Evaluation Of The Effect Of Smoking On Select Cytomorphometric Indices Of Buccal Mucosal Cell In Middle Aged Individuals

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Abstract: Background: The aim of the present study was to evaluate the effect of smoking on select cytomorphometric indices of buccal mucosal cell in middle aged individuals. Selected measurements of cytomorphometric indices such as nuclear area (NA) (μm^2), nuclear perimeter (NP) (µm), minimal nuclear diameter (Dmin) (µm) and maximal nuclear diameter (Dmax) (µm) were observed and assayed through light microscopy making use of digital image processing software TS view version 7.3.1.7. Methods: 60 smokers and an equal number of age and sex matched control in the age range of 40 - 50 years were recruited in the present study. On exfoliative buccal mucosal cytology average nuclear area (NA), nuclear perimeter (NP), minimal nuclear diameter (Dmin) and maximal nuclear diameter (Dmax) values of cell nuclei were measured using the software TS view version 7.3.1.7. Results: Statistically significant increased cytomorphometric mean values of nuclear i.e. measurements NDmax (µm) (P=0.000), ND_{min} (µm) (P=0.000), NA (μm^2) (P=0.000), and NP (µm) (P=0.004) in smokers as compared to that seen in non-smokers. Conclusion: In the present study, statistically significant increase in the cytomorphometric nuclear parameters, namely minimum nuclear diameter (NDmin), maximum nuclear diameter (NDmax), nuclear area (NA) and nuclear perimeter (NP), could be appreciated in smokers as compared to that observed in non-smokers, underscoring the relevance of exfoliative cytology as a potential screening assay for the diagnosis of dysplastic changes in the oral mucosal cells. [Alka M NJIRM 2017; 8(1): 66-70]

Key Words: Oral exfoliative cytology, Light microscopy, Smokers and Non Smokers.

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Introduction: Tobacco is a menace that has afflicted millions of people all over the world, cutting across the nation and social barriers.[1] The World health organization reported that tobacco smoking killed 100 million people worldwide in the 20th century and warned that it could kill one billion people around the world in the 21st century also. [2]

WHO (1998) has laid down guidelines for stratification of the population into smokers and non-smokers. [3] Oral cancer affects as many as 274,000 people worldwide annually, and the frequency of oral cancer around the world is often indicative of the patterns of use of tobacco products.[4-7] It is prevalent among middle-aged adults and is associated with low survival rate.[8-10] Oral exfoliative cytology is a simple, non-invasive and painless method that involves microscopic analysis of cells collected from the surface of the oral mucosa and is seemingly a potential tool to evaluate the malignant changes in the mucosal cells, if any at an early stage.[11,12] Today, cytomorphometric analysis is gaining in popularity and recognition as an effective tool of assessing the dysplastic, premalignant and malignant changes in the sample tissue.[12] Normal epithelium undergoes continuous exfoliation or shedding of its superficial cells, and it is replenished by new crop of cells from the basal layer. Thus the thickness of the epithelium is maintained. Under normal conditions the epithelial cells strongly adhere to each other. When the epithelium becomes the seat of malignant disease or of some benign conditions the cells may lose their cohesiveness so that the deeper cells may be exfoliated along with the superficial cells.[13] Morphological examination of these cells reveals that buccal cells have large size, a pancake-like shape, and non-granular voluminous and centrally located small and oval nuclei. With the help of these features, these cells can be easily distinguished from polymorph nuclear leukocytes and other cells present in the oral cavity.[14] In exfoliative cytology various parameters such as nuclear size, cell and nuclear pleomorphism, nuclear membrane discontinuity, degenerative changes of nucleus and nuclear cytoplasmic ratio are analyzed.[15] As nuclear changes are the most important criteria for diagnosing precancerous and cancerous lesions and since no single structural change is diagnostic by itself, a combination of several abnormalities is always necessary. Because quantitative procedures are objective and reproducible, they may be important aids in making a cytopathologic diagnosis.

The aim of this study is to compare the exfoliated buccal mucosa cells among smokers and non-smokers cytomorphometrically, which includes measurements of parameters such as nuclear area (NA), nuclear

perimeter (NP), minimal nuclear diameter (D-min), and maximal nuclear diameter (D-max).

Methods: The present study was carried out in the department of Physiology in collaboration with the department of Medicine, at S.M.S. Medical College, Jaipur Rajasthan. 60 smokers and an equal number of age and sex matched control in the age range of 40 - 50 years were included in the present study. Subjects were selected by simple random sampling. After getting clearance from the Institutional Ethic Committee, written informed consent was obtained from the participants before the onset of the study and oral exfoliative cytology was performed. The nuclear cytomorphometric measurements evaluated in the present study were, maximum nuclear (ND max), minimum nuclear diameter (ND min), nuclear area (NA) and nuclear perimeter (NP) and the cytomorphometric assay was done by using Radical USB Digital Microscope fitted with Nikon 5.1 megapixel camera using the software TS view version 7.3.1.7 provided by Radical Instruments, India. The nuclear dimensions were give credence and nuclear : cytoplasmic ratio was not included in the present study, a parameter that is intended to be included in the next proposed prospective study.

