Cytomorphometric Study of Effect of Tobacco Smoking on Buccal Mucosa: A Case control study

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Abstract—*Tobacco smoking is associated with increasing risk of oropharyngeal cancer. Oral cytology, mainly based on the presence of nuclear or cytoplasmic alterations, can easily be performed to detect cancer at an early stage and provides a quantitative technique. The objective of the present study was to assess the effect of smoking on buccal mucosa using cytomorphometry. This case control study included 36 smoker subjects and 36 non-smoker controls between the age of 30 and 70 years. Buccal epithelial cells were collected with a brush and fixed smears were stained with Papanicolaou stain and cytomorphometric analysis performed using image analysis software (Image J v 1.47). Smoker group was found to have higher mean nuclear diameter (MND), lower in mean Cellular diameter (MCD), and higher nucleo-cytoplasmic ratio as compared to normal subjects. This change in nuclear parameters indicates smoking-related cellular adaptation, leading to progression towards dysplasia. Cytomorphometric changes could prove to be the earliest indicators of these cellular alterations.*

Keywords: Cytomorphometry, Smoker, Buccal Mucosa.

I. INTRODUCTION

Oral cancer is one of the six common cancers in the world[1,2] and it is also one of the ten major causes of death across the globe.[3] Tobacco smoking has been associated with increasing risk of oropharyngeal cancer and oral leukoplakia. Most of the oral cancers are preceded by precursor lesion, which could be of great help in early diagnosis. Oral cytology, which is mainly based on the presence of nuclear or cytoplasmic alterations, can easily be performed to detect cancer at an early stage and to establish quantitative techniques.[4]

Tobacco is of two main types: Smoked and Smokeless tobacco. Commercially it is available in dried, cured, and natural forms. In India Bidies majorly account for about 40% and Cigarettes about 20% of total consumption followed by Cherrut or Chutta, Chillum, Hukli and Hukkah and rest divided among different forms of chewing tobacco.[5]

Many carcinogenic substances, mostly DNA-toxic carcinogens are present in cigarettes which cause genetic mutations and chromosomal abnormalities and micronuclei.

Cytological staining techniques developed by Papanicolaou & Traut is now widely used in the detection of asymptomatic cancers,[6] Recent advancements in the field of quantitative oral exfoliative cytology have lead to evaluation of various parameters such as nuclear size, cell size, nuclear-to-cytoplasmic ratio, nuclear shape, nuclear discontinuity and optical density.[7] The two significant morphologic changes known to occur in actively proliferating cells of oral lesions are, decrease in the cellular diameter and increase in the nuclear size.[8]

Present study was undertaken to find out any difference in cytological parameters like cell diameter (CD), nuclear diameter (ND) and nuclear diameter to cell diameter ratio (ND/CD) between tobacco smokers and non-smokers.

II. METHODOLOGY

A cross sectional case control study was conducted at SMS Medical College and attached hospitals, Jaipur, Rajasthan from May to December 2015. Present study included 36 subjects aged 30 to 70 years in each of the two groups, Group 'A' included subjects with tobacco smoking habit (for a minimum of 10 years and atleast 20 times per day) and Group 'B' included subjects without tobacco smoking habit. In both these group subjects with habit of alcohol intake or tobacco chewing and those with family history of oral cancer and even subjects with any lesion were excluded from study.

Sample size was calculated at alpha error 0.05 and study power 80% assuming detectable difference in mean of maximum cellular diameter to be 5.73 µm and SD 8.22 µm (as per a previous study[1])

Ethical clearance was obtained from the Institutions Research Review board and informed consent was taken from each participant.

Collection of samples:- Subjects were asked to rinse their mouth with water and a pre-moistened wooden spatula was used for collection of sample from buccal mucosa of both sides. Using a gentle scraping motion cells were scraped from the clinically normal appearing buccal mucosa. The sample was immediately smeared on the microscopic slides and fixed with ethyl alcohol.

