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Exfoliative Cytology of Buccal Mucosa in Smokers and Non Smokers

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ABSTRACT

Background: Tobacco smoking have been attributed to as major risk factor for carcinogenesis. The oral mucosa of smokers exhibits many changes. Exfoliative cytological methods have been employed to examine these changes, especially in cells collected from the buccal mucosa. Atypical cells are present on surface of mucosa in smokers compared to person who doesn't have any deleterious habit. Oral exfoliative cytology is popular as an oral cancer screening tool, as it is a painless, non-invasive procedure, and is well accepted by patients as it causes little discomfort.

Materials and Method: The aim of this study was to compare the cytology of cells collected from apparently normal buccal mucosa in tobacco smokers, with results obtained for nonsmokers. Objective was to evaluate presence of atypical cells in apparently normal mucosa among smokers using Cytomorphometry. The study population comprised of study group and control group.

Result: Results were obtained by measuring nuclear diameter, cytoplasmic diameter, nuclear and cytoplasmic ratio in each sub group and comparing between study and control group. There was statistically significant difference between study group and control group.

Conclusion-The effect of smoking from this study convince evidenced that tobacco have definitively deleterious effect on oral mucosa in terms of nuclear cytoplasmic changes, which are the key features for early diagnosis of malignancy.

Keywords: Smoking, Oral exfoliative cytology, Nuclear-cytoplasmic ratio, Cytomorphometry.

INTRODUCTION

Oral cancer is one of the most common cancer according to WHO and each year 5,75,000 new cases and 3, 20,000 deaths occur worldwide.[1] Oral cancer is currently the sixth most common malignancy in the world.[2] In India it is the most common malignancy among men and one of the five most common malignancies among women. In India oral cancer is a major health problem, which accounts for 50-70% of all carcinomas diagnosed.[1, 2]

There is a geographic variation in the incidence of oral cancer among different countries of the world and among different regions within a country. This indicates that environmental factors may play an important role in the pathogenesis of cancer of the head and neck. Effect of smoking tobacco on oral health is well documented. Tobacco smoking has been attributed to as major risk factor.

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In the early stages, oral cancer may disguise itself and appears only as a benign and asymptomatic lesion. Patients usually reports to the clinician at a time when the tumor is at an advanced stage. Although a visible lesion precedes the development of majority of oral cancers, it may be possible for a tumor to develop within apparently normal appearing mucosa.[3]

Despite advances in surgery, radiotherapy and chemotherapy, the 5-year survival rate of OSCC patients has remained approximately 50%. This poor survival rate among oral cancer patients can be attributed to several factors; one prominent factor being the lack of early detection and treatment. Hence, it is necessary to detect potentially malignant lesions at their incipient stage. Early diagnosis and initiation of appropriate treatment of early malignant lesions offer the best hope of improving the Prognosis.

As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated from the surface of the oral mucosa. [2, 3]

Exfoliating superficial cells from can be been scraped or pulled from the surface through gentle excavation.[4] Oral exfoliative cytology is a simple and non-invasive diagnostic technique that could be used for early detection of initial mucosal changes, oral premalignant and malignant lesions.[5] Early detection of a premalignant or cancerous oral lesion promises to improve the quality of life and the morbidity of patients suffering from such conditions. Cytological study of oral cells is a non-aggressive technique that is well accepted by the patient, and is therefore an attractive option for the early diagnosis of oral cancer, including epithelial atypia and squamous cell carcinoma.[6] In healthy individuals, epithelial cells of the buccal mucosa in the oral cavity are naturally exfoliated everyday.[7] Thus, buccal mucosa cells can be collected through excavation. Many factors affect the cytomorphology of the cells collected from the oral mucosa. Some of these factors are systemic diseases, e.g., anemia and diabetes mellitus; radiotherapy; alcohol consumption; and smoking. Cigarettes contain many carcinogenic substances, mostly DNA-toxic carcinogens. It is well known that these carcinogenic substances cause genetic mutations and chromosomal abnormalities and micronuclei.[5,7]

Cytomorphology has been utilized widely in exfoliative cytology and analyzes parameters like cell and nucleus diameter, nucleus and cytoplasm area and their ratio, nucleus morphology, nucleus membrane integrity, and its aggregation and texture. These parameters especially nucleus area and its ratio to cytoplasm can provide significant data in diagnosis of oral lesions.[8, 9]

Ogden et al (1999), showed that quantitative techniques based on parameters like nucleus and cytoplasm area and their ratio, due to their precision and repeatability, can enhance exfoliative cytology's sensitivity in rapid diagnosis of oral cancers.[10]

Hence, the proposed study was designed to review and summarize the evidence associating habitual tobacco smoke exposure with mucosal changes and early detection of histopathilogical changes associated with smoking among people living in and around the city of Udaipur.

MATERIAL AND METHODOLOGY

The aim of this study was to compare the cytology of cells collected from normal buccal mucosa in tobacco smokers, with results obtained for nonsmokers. Objective was to evaluate presence of atypical cells in apparently normal mucosa among smokers using Cytomorphometry.

