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ORIGINAL RESEARCH

Cytomorphological Changes in Buccal Mucosa among Tobacco Smokers and Tobacco Chewers and Level of Awareness towards their Adverse Effects on Health

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ABSTRACT

Background: Tobacco contains chemical constituents which are known as carcinogens and have been attributed as a major risk factor to cause oral cancer. Quantitative parameters such as nuclear size, cell size, nuclear to cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density and nuclear texture could be early indicators of malignant change. **Aim & Objective:** To evaluate the cytomorphometric changes in clinically normal buccal mucosa of habitual tobacco smokers and chewers and to assess their level of awareness towards adverse effects of tobacco on health. **Material & Methods:** The present study included 30 individuals each of habitual tobacco smokers and chewers in the study group of an age range 15-70 years and 15 age matched individuals without tobacco habit in the control group. Questionnaire was used to record the subject data and habits. Following questionnaire, smears were prepared from buccal epithelial cells, stained with Papanicolaou stain and cytomorphometric analysis performed using Image J analysis software. **Results:** The mean of above nuclear and cell morphometric parameters and their ratio were found to be statistically significant by Univariate analysis of variance (ANOVA) with p value being < 0.001. No significant difference was observed on comparison of reasons for getting habituated between tobacco smokers and chewers as well as on comparison of knowledge pertaining to the health problems between smokers and control group. **Conclusions:** There was progressive increase in nuclear area, decrease in cytoplasmic area and increase in nuclear to cytoplasmic ratio in smears from tobacco smokers and chewers, as compared with control group.

Key words: Cytomorphometry, oral mucosa, tobacco chewing, smoking.

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INTRODUCTION

Tobacco is a menace that has grabbed millions of people all over the world, cutting across the nation and social barriers.^[1] Betel quid chewing is one of the most popular habits among Indians, in which tobacco is used along with betel leaf, areca nut, slaked lime etc.^[2]

One of the commercial replacements for betel quid is Pan Masala and Gutkha where tobacco along with other ingredients is dispensed in ready to use packets. The packaging revolution has made tobacco products portable,

cheap and convenient, with the added advantage of a long shelf-life. As of these reasons use of commercial tobacco product has become highly prevalent among youngsters.^[3] Tobacco contains carcinogens which are known to cause oral cancer. During malignant transformation, changes occur at the cellular level before clinical changes become evident.^[4]

Quantitative parameters such as nuclear size, cell size, nuclear to cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density and nuclear texture evaluated

could be earliest indicators of malignant change. Of these parameters, nuclear and cytoplasmic area and nuclear to cytoplasmic ratio have been shown to be significant in the diagnosis of malignant changes.^[5]

Therefore a need was felt to carry out a study with an aim to evaluate the cytomorphometric changes in clinically normal buccal mucosa of habitual tobacco smokers and chewers and to assess their level of awareness towards adverse effects of tobacco on health.

MATERIALS AND METHODS

The present study included a total of 75 individuals; with 60 individuals (30 each of habitual tobacco smokers and chewers) in the study group of an age range 15-70 years and 15 age matched individuals without tobacco habit in the control group. Patients with systemic disease such as anemia or diabetes, previous benign or malignant lesions and mental abnormality were excluded from this study. Ethical clearance from the institute ethical committee and informed consent from all patients before taking the cytological smears were obtained. Questionnaire was used to record the subject data and habits as per given by **Ruchi Nagpal et al, 2014**. Following questionnaire, general mucosal screening was carried out using mouth mirror and straight probe. Individuals were asked to rinse their mouth with water so as to remove the residual food particles and then buccal mucosal scrape was collected using a cytobrush, the smears were prepared, fixed and stained with PAP stain.