Staining was done with Papanicolaou stain. The stained slides were studied under 400X magnification. From each slide, Nuclear and Cytoplasmic diameter of 50 cells with well-preserved cytoplasm were measured along both X-axis and Y-axis along the maximum dimension observed. Then the mean of the values of X-axis and Y-axis for each cell was calculated and was taken for analysis.

Statistical analysis:- Categorical data was expressed as percentage/proportion and was analyzed using Chi square test**.** Continuous variables were expressed as mean and standard deviation and the difference in means was inferred using unpaired t test. P value <0.05 was taken as statistically significant. All calculations were done by using Medcalc trial version 14.0.0 versions. $p<0.05$ was taken as significant.

III. RESULTS

This study was conducted on 72 eligible subjects, which included 36 smokers (Group 'A') who smoked at least 20 times a day for the last 10 years and 36 non-smokers (Group 'B'). Both groups were without any local or systemic diseases.

Both these groups were well comparable as per age ($p=0.768$) and sex ($p=1$). Although in smoker group maximum subject were in 30-39 years followed by 40-49 years, 50-59 years and 60-69 years whereas in non smoker group maximum subject were in 40-49 years followed by 30-39 years, 50-59 years and 60-69 years but this variation in distribution was not found significant. In both the group, males and females were equally distributed. (Table 1)

When type of smoking was assessed, it was found that Bidi smoker was maximum (58.3%) followed by Cigarette and Hukah. (Table 1)

To compare both the groups, measured parameters were Mean Nuclear Diameter (MND), Mean Cytoplasmic Diameter (MCD) and Ratio of MND to MCD of buccal mucosa.

Table 1
Comparison of Baseline characteristics of Group 'A' and group 'B'

Parameter	Subgroup	Non-smoker (Group 'A')	Smoker (Group 'B')	*p Value	**LS
Age Groups (in Years)	30-39	13 (36.1%)	8 (22.2%)	0.768	NS
	40-49	12 (33.3%)	17 (47.2%)		
	50-59	7 (19.4%)	7 (19.4%)		
	60-69	4 (11.1%)	4 (11.1%)		
Sex	Male	18 (50%)	18 (50%)	1	NS
	Female	18 (50%)	18 (50%)		
Type of smoking	Bidi	0 (0%)	21 (58.3%)	-	
	Cigarette	0 (0%)	11 (30.6%)		
	Hukah	0 (0%)	4 (11.1%)		

***p Value is calculate by Chi-square test** ****LS is level of significance**

When both groups were compared as per Mean Nuclear Diameter (MND), Mean Cytoplasmic Diameter (MCD) and Ratio of MND to MCD of buccal mucosa, Mean Nuclear Diameter was found to be significantly higher in Smokers (11.97 µm) as compared to Non-smokers (10.83 µm). Mean Cytoplasmic Diameter was significantly smaller in Smokers (68.29 µm) as compared to Non-smokers (75.38 µm). Ratio of Mean Nuclear to Cytoplasmic Diameter was significantly higher in Smokers (0.1778) as compared to Non-smokers (0.1441). ($p<0.05$). (Table 2)

Table 2
Comparison of cytological parameters of Group 'A' and group 'B'

Parameter	Smoker (Group A)	Non smoker (Group B)	*p Value	**LS
Mean Nuclear Diameter (µm)	11.97 ± 1.204	10.83 ± 1.043	<0.001	**S**
Mean cytoplasmic diameter(µm)	68.29 ± 12.11	75.38 ± 8.669	0.006	**S**
Ratio of MND to MCD	0.1778± 0.015	0.1441± 0.0013	<0.001	**S**

***p value calculated using unpaired t test** ****LS is level of significance**

IV. DISCUSSION

All major forms of tobacco use like cigarettes, cigars, pipes and smokeless tobacco are known to cause oral cancer. Carcinogens that influence the DNA repair, cell cycle control and may produce chromosomal aberrations are found in tobacco. There is a strong association between cancer of the oral cavity and the use of tobacco. This risk tends to increase with the duration of smoking.

