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STUDY OF ORAL EXFOLIATIVE CYTOLOGY IN TOBACCO CHEWERS OF WESTERN INDIA

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ABSTRACT

Aim: *The aim of this project is to study the cytomorphometric features of effect of tobacco chewing on buccal mucosa and to identify its role in early diagnosis of oral cancers in tobacco chewers.* ***Methods:*** *Total 100 patients, including 20 control and 80 patients with history of tobacco chewing were included in the study. They were divided into five groups as Group N: Control; Group A: subjects with tobacco chewing habit but without any lesion; Group B: subjects with tobacco-lime lesion; Group C: leukoplakia; Group D: oral squamous cell carcinoma. Oral cytology smears were obtained using cytobrush technique. Smears were stained with Papanicolaou stain. The cellular and nuclear diameter were measured using ocular micrometer. Statistical analysis was carried out using Analysis of Variance (one way ANOVA)* ***Results:*** *The cellular diameter was progressively reduced from normal, through history of tobacco chewing but without lesion, tobacco-lime lesion and leukoplakia to oral squamous cell carcinoma. We found decrease in the mean cellular diameter, increase in the nuclear diameter and ratio of nuclear diameter to cellular diameter. On statistical analysis P value of cellular diameter was 0.003 and for nuclear diameter was 0.000, which was highly significant.* ***Conclusion:*** *Cytomorphometry is a simple, noninvasive diagnostic technique to assess the influence of tobacco on buccal mucosa. This helps in early detection of premalignant changes and improves the prognosis by early intervention.*

KEY WORDS

Oral cytology, tobacco chewers, Papanicolaou stain, Ocular micrometer

INTRODUCTION

Oral cancer affects as many as 274000 people worldwide annually.[1] It accounts for 5% of all malignant tumors and 60% of these lesions are well advanced at the time of diagnosis. The early diagnosis and subsequent treatment of oral cancer can prevent large number of these diseases.[2]

There is geographic variation in the incidence of oral cancer among different regions within a country. This indicates that environment factors may play an important role in the pathogenesis of oral cancer. Tobacco chewing, smoking and alcohol intake have been attributed to as major risk factors.[3]

Different forms of tobacco usage are prevalent in India and many of them are specific to certain areas. The habit of placing tobacco mixed with lime, usually in the canine-premolar region of the mandibular sulcus is widespread in the rural population of Central Maharashtra, India.

Thirty percent to 80% of the malignancies of the oral cavity arise from premalignant lesions, such as

leukoplakia, erythroplakia, and oral submucous fibrosis.[4,5,6]

Exfoliative cytology is a simple, noninvasive diagnostic technique, increasing the chances of early detection of premalignant and malignant lesions.[7] The use of cytobrush has a wide clinical application in assessment of surface oral mucosa. In this technique, the quantitative parameters are objective and reproducible that can be used for cytopathological diagnosis. One such quantitative parameter is morphometry which measures nuclear size, cell diameter, nuclear-to-cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density, and nuclear texture. Cytomorphometric analysis of buccal mucosa can help in identifying early dysplastic changes which helps to diagnose the malignancy. This is an easy, cheap and rapid method for early detection of premalignant and malignant lesions in tobacco chewers.[8]

Taking this into consideration, the present study has been carried out to assess the effect of tobacco chewing on buccal mucosa and compare the cytomorphology of cells collected from buccal mucosa of tobacco non-chewers.

MATERIAL AND METHODS

The study group consisted of 100 patients divided into 5 groups:

- Group N: subjects without tobacco chewing habit and without any lesion;
- Group A: subjects with tobacco chewing habit but without any lesion;
- Group B: subjects with tobacco chewing habit and tobacco-lime lesion;
- Group C: subjects with tobacco chewing habit and leukoplakia;
- Group D: subjects with tobacco chewing habit and oral squamous cell carcinoma.

People with history of smoking cigarettes, cigars, pipes and snuff were excluded from the study.

The detailed information about the habit of tobacco chewing with lime (duration and frequency of the tobacco chewing habit) was recorded for each individual.