INCLUSION CRITERIA

All the patients who had habit of smoking more than 1 year were included in study.

EXCLUSION CRITERIA

1. People who presented any mucosal lesion were excluded from study.
2. Any tobacco related habits other than smoking were excluded from study.
3. Participant with any known systemic illness such as anemia, diabetes etc. were excluded as these factors may alter nuclear cytoplasmic ratio.[11]

4. Female participants were excluded as hormonal changes may result in alteration in exfoliating mucosal cells.[12]
5. Patients who have exposed to ionizing radiation within past 6 months were excluded from present study.

The Department of Oral Medicine and Radiology and Oral health and screening camps conducted by Department of Community Dentistry, Darshan Dental College and Hospital. The study sample size consisted of 30 males with smoking habit and 30 non-smokers. Ethical clearance was obtained from ethical committee of the institution.

Study participants were examined irrespective of sex, caste and socioeconomic status. General mucosal screening was done. Questionnaire/ interview were used to record the subject data and habits. In the presence of habit detailed history was recorded as to the type, period and frequency of the habit and a written consent was signed by the patients. All the individuals with smoking habit were considered as the study group population. Individuals without any habit of tobacco served as the control group population of the study.

Following informed consent, buccal mucosal scrape were collected using a custom made cytobrush. Cells were sampled using a gentle scraping motion with little pressure until pin-point bleeding appeared, and were spread on the glass slide and were immediately fixed using spray fixative by a distance of 25 cm by maximum 2 times spray pressure and the code related to every patient was written on each slide and in order to prevent the possible error. [13] Papanicolau technique (RAPID PAP® stain, Biolab Diagnostics (I) Pvt. Ltd., India) was used to stain the smear.[14]

Smears were observed under microscope and randomly selected 50 non-overlapping cells with well defined borders were selected. Microscopic examination of each slide was done starting from left to right in horizontal manner. The images were captured with a camera attached to the microscope. One clear image was captured for each cell (Figure 1). All the images of the cells were captured with a 40x achromatic objective. Captured images were stored on the computer and analysis was done using the software Magnus image analyzer version 3.0.

Nuclear and cellular diameter

The nuclear and cell diameter were calculated taking the mean of maximum diameters in two perpendicular plane in microns (µm).[2]

Nuclear cytoplasmic ratio=nuclear diameter/cellular diameter

Statistical analysis

The data was statistically analyzed using unpaired t-test and the value of statistical significance was set at p value <0.05. The statistical significance within and between the groups was analyzed using One-way Analysis of Variance (ANOVA) with a value of p <0.05 being statistically significant.

RESULTS

Study comprised of study group and control group. Habitual cigarette smokers were included in study group and people without any tobacco related habit were included in control group. 42 people, who had habit of cigarette smoking were screened out of 12 patients were excluded according to exclusion criteria. 39 people were screened for control group out of which 9 were excluded. 30 participants were included in both groups. In presence of habit participants were further divided in sub-groups according to age, duration of habit, quantity of cigarette smoking and quality of cigarette smoking.

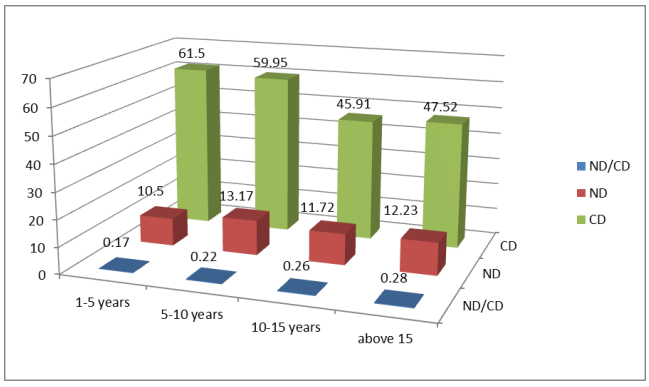
Age ranging in study group was 21 to 72 years (mean age 36 years) while in control group it was 22 to 68 years (mean age 34.5 years). Mean duration of smoking habit in study group was 12 years. Mean quantity of cigarette smoking per day was 9 cigarettes per day. Analysis of nuclear, cytoplasmic diameter (microns) and nuclear-cytoplasmic ratio were done on the basis of age, duration and frequency.

Table 1: Comparisons of mean nuclear cytoplasmic ratio (ND/CD) of study group and control group based on different age group.