Previous study like Ramaesh T et al [9] suggested that, of various cellular and nuclear parameters, nuclear and cytoplasmic area and nuclear to cytoplasmic ratio are significant in the diagnosis of oral lesions. Cowpe JG et al[10] reasoned that increase in the nuclear diameter could be due to increased DNA content of the nucleus and increase in ratio of nuclear diameter to cellular diameter is due to the changes in nuclear size and cytoplasm.

Present study also showed a significant quantitative alteration in the form of decreased cellular diameter, increased nuclear diameter and increase in ratio of nuclear diameter to cellular diameter in the

tobacco smokers as compared to non-smokers. Similar findings were reported by Ogden et al[11] in study of the effect of smoking on the oral mucosa in individuals over 40 years of age using cytomorphological methods and reported a 5% average increase in the NA values of smokers when compared to non-smokers.

Deepankar Parmar et.al.[11] studied the effect of smoking on buccal mucosa by using cytomorphometry on 50 smoker subjects and 50 non-smokers between the age of 28 and 71 years and reported that the MNA, MNP, Max-ND and Min-ND values of the buccal mucosa cell nuclei of smokers were significantly higher than those of non-smokers. Similar findings were observed in the present study between tobacco smokers and non-smokers. The effect of smoking and betel quid chewing on the oral mucosa using cytomorphological methods was analyzed by Einstein and Sivapathasundraham,[12] and reported an increase in the average value of nuclear diameter, and a decrease in cytoplasmic diameter values of smokers. Ramaesh et al[13] also reported that the cytoplasmic diameter of individuals who smoked cigarettes and chewed betel quid and practiced both these habits was significantly smaller than that of the control group individuals, and the nuclear diameter of the buccal mucosa cells in individuals who smoked cigarettes, chewed betel quid was significantly greater than that of the control group individuals.

Franklin CD and Smith CJ[14] reported that the N:C ratio has the advantage of relating nuclear volume to cytoplasmic volume and possibly represents the significant changes that occur in the cell, more accurately at a morphological level. A significant difference was reported in nuclear diameter of the two groups. It can be inferred from above evidence that tobacco use in any form causes alteration in the nuclear size, indicating the detrimental effect of tobacco on oral mucosal cells.

Increase in nuclear size resulting from the increase of nuclear DNA content could be cell adaptation in response to the oral mucosa epithelium lesion. By cell irritation, smoking facilitates ageing process of oral mucosal cells. Epithelial cells of oral mucosa have a decreased turnover, so cells remain in cell cycle for longer periods resulting in a delayed cell division. As a result, proteins which are synthesized within the nucleus divide slowly, which in turn, it increases the nuclear size. The sizes of nuclear and cytoplasm decline following aging process as a result of degeneration of Golgi apparatus and endoplasmic reticulum in aged cells.[15] it has also been demonstrated that exfoliative cytology is valuable for monitoring clinically suspect lesions and malignant lesions after definitive treatment.[16,17]

Present study included linear measurements only and did not give the ploidy status of the cell or three dimensional view.

V. CONCLUSION

This study concludes that reduction in cell diameter, increase in nuclear diameter and increase in nucleo-cytoplasmic ratio among cytomorphometry finding found in smokers. These findings may be an early indication of malignant change. The association of these cytomorphometric effects with tobacco exposure gives important information about risk assessment process and may be used to assess health risks for exposed persons. The simple, inexpensive and easy cytomorphometric analysis method can make the cytological study of subjects with tobacco smoking habit a more objective and practical tool for the early detection of oral cancer. To further confirm the cytological alterations and its role, further studies are required with prospective design, more sample size and standardized parameters.

CONFLICT OF INTEREST

None declared till now.

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