Twenty individuals without tobacco chewing habits (group N) served as controls.

Tobacco-lime lesion is a yellowish white to brown lesion, which unlike leukoplakia could be scraped off.[9] Patients with such lesions were included in group B. Leukoplakia is defined by *WHO Classification* as predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion.[10]

Informed consent was obtained from all patients to obtain a cytological smear.

Scrapings were obtained by using a cytobrush moistened with normal saline. Using a gentle scraping motion, exerting little pressure, cells were scraped from the clinically normal appearing buccal mucosa of the study group N and A. In the B group, the entire lesion was scraped if possible. If not, a representative area was scraped. In the C group, in cases where a heavy keratinized surface was present, fissured or reddish areas were scraped to obtain the sample. In the D group, smears were obtained from the ulcerated or erythematous areas. The scrapings were smeared on to the center of glass slide, over an area of approximately 2.5×2.5 cm. The slides were immediately sprayed with commercially available spray fixative to ensure proper fixation. All cytological smears were stained by Papanicolaou staining technique using a commercially available staining kit.

The cellular diameter and the nuclear diameter of the cells were measured using calibrated eye piece (ocular) micrometer. On high power one marking was calibrated as 0.025µm. Measurement was obtained in the longest axes of the cells and the nuclei by superimposition of the eyepiece micrometer on the smear. One hundred cells were evaluated by morphometry and the average of the values from long axes was taken as the diameter of that cell and nucleus and N:C ratio was calculated and statistical analysis was done.

RESULT

Total 100 cases were studied. All the subjects in the study groups except group N had history of tobacco chewing ranging from 2 years to 20 years or more. Their frequency of tobacco chewing was minimum of 3-5 times/day. Age and sex wise distribution of the total number of cases in each group is as shown in **Table 1.**

The maximum numbers of cases were of leukoplakia in the age group of 30-49 years. On Ocular micrometry, 100 cells were measured for cellular diameter and nuclear diameter in each case and their mean was calculated. The cellular diameter was progressively reduced from normal (group N), through history of tobacco chewing but without lesion (group A), tobacco-lime lesion (group B) and leukoplakia (group C) to squamous cell carcinoma (group D) as shown in **Table: 2**. The cytomorphology of group N showed benign squamous cells against a clean background. (**Fig: 1A**). Group A showed normal cells with occasional neutrophils in the background. (**Fig 1B**)

Group B showed mild nucleomegaly (**Fig 1C**). Group C showed increased cellularity with few cells showing high N:C ratio with few nuclei showing irregular nuclear chromatin. (**Fig 1D, 2A**) The background showed more neutrophilic infiltrate. Smears of Group D showed clusters of squamous cells with large hyperchromatic nuclei and moderate eosinophilic cytoplasm with high N: C ratio suggestive of dysplastic changes. The background showed dense neutophilic infiltrate and haemorrhages at places. (**Fig 2B**)

Analysis of variance (one way ANOVA) was performed for the 5 groups to compare the mean of cellular diameter, nuclear diameter and ratio of nuclear diameter to cellular diameter. Standard deviation was calculated for all groups. P value of cellular diameter is p=0.003 and for nuclear diameter is p=0.000, which is highly significant.

Table: 1 Age and sex wise distribution of the total number of cases in each group.

GROUP	TOTAL	SEX		AGE			
		Male	Female	<30 years	30-49 years	50-70 years	>70 years
N	20	19	1	16	1	1	2
A	20	16	4	3	15	2	0
B	22	16	6	2	7	11	2
C	28	19	9	4	15	4	5
D	10	10	0	1	6	2	1

Table: 2 Mean cellular diameter, nuclear diameter and ratio of nuclear diameter to cellular diameter in all study groups.