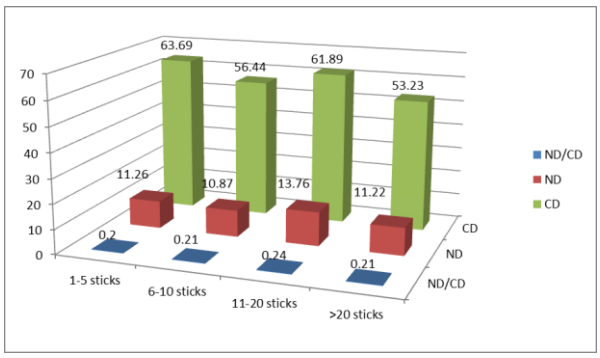
Age groups	Study group	Control group	P value
20-30 YEARS	0.18	0.17	>0.05 (NS)
31-40 YEARS	0.17	0.19	>0.05 (NS)
41-50 YEARS	0.23	0.21	>0.05 (NS)
51-60 YEARS	0.31	0.24	<0.05 (S)
ABOVE 60	0.33	0.26	<0.05 (S)

Table 2: Comparisons of nuclear, cytoplasmic diameter and nucleus-cytoplasmic ratio between study and control group.

	ND		CD		N/C Ratio	
	Mean	±SD	Mean	±SD	Mean	±SD
Study	11.94	0.24	45.24	9.69	0.24	0.06
Control	9.93	0.14	55.49	9.75	0.21	0.04
P Value	>0.05 (NS)		>0.05 (NS)		<0.05 (S)	



Graph 1: Intra group comparisons in study group on the basis of duration of habit.



Graph 2: Intra group comparisons in study group on the basis of quantity of cigarette smoking/day.

Comparisons of mean nuclear cytoplasmic ratio (ND/CD) of study group and control group based on different age group showed statistical significant difference in age group 4(51-60 years) and age group 5(above 60 years). (Table 1)

Comparisons of nuclear, cytoplasmic diameter and nucleus-cytoplasmic ratio between study and control group revealed statistical significant difference in mean nuclear-cytoplasmic ratio between study group and control group. (Table 2)

Intra group comparisons in study group on the basis of duration of habit revealed gradual increase in nuclear-cytoplasmic ratio as the duration of habit increases (Chart 1). Nucleus to cytoplasmic ratio was statistical significant between group 1 (1-5 years) and group 4 (above 15 years). Intra group comparisons in study group on the basis of quantity of cigarette smoking/day showed nuclear to cytoplasmic ratio was statistically non-significant between all groups based on quantity of cigarette smoking per day. (Chart 2)

DISCUSSION

Results of the present study demonstrated that, there was a significant difference in nuclear and cytoplasm diameter, nuclear to cytoplasm ratio in habitual cigarette smokers compared to the non-tobacco users. It seems that smoking results increasing cytomorphometric changes, the results of our study is similar to the results of other studies by Sharma VL, Hande A. [1, 3,]

In the present study, nuclear-cytoplasmic ratio was analyzed between study and control group on the basis of different age groups to eliminate age related changes in exfoliating mucosal

cells. Participants were divided in five different groups according to their age. It was observed that there was significant difference in nuclear-cytoplasmic ratio in age group 4 (51-60 years) and age group 5 (above 60 years). (Table 1)

It was observed that most of the participants in study group had started habit of smoking in their teen age. Participants above age of 50 years may be exposed to chronic smoke for longer duration compared to young participants.

Overall comparisons between study group and control group revealed statistical significant different in nuclear cytoplasmic ratio. (Table 2)

It is believed that chronic exposure to smoking causes alteration in mucosa specially buccal, labial and tongue mucosa, as the fact established that tobacco smoke contains more than 40 known carcinogens such as tar, nitrosamines etc. as well as heat generated by smoking plays important role in mucosal changes. Alteration in nuclear as well as cytoplasmic diameter is one of the earliest changes which take places in process of carcinogenesis.

In a study conducted by Maryam A. in 2013 on Exfoliative Cytology of Oral Mucosa among Smokers, Opium Addicts and Non-smokers found decrease in cellular diameter as well as an increase in nuclear diameter and nuclear/cytoplasmic ratio in smears taken from both smokers and opium addicts.[15]

In present study, participants were divided according to duration of smoking habit and quantity of cigarette smoking per day to correlate effect of smoking and alteration in nuclear-cytoplasmic ratio. It was observed that nuclear diameter is increasing and cytoplasmic diameter is decreasing with longer duration of habit. There was statistical significant different between participants who started habit 5 years back compared to participants who smokes since 15 years or more (Chart 1). This findings were supported by various studies.[15,16]

Present study did not demonstrate any statistical significant difference between groups based on quantity of smoking cigarettes per day (Chart 2). Most of the participants in these groups were of different age and their duration of habit was also different. Future study with standardized age group and duration of habit may analyze exact relation between quantity of cigarette smoking per day and mucosal changes.

CONCLUSION

The effect of tobacco from this study convince evidence that tobacco have definitively deleterious effect on oral mucous membrane in terms of nuclear cytoplasmic changes, which are the key features of malignancy. Such evidence could be used for early diagnosis of mucosal changes. Thus, added responsibility upon us to educating the population regarding the adverse effect of tobacco as well as to encourage quitting habit.

However, more longitudinal study with large sample size has to be undertaken to establish the findings of the present study.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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