 times/month	1 (2.2)		
Once a week	3 (6.7)		
2-6 times/week	18 (40)		
Once a day	20 (44.4)		
Twice or more a day	2 (4.4)		
Uses toothpaste	Yes 34 (75.6)		
	No 11 (24.4)		

When a previous history of 12 months was taken n=21 (46.7%) of the subjects reported discomfort within the oral cavity When a previous history of 12 months was taken n=21 (46.7%) of the subjects reported discomfort within the oral cavity, whereas sensitivity was reported by n=20 (44.4%), halitosis by n=8 (17.8%), and any type of jaw pain by n=7 (15.6%). When the cleaning habits and tooth paste use for teeth cleaning were asked n=20 (44.4%) brushed once with a regular dentifrice (Table 4).

The sugar intake of the participants was identified by asking the frequencies of beverages and tea usage (Table 5). Different addictive habits were also common in the studied area with pan and tobacco chewing most common in 35 subjects (77.8%) and were used on a daily basis. The other addictive habits are summarized in Table 6.

Table 5: Common study variables of subjects with addictive habits.

Variables	Results (%)
Soft drinks	
Never	4 (8.9)
Several times a month	2 (4.4)
Once a week	3 (6.7)
Several times a week	12 (26.7)
Everyday	19 (42.2)
Several times a day	5 (11.1)
Tea with sugar	
Never	8 (17.8)
Once a week	1 (2.2)
Several times a week	1 (2.2)
Everyday	16 (35.6)
Several times a day	19 (42.2)

Table 6: Types and frequency of different addictive oral habits.

Different addictions	Results (%)			
Cigarettes usage				
Never	24 (53.3)			
Everyday	21 (46.7)			
Chewing tobacco or pan chewing				
Never	10 (22.2)			
Everyday	35 (77.8)			
Snuff or Niswar				
Never	28 (62.2)			
Everyday	17 (37.8)			
Marijuana				
Never	40 (88.9)			
Everyday	05 (11.1)			
Gukta				
Never	36 (80)			
Everyday	09 (20)			

Cytology slides were prepared by the saliva collected from the subjects and was studied for different cytological parameters. Karyolysis was observed in n=41 (91.1%) and micronuclei in n=40 (88.9%) subjects. However, opportunistic fungal infection in the form of hyphae was observed in only n=4 (8.9%) subjects. Other observed cytological features of the subjects are given below (Table 7).

When cross relations were studied among the demographics, addictive habits and cytological variables, soft drink usage were associated with epithelial atypia

(p=0.05), marijuana usage was associated with inflammatory infiltrate on cytology (p=0.047) and snuff/naswar usage was associated with inflammatory atypia (p=0.048). No associations were found among the other studied variables.

Table 7: Common cytological variables of subjects with addictive habits.

Variables	Present (%)	Absent (%)
Inflammation (mild)	36 (80)	09 (20)
Hyperchromatism	03 (6.7)	42 (93.3)
Prominent nuclei	30 (66.7)	15 (33.3)
Binucleation	38 (84.4)	07 (15.6)
Karyolysis	41 (91.1)	04 (8.9)
Karyorrehxis	33 (73.3)	12 (26.7)
Pyknosis	32 (71.1)	13 (28.9)
Micronuclei	40 (88.9)	05 (11.1)
Swelling of organelles	02 (4.4)	43 (95.6)
Elongated nuclei	14 (31.8)	31 (68.9)
Mitosis	32 (71.1)	13 (28.9)
Adequate squames	38 (84.4)	07 (15.6)
Adequate keratinization	27 (60)	18 (40)
Inflammatory atypia	03 (6.7)	42 (93.3)
Epithelial atypia	07 (15.6)	38 (84.4)
Nuclear atypia	32 (71.1)	13 (28.9)
Fungal hypae	04 (8.9)	41 (91.1)

DISCUSSION

Oral health is a condition of being free from birth defects such as cleft lip and palate, facial pain, chronic mouth, oral sores, oral and throat cancer, tooth loss, periodontal (gum) diseases, tooth decay and other disorders and diseases that involve the oral cavity. Risk factors for oral diseases comprise of tobacco use, alcohol use, poor oral hygiene and unhealthy diet.¹⁶

In diverse parts of the world, the main incidence of smoking is reported between the younger age groups and descends gradually in older age. ^{17,18} These results are consistent with our study where all the participants were males with a mean age of 28.88±9.50 years and a range of 15 to 58 years.