CYTOMORPHOMETRIC ANALYSIS

All the stained smears were observed under binocular microscope and 30 non-overlapping cells with well defined borders were randomly selected in each smear as per the criteria given by **Ignacio Gonzalez Segura et al, 2014**. The images of these cells were captured with a digital camera under 40x objective. Images thus captured were transferred to the personal computer and nuclear, cell boundaries were drawn to calculate Nuclear area (NA), cytoplasmic area (CA) using the digitizer cursor as per the criteria given by **Juliana Umetsu Paraizo et al, 2013 (Fig 1)**. The measurements were recorded using measurement tool represented in square microns (μm^2). All the samples were evaluated by two observers who were blind to the particulars of the study; if they did not agree on their readings, a third observer evaluated the sample.

STATISTICAL ANALYSIS

The data obtained was statistically analyzed by using one-way ANOVA (Analysis of Variance) for comparing the parameters. Comparison of the mean NA, CA and N/C values in between the groups was done using

multiple comparison by Bonferroni test. The Chi Square test was applied to inter compare the categorical variables between control and study groups.

RESULTS

The comparison of mean values of parameters NA, CA and N:C ratio revealed statistically significant difference among tobacco smokers, chewers and individuals without tobacco habit with p value being 0.00 (Table 1).

Multiple comparisons of parameters (NA, CA, N:C ratio) between the study and control groups for NA between tobacco smokers versus individuals without tobacco habit, tobacco chewers versus individuals without tobacco habit revealed statistically significant difference ($p=0.00$). Significant difference in CA was observed between tobacco smokers versus tobacco chewers, tobacco smokers versus individuals without tobacco habit, tobacco chewers versus individuals without tobacco habit ($p<0.05$). Significant difference in NA:CA was observed between tobacco smokers versus tobacco chewers, tobacco smokers versus individuals without tobacco habit and tobacco chewers versus individuals without tobacco habit (<0.05).

Most of the individuals among tobacco smokers (9 out of 30) were illiterates whereas among control group, majority of the individuals (8 out of 15) were with education at college level or above. Statistical evaluation revealed non-significant difference with p value being 0.22.

Majority of the individuals in tobacco chewers and control group were illiterates and college or above respectively. Statistical evaluation revealed non-significant difference with p value being 0.16.

Among tobacco smokers and chewers, the most common reasons for getting habituated to the tobacco habits answered were to be time pass and pleasure followed by curiosity and stress. Statistical evaluation revealed non-significant difference with p value being 0.39.

Among tobacco smokers, oral cancer (13 out of 30 individuals) was the most commonly answered health problem followed by general health problems, mouth ulcers and gum diseases. No statistically significant differences was found between persons with habit and without habit. Among tobacco chewers and individuals without tobacco habit oral cancer was the most commonly answered health problem. Statistical evaluation revealed significant difference with p value being 0.04

Majority of the individuals among tobacco smokers, chewers and individuals without tobacco habit believed that the community residents would quit the addiction through family pressure. No statistically significant differences was found between study and control group.

Table 1: Comparison Of Mean Values Of Nuclear Area, Cytoplasmic Area And Nuclear: Cytoplasmic Ratio Between Study And Control Groups

		Sum Of Squares	df	Mean Square	F	p value
NA	Between groups	5.209	2	2.604	40.290	0.00 (S)
	Within groups	4.654	72	0.065		
	Total	9.863	74			
CA	Between groups	3953.557	2	1976.778	19.151	0.00 (S)
	Within groups	7431.735	72	103.219		
	Total	11385.292	74			
N:C Ratio	Between groups	0.003	2	0.002	21.217	0.00 (S)
	Within groups	0.005	72	0.000		
	Total	0.009	74			

ANOVA test, p<0.05 significant, S=Significant, df= Degree of Freedom.