GROUP	TOTAL NO. OF CASES	CELLULAR DIAMETER [µm]	NUCLEAR DIAMETER [µm]	RATIO OF NUCLEAR DIAMETER TO CELLULAR DIAMETER
N	20	58.62	7.25	0.12
A	20	57.5	9.05	0.15
B	22	55.87	10.17	0.18
C	28	54.12	12.4	0.22
D	10	52.25	15.3	0.29

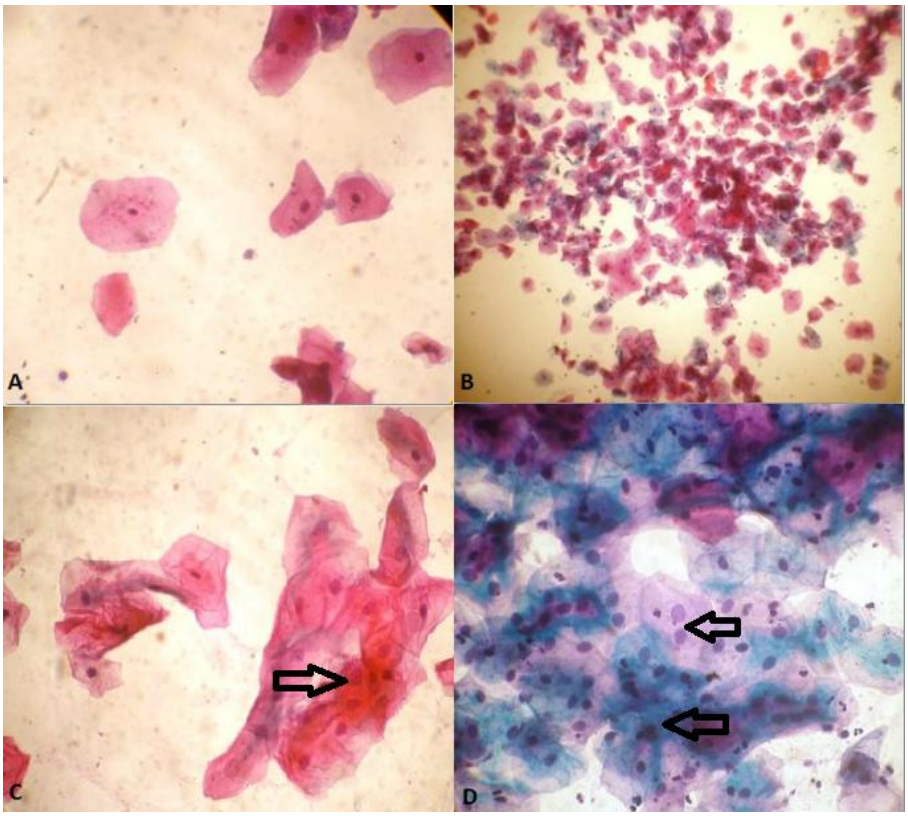

Fig: 1. Photomicrograph of oral cytology smears of
A. Group N showing normal cytology (Pap, X 400)
B. Group A showing normal cells with occasional neutrophils in the background. (Pap, X 100)
C: Group B showing mild nucleomegaly (arrow) (Pap, X 400)
D. Group C showing high N:C ratio(arrow) (Pap, X 400)