Soft drinks have a lot of probable health harm. The sugars and inherent acids have equally cariogenic and cariogenic prospective, following in possible enamel erosion and dental caries. ¹⁹ As we know that in current years there has been elevated importance in the part of soft drinks in dental diseases that is erosion and. dental caries. Bertoldi and his colleague reported that not only the surface but as well as the subsurface enamel were damaged by soft-drink erosion and Sauro et al stated that acidic soft drinks stimulate enamel erosions. ^{20,21}

Sreebny interrelated the dental caries experience (DMFT) of 12-year-olds with sugar supplies data of 47 countries to the dental caries occurrence of primary dentition (DMFT) of 5 and 6-year olds with sugar supplies data of 23 countries. For both age groups, noteworthy association were noted.²² In countries where intake of sugar is below 18 kg/person/yr (equal to, 50 g/person/d), occurrence of caries was constantly below DMFT 3, whereas the countries where sugar supplies is over indulgence of 44 kg/person/yr (120 g/person/d), had significantly greater intensity of caries. 23 In present study the higher proportion of participants reported to have hidden sugar every day in the form of soft drinks as well as tea (42.2%), their consumption is on daily basis and several times a day accordingly. Hence, we conclude that oral health education is significant in educating people regarding a healthy diet and this is not only healthy for their general body but in addition also for the oral health

However, Bangesh and his colleagues and Parveen et al reported that merely 45.9% and 42.5% visited the dentist and majority among them only visited when they experienced any dental problem. These results are better as compared to the present study. Overall, 90% of all untreated oral diseases, including periodontal disease and caries are due to lack of oral health awareness. Barker and Horton reported that the hindrance in search of dental care could be due to multiple factors like accessibility of dental services and lack of resources. From the findings of the present study and previously conducted studies in Pakistan, we conclude that the dental visits and dental awareness is not so frequent in our population and a major reason for dental visits are basically unbearable dental pain.

The toothbrush was observed to be an essential thing in the studied subjects. Females were noted to spend increased time throughout brushing and clean their teeth more as compared to males.²⁷ Studies have reported that the incidence of tooth brushing had a noteworthy relationship with caries incidence.²⁸ In the present study, 75.6% participants use tooth paste for tooth brushing, this is in accordance with the other study where 75% participants brush their teeth with toothpaste.²⁹

In the present study, 46.7% of subjects visited a dentist because of oral cavity discomfort and pain, followed by sensitivity (44.4%). The study conducted by Devaraj and Eswar reported that the previous dental visit was more than 2 years in 38.5% of individuals and the dental pain (35.3%) was major cause for visiting a dentist followed by decayed teeth (27%) and none of them said they had visited the dentist for schedule oral examination. Multiple studies reported that the predominant reason for dental visits are pain. Thus these all previous conducted studies are in accordance with the present study.

In South Asia, tobacco is also available in different forms such as, chewed with betel nut, pan and snuff [smokeless tobacco (ST)] which has been recognized as dangerous and reason for oral and esoghageal cancer. ^{32,33} In Pakistan, the tobacco industry is growing at a rate of 5% per year. ³⁴ Among the world, Pakistani cigarettes have the maximum levels of nicotine and tar. ³⁵ Tobacco use or cigarette smoking has been significantly related with a large range of diseases, including cancers of mouth, larynx, throat, lungs, emphysema and coronary heart disease. The prevalence of tobacco smoking in Pakistan is estimated to be 9% for females and 36% for males. ³⁶

Cytology screening is the most excellent technique for early identification of cancer, it is believed to be as an additional technique which is safe, non-invasive, inexpensive, fast with high sensitivity and with no need of anesthesia, while it can be completed in the form of any brush cytology or exfoliative cytology. Only a small number of studies have been carried out on exfoliative cells acquired from oral mucosa exposed to cigarette smoking.

The occurrence of two or further subsequent features were constant with atypia: related to the increased nuclear/cytoplasmic ratio, nuclear hyperchromatism, increased keratinization, nuclear enlargement, irregularity of nuclear membranes, chromatin clumping with prominent nucleation and bi or multi-nucleation.³⁹ Assessment of these changes was included in our study for atypical changes.

The incidence rate of micronuclei is calculated by chromosomal breakage in early cell divisions. The figure of micronuclei is identified to increase with carcinogenic stimuli. 40 Buccal cells micronuclei were more numerous between cigarette smokers as shown by previous studies. 41,42 Wu et al stated that buccal cell micronuclei were positively linked among micronuclei frequency and each cumulative smoking (packs/year) or smoking intensity (e.g., daily cigarette consumption).⁴³ Similarly, in a study of 120 healthy subjects, Konopacka reported that in smokers the incidence of micronuclei in oral epithelial cells was three times greater as compared to nonsmokers, the recorded micronuclei frequency values were 0.47% and 0.32%, respectively (p<0.05).44 Sarto et al reported that the frequency of micronuclei was about twice as elevated in smokers in contrast with nonsmokers (p<0.01). No statistically noteworthy association was established for several of the variables examined; consisting of gender, age, hot food, spicy food, coffee, oral antiseptics, teeth brushing, alcohol consumption, oral infection and oral prosthesis.⁴⁵ Elevated occurrence of micronuclei in cells collected from smokers was also noted in our study and the previous studies were in compliance with the present study.