DISCUSSION

All of the major forms of tobacco such as cigarettes, cigars, pipes, and smokeless tobacco (chewing tobacco and snuff) are known to cause oral cancer. Identification of high risk oral premalignant lesions and intervention is one of the keys to reducing the mortality, morbidity, and cost of treatment associated with oral squamous cell carcinoma.^[7,8] In the present study, evaluation of mean values of NA, CA and N:C RATIO revealed an increase in mean nuclear area, decrease in mean cytoplasmic area and increase in mean nuclear to cytoplasmic ratio in the study group as compared to control group. These findings were consistent with the results of Swetha Acharya et al. 2013^[9] who observed a gradual decrease in CD, CA, increase in ND, NA, ND:CD and NA:CA in smears from all gutkha chewers, as compared to healthy controls. Babuta S et al. 2014,^[10] Khot K. 2015^[11] and D Parmar et al. 2015^[12] also reported similar findings in tobacco users as compared to healthy controls. The results of our study were in contrast with the study carried out by Cowpe JG et al. 1985,^[13] Deepti Sharma et al. 2015^[14] who observed no significant reduction in the cellular diameter, cellular area and increase in the nuclear diameter, nuclear area and nuclear/ cytoplasmic ratio in tobacco users as compared to normal individuals. Variations in our findings from other studies could be attributed to certain factors. Also comparison of mean NA, CA and N:C ratio with duration of tobacco habits revealed statistically non-significant results in the study group. Variation in the mean number of NA, CA and N:C ratio explains about their cytogenic and dysplastic effect on oral mucosa when compared with respect to type of habits. The results of our study were in contrast with the study carried out by Hashemipour et al. 2013^[15] and Babuta S et al. 2014,^[16] according to which with increasing heavy exposure in duration of years, cytomorphometric changes show significant altered values for all three measured parameters (NA, CA and N/C ratio).^[17] In the present study, maximum individuals in the study and control group were illiterate and college or above level respectively. The results of our study were in accordance with the study carried out by Daga MK et al.

2012,^[18] Bhuputra Panda et al. 2012^[19] and Nathan John Grills et al. 2015^[20] who reported that tobacco use was highest amongst the illiterates. Other studies by Rani et al. 2006^[21] and Chhabra et al. 2001^[22] have also shown lower literacy level to be a strong predictor of smoking. Thus, poor literacy status suggestive to be associated with poor awareness of the health hazards of tobacco consumption, increased likelihood of exposure to conditions favoring initiation of smoking and chewing of tobacco, and higher overall risk taking behavior.

Among tobacco smokers and healthy individuals, the most common reasons was found to be time pass and pleasure while individuals with the habit of tobacco chewing perceived time pass as the most common reason. The results were in accordance with Raj Kumar et al. 2010,^[23] Khan NS and Ravishankar TL. 2008,^[24] Nagpal R et al. 2014^[25] who observed pleasure as the most commonly reported reason for tobacco consumption. These results suggest that pleasure and time pass has become the major problem and also an important factor leading to tobacco consumption.

Among tobacco smokers, chewers and individuals without tobacco habit, oral cancer was the most commonly reported health problem. Similar findings were observed by S Ahmed et al. 1997,^[26] Anuradha Pai et al. 2014^[27] and Nagpal R et al. 2014.^[25] These findings corroborates earlier findings which report that mass media is a common source of information regarding oral cancer (Ariyawardana and Vithanaarachchi 2005; Petty and Scully 2007; Amarasinghe et al., 2010; Srikanth et al., 2011).^[28]

CONCLUSION

Our study concludes that cytomorphometric changes such as could be the earliest indicators of cellular alterations an increase in nuclear area, decrease in cytoplasmic area and increase in nuclear to cytoplasmic ratio indicating that there could be cause-effect relationship between tobacco consumption and quantitative cellular alterations. Hence it is possible to suggest that this adaptive change in the cell nucleus tends to be a progression towards dysplastic change.

Also, it could strongly influence the participants to quit the habit of smoking and chewing tobacco, thus encouraging us to adopt this modality of very accessible and easy to perform procedure to educate the general and addicted population about the deleterious effects of tobacco smoking and chewing.

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