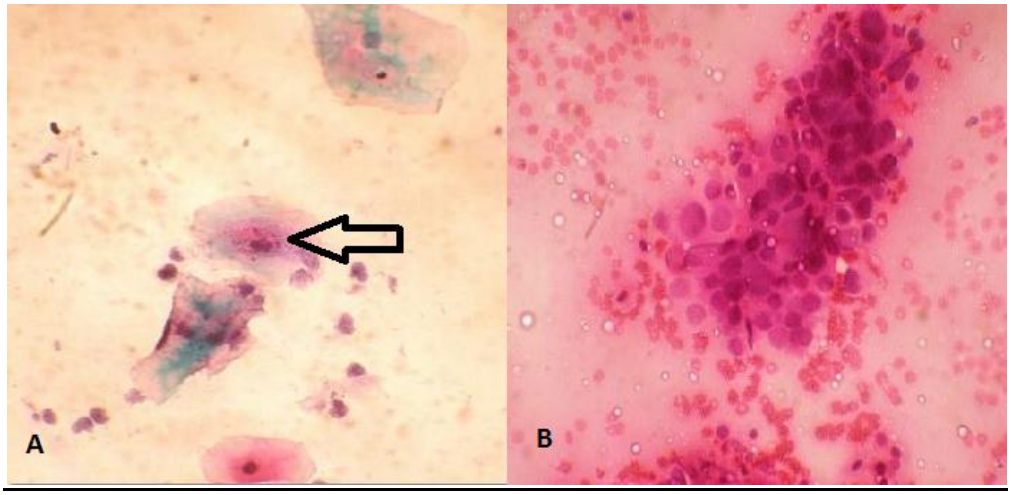

Fig 2: Photomicrograph of oral cytology smears of
A. Group C showing irregular nuclear chromatin (Arrow) (Pap, X 400)
B. Group D showing marked dysplasia of the squamous cells (Pap, X 400)

DISCUSSION

All the major forms of tobacco are known to cause oral cancer. This is evident by the magnitude of the risks associated with greater amounts or longer duration of tobacco usage. We found that the cytomorphological features showed increasing N:C ratio with increase in duration and frequency of tobacco usage. Users of smokeless tobacco exhibit oral cancer preferentially in areas where the quid is held, that is the buccal or alveolar surfaces. The habit of placing tobacco mixed with lime (Khaini) in the mandibular sulcus, usually in the canine premolar region is widespread in the rural population of Central Maharashtra, India [9]. This is characterized on gross appearance by a thick, yellowish white intra oral lesion known as tobacco lime lesion which is 4 times more common than leukoplakia.

During transformation of normal tissue to premalignancy or malignancy, cellular changes occur at the molecular level before they are seen under the microscope and much before clinical changes become evident.

Identification of high-risk oral premalignant lesions and intervention at premalignant stages could constitute one of the keys in reducing the mortality, morbidity and cost of treatment associated with oral squamous cell carcinoma.

Tobacco induced mucosal changes have been identified in exfoliative cells. The nuclear size, cytoplasmic size and their ratio have been shown to be significant in evaluation of oral lesions.[2]

Einstein TB and Siva pathasundharam B reported cytomorphologic alterations in the form of reduction in cellular diameter and increase in nuclear diameter in buccal squames of tobacco users in the south Indian population.[10]

Our study showed significant quantitative alterations in the form of decreased cellular diameter, increased nuclear diameter and increase in ratio of nuclear diameter to cellular diameter in the A, B, C, and D groups, compared to N group. This significant progressive reduction in cellular diameter shows that the reduction in cell size could be an early indication of malignant change, as suggested by Cowpe JG *et al.*[2]

Increase in the nuclear diameter could be due to increased DNA content of the nucleus. Increase in ratio of nuclear diameter to cellular diameter is due to the changes in nuclear size and cytoplasm, same as reported by Cowpe JG *et al.*[2] Franklin CD and Smith CJ (1980) 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.[11]

These observations suggest that tobacco chewing is responsible for the significant cellular and nuclear alteration in the A, B, C, and D groups. In group A, that is tobacco users but without any lesion, though the oral mucosa appears clinically normal, the mean difference of cellular diameter shows statistically significant difference as compared to group N, B, C, and D. This indicates that the alterations are probably due to changes at the molecular level, which is not apparently appreciable at the clinical level.

This study, therefore, confirms only the cause–effect relationship between tobacco chewing and quantitative cellular and nuclear alterations. In study groups N, A, B, and C the mean values of cellular diameter and nuclear diameter differed significantly from the values obtained for the oral squamous cell carcinoma group (D).

CONCLUSION

Our study concludes the importance of early recognition of cellular alterations for identification of pre malignant changes in the patients with tobacco chewing.

We emphasize that cytomorphology is an invaluable parameter to assess the influence of tobacco on buccal mucosa. Our study restricts itself to performing linear measurements on exfoliative cytology and hence, we propose that further studies using automated image analysis need to be carried out.

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