Subjects in the age-range of 40- 50 years of either sex, male or female, smoking at least 10 cigarettes a day were included in the study and subjects with history of any systemic diseases such as anaemia or diabetes, who have received radiotherapy and/or chemotherapy in the last month, pregnant or menstruating women, alcoholics were excluded from the sample population.

The subjects were examined orally in the morning using a mouth mirror and artificial light and were asked to rinse their mouths with water before samples were taken to remove and eliminate debris and excess saliva from the oral mucosa. Exfoliated epithelial cells were obtained from the right buccal mucosa with the help of a moistened wooden spatula. The smear was spread on a clear glass slide and immediately fixed in 95% ethanol for a minimum of 15 minutes to avoid exposure to dry air (otherwise the cells would degenerate). The smears were stained with hematoxylin and eosin and were observed under microscope and 50 non-overlapping cells with well-defined borders were sequentially selected. The sampling was done in a stepwise manner, moving the slide from the left upper corner to the right, and then down in order to avoid measuring the same cells twice. The images were captured with a camera attached to the microscope. All the images of the cells were captured with a 100x oil immersion objective. Images thus captured were stored on the computer and analysis was done using the software TS view version 7.3.1.7.[16] The average nuclear area (NA), nuclear perimeter (NP), minimal nuclear diameter (Dmin), and maximal nuclear diameter (Dmax) values of cell nuclei were obtained for each subject. The data was analyzed in terms of mean and standard deviation and the measures were evaluated for statistical significance through student's 't-test'.

Result: The present study was a comparative evaluation on 60 smokers and an equal number of aged matched non-smoker population. The number of male subject was 74 in which smokers and non-smokers ratio was approximately 2:1, whereas the total number of female subject was 46 wherein the smoker and non-smokers ratio was approximately 1:3 as can be deduced from table number 1.

It can be concluded from table number 2 that all the cytomorphometric nuclear values in smokers were observed to be statistically increased.

Table number 3 depicts a similar trend wherein the cytomorphometric nuclear values are significantly increased in male smokers as compared to that seen in male non-smokers.Table number 4 gives an insight into the role that gender plays in the cytomorphometric nuclear parameters, wherein significant changes could be appreciated in mean values of ND_{MIN} (μm) and NA (μm^2) in smoking females as compared to that of non-smoking females.

Table No. 1: Gender distribution of the sample population

	Smoker	Non-Smoker	Total
Male	48	26	74
Female	12	34	46
Total	60	60	120

Chi-square = 15.546 with 1 degree of freedom;
P = 0.000

Table No. 2: Comparison of mean cytomorphometric values between smokers and non-smokers (n = number of subjects and the values represent mean ± standard deviation).

Parameter	Smoker (n = 60)	Non-Smoker (n = 60)	'P' Value*
Maximum Nuclear Diameter (µm)	11.11 ± 0.43	10.41 ± 1.31	0.000*
Minimum Nuclear Diameter (µm)	8.23 ± 0.55	7.39 ± 0.73	0.000*
Nuclear Area (μm^2)	75.54 ± 6.35	64.32 ± 5.73	0.000*
Nuclear Perimeter (µm)	32.13 ± 1.34	30.48 ± 4.13	0.004*

[P < 0.005 (Significant, S) NS = Not Significant]

Table No. 3: Comparison of mean cytomorphometric values between male smokers and non-smokers (n = number of subjects and the values represent mean ± standard deviation).

Parameter	Smoker (n = 48)	Non-Smoker (n = 26)	'P' Value*
Maximum Nuclear Diameter (µm)	11.11 ± 0.46	10.5 ± 0.32	0* (S)
Minimum Nuclear Diameter (µm)	8.27 ± 0.44	7.43 ± 0.44	0* (S)
Nuclear Area (µm2)	75.82±6.25	64.05±3.93	0* (S)
Nuclear Perimeter (µm)	32.22 ± 1.35	29.8 ± 0.76	0* (S)

[P < 0.005 (Significant, S) NS = Not Significant]

Table No. 4. Comparison of mean cytomorphometric values between female smokers and non-smokers (n = number of subjects and the values represent mean ± standard deviation).