The biological importance of the micronuclei in buccal cells of the oral mucosa is that the micronuclei are signs

of a readily particular clastogenic event, as reported here in, have been linked with smoking. The clinical importance of micronuclei in buccal cells is that the manifestation of the micronucleated buccal cells is helpful biomarkers that are associated with oral cancer.

Binucleation is the occurrence of two nuclei within a cell. Binucleation is believed as a sign of cytotoxicity.³⁹ A study reported that it is a nuclear abnormality seen in dysplastic cells and is seen to be increased in smokers.⁴⁶ In the present study significant increase in binucleation frequency was observed in smokers group.

In the present study, pleomorphism was reported in smoker group. It appears that a raise in nuclear size is a type of cell alteration in reaction to the oral mucosal epithelium lesion. This study; consequently, validate the association among quantitative cellular and nuclear alterations and tobacco chewing this is in accordance with the multiple international and national studies. Einstein and Siva Pathasundharam noted that among tobacco users, in the south Indian population, cytomorphologic modifications in the appearance of decrease in cellular diameter and raise in nuclear diameter in buccal squames.⁴⁷ Hosseini et al have reported that pleomorphism and increased number of multi-lobed nuclei in epithelial cells of smokers were observed as compared to non-smokers.⁴⁸

In present study adequate keratinization were observed in 60% of smears. This is in accordance with the study conducted on smokers with clinically healthy oral mucosa and it was reported that an increase in the quantity of keratinized cells a higher rate of proliferation of epithelial cells as well as nuclear and cytoplasm alterations was observed. 49-51 This raise in the figure of ortho-keratinizated superficial cells or enucleated in oral mucosa of smokers has been observed by various authors. 52-54 Bustoc et al noted that smokers with clinically normal mucosa displayed a larger proportion of keratinized cells. 55 It is hypothesized that the chemical action of volatile products of the tobacco and the direct stimulation of the heat of the cigarette would cause an increase in keratinizated cells, is a defensive mechanism of epithelial cells to these harmful agents. 52

In the present study, the epithelial atypia was reported in 15.6% of smears and absent in the remaining of the smears. Mohammed and Tagwa reported that cell atypia was detected in 12% of the smokers smears. The Present study reported that cytological atypia among the tobacco users is considerably elevated than the non-tobacco users, which is a similar finding with the study of Ahmed et al, Babu et al and Idris et al. Their findings recommended that cigarette smoking is connected with a possibility for incidence of oral epithelial atypia, which can be identified by the use of simple cytological methods. Thus, our results are in accordance with multiple international studies.

Summarily, this study adds knowledge to the literature by recognizing tobacco-associated genetic and non-genetic buccal cell changes, that these changes might demonstrate useful for smoking cessation. These changes have been evaluated by means of different assays and cancer prevention, that buccal cell micronuclei have been recognized as helpful biomarkers in clinico-pathologic analysis and for the early detection of oral cancer. Additional studies in oral smears are compulsory for the early detection of malignancies in the oral mucosa of smokers and to recognize the role of cytology.

CONCLUSION

For screening in high risk groups oral smears stained with PAP are easy procedures and non-invasive techniques. The objective is to make sure that all patients who smoke are regularly identified, examined and properly taken care of

Our study concludes the significance of early detection of cellular alterations for recognition of pre-malignant variations in the patients with smoking habits. The results of cellular changes in the smears from smokers can be utilized as an educational and screening tool in smoking cessation therapy. The results attained in this study proposed that tobacco smoking produces cellular modifications in the clinically normal mucosa in exfoliation cytology.

ACKNOWLEDGEMENTS

All the authors acknowledge the co-operations of study participants, Mr. Sameer Anjum (Lab Technician) Morbid Anatomy and Histopathology Departments, UHS, Lahore.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the

Institutional Ethics Committee

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Cite this article as: Gul H, Asif F, Samad A, Minhas S, Anwar MA, Tayyab MA, et al. Self-perceived oral health status and cytomorphological changes in individuals with addictive oral habits: assessed by WHO oral health questionnaire for adults. Int J Community Med Public Health 2017;4:4393-400.