Parameter	Smoker (n = 12)	Non-Smoker (n = 34)	'P' Value*
Maximum Nuclear Diameter (µm)	11.09 ± 0.29	10.34 ± 1.72	0.146 (NS)
Minimum Nuclear Diameter (µm)	8.11 ± 0.87	7.35 ± 0.89	0.014* (S)
Nuclear Area (µm2)	74.42 ± 6.92	64.52 ± 6.85	0* (S)
Nuclear Perimeter (µm)	31.8 ± 1.32	31 ± 5.42	0.617 (NS)

[P < 0.005 (Significant, S) NS = Not Significant]

Discussion: Oral cavity is a miniature screen of whole body and a lesion present in oral cavity may give an insight into and reflect an individual's health.17 Oral cavity and oral mucosa is the first site to get exposed to any substances including noxious substance like tobacco products. Exfoliative cytology, a method initiated and developed by Miller and Montogomery, is based on the study of cell and molecular Physiological aspect of the epithelium.18 The oral epithelium/ mucosa is a stratified squamous epithelium inclusive of the basal cell, intermediate prickle cells and apical cornified cells.18 The nucleus of the apical cells is round and small as compared to that of the basal cell. As the epithelium cell matures, the cell compress and flatten and assumes a wafer shape and nuclear chromatin condenses to a dense physiologically hypoactive entity (pyknosis).18 In normal dynamic healthy situation the buccal cells strongly adhere to each other maintaining an optimal thickness of buccal epithelium. However, in the presence of a disease state or malignancy changes take place within the epithelial cells, wherein the epithelial cells tend to lose cohesion resulting in exfoliation that result in easy collection and examination of the exfoliated cells.19

Cytomorphology is the most widely used method of oral exfoliative cytology and assesses parameters such as cellular diameter (CD), nuclear diameter (ND), nuclear area (NA), cytoplasmic area (CA), NA/CA ratio, nuclear shape, nuclear membrane continuity, optical density and nuclear texture.19-21 These parameters, especially nuclear area (NA) and NA/CA ratio, have been shown to provide meaningful results in the diagnosis of oral lesions.19,22 Quantitative cytomorphometric evaluation of exfoliated buccal mucosa cells obtained from premalignant and malignant lesions has revealed significant differences at the cellular level. 23-25 Individuals who have been smoking one or more than one pack a day for 10 years or more have been defined as heavy smoker.26,27 Smoking and tobacco chewing has been implicated mired changes in the oral mucosa of many individuals ranging from harmless and reversible lesions to malignant change in oral mucosa.28-31 Since the present study focused on the effect of smoking of buccal mucosal cells, buccal mucosal lesions such as

epithelial dysplasia, leukoplakia, erythroplakia and squamous cell carcinoma were not included in the study. Considering the initial sensitive and specific changes that take place within the nuclear function mirrored as changes taking place in the nuclear morphology and morphometry. It was observed that significant changes in the buccal mucosal cytomorphometric characters could be appreciated in smokers as compared to that observed in non-smokers. Ramaesh et al(1999)32 reported that the nuclear diameter of the oral mucosa cells in individuals who smoked cigarettes, chewed betel quid, or practiced both these habits, was significantly greater than that of the control group individuals.

They also reported that the cytoplasmic diameter of individuals who chewed betel quid and smoked cigarette was significantly smaller than that of the control group individuals. Similarly Einstein et al(2005)33 also analyzed the effect of smoking and betel quid chewing on the oral mucosa, using cytomorphological methods and determined an increase in the average value of nuclear diameter (ND) and a decrease in cytoplasmic diameter values of smokers and individuals who smoked and chewed tobacco. The result of present study are consonance with those of Ramaesh et al (1998) wherein maximum nuclear diameter (NDmax) (P = 0.000), minimum nuclear diameter (NDmin) (P = 0.000), nuclear area (NA) (P = 0.000) and nuclear perimeter (NP) (P = 0.004) were found to be significantly increased in smokers as compared to that observed in non-smokers. Ogden et al 34 studied the effect of smoking on the oral mucosa in individuals in the age ranging 40 - 50 years using cytomorphological technique and reported a 5% average increase in the nuclear area (NA) values of smokers as compared to that of non-smokers. In the present study an increase of 17.5% in the nuclear area (NA) in the smoker population was observed as compared that seen in non-smoker. The increase in nuclear area (NA) of the smoker reflects plausible compensatory mechanism taking place at the molecular level within the nucleus to counteract the modulatory influence(s) of the contents of cigarette smoke on the buccal mucosal level mirrored at the macroscopic level as an increase in nuclear area so observed in the smokers.

It could be concluded from the result of present study that cytomorphometric analysis of the study of exfoliative buccal mucosal cells could act as a sensitive and specific indicator of the changes, benign, premalignant and malignant, so induced by the contents of cigarette on the buccal mucosa. Henceforth, exfoliative buccal mucosal cell cytomorphometry could act as a low-cost, sensitive, specific and effective screening assay for pre-malignant and malignant changes in high-risk population.

Conclusion: In the present study, statistically significant increase in the cytomorphometric nuclear parameters, namely minimum nuclear diameter (NDmin), maximum nuclear diameter (NDmax), nuclear area (NA) and nuclear perimeter (NP), could be appreciated in smokers as compared to that observed in non-smokers, underscoring the relevance of exfoliative cytology as a potential screening assay for the diagnosis of dysplastic changes in the oral mucosal cells.

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