Original Article

A Study of Buccal Mucosa of Smokers to Detect Precancerous Lesions

Daksha Dixit*, T.C. Singel**, Shyambabu Rauniyar***, S.M. Bhimalli****

Abstract

Introduction: Oral cancer is one of the 10 most common cancers in the world. Oral habits like smoking, chewing tobacco, gutkha, etc. are documented as initiators of dysplastic changes in the oral mucosa. **Aims:** To study the buccal mucosa of smokers by exfoliative cytology and to assess the cytological and nuclear changes therein. **Objectives:** To study the buccal mucosa of smokers for early detection of precancerous lesions of oral cancer thus helping in better treatment and prognosis. **Materials & Methods:** The study included examination of buccal mucosa followed by scrapping and making smears thereof. The study sample consisted of 100 smokers and 100 controls. The buccal smears thus prepared were stained by Papanicolaous method. The nuclear changes like micronucleation, binucleation, karyorrhexis, karyolysis, pyknosis and condensed chromatin were observed using binocular microscope. **Results:** A significant increase in micronucleation and binucleation of cells was observed in smokers. **Conclusion:** We conclude that tobacco smoking produces cellular alterations in the buccal mucosa. These precancerous lesions can be picked up using exfoliative cytology as early as 10-15 years prior to their malignant transformation. Exfoliative cytology is a non-invasive method which can be used for mass screening of the population for early detection of precancerous lesions of the buccal mucosa.

Keywords: Buccal mucosa; Exfoliative cytology; Smoking; Tobacco; Micronuclei.

Introduction

Cancer is the second most leading cause of mortality in economically developed countries (following heart diseases) and the third most leading cause of death in developing countries (following heart diseases and diarrhoeal diseases).[1] Oral cancer is 1 of the 10 most common cancers in the world. For the year 2008, with estimated incidences of 9.8 cases per 1 lakh population for males and 5.2 cases per 1 lakh population for females, oral cancer is now a major problem in India.[2]

Oral habits like smoking, chewing tobacco, gutkha, etc. are documented as initiators of dysplastic changes in the oral mucosa. Despite numerous advances in treatment taking advantage of most recent protocols for surgery, radiation therapy and chemotherapy, the overall long term survival has remained at less than 50% for the past 50 years.

Tobacco is the most common drug of abuse and is consumed as one of chief source of pleasure by all socio-economic strata in developing countries like India. Tobacco in its many forms (smoking and smokeless tobacco) and alcohol consumption are risk factors for oral cancers and oral mucosal lesions also giving rise to typical cellular changes in oral mucosa.[3,4]

An increased frequency of micronuclei is

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found in smokers and/or tobacco chewers with oral carcinomas.[5,6,7] Hence, micronucleus test, currently known as micronucleus assay has been used for screening populations under risk of mutagenic agents that cause oral neoplasias, especially for the detection of pre-clinical stages of carcinogenic process.

Exfoliative cytology helps in early diagnosis even before the clinical changes occur and is the best method for identifying the precancerous lesions thus remarkably reducing the mortality and morbidity associated with oral squamous cell carcinoma.

Need for the study

Oral lesions constitute major public problem in South Asian countries including India. People in these areas are habitual of taking spicy food, *pan, sopari* (areca nut), smoking and *naswar* (snuff). Prevention and early detection of such potentially malignant disorders have the potential of not only decreasing the incidence, but also in improving the survival of those who develop oral cancer. In view of this, the present study was undertaken to assess the levels of micronuclei in the oral exfoliative cytology of healthy control subjects and the subjects who were in the habit of consuming tobacco in the form of smoking.

Aims & Objectives

To study the buccal mucosa of smokers by exfoliative cytology, and to assess the cytological and nuclear changes therein, for early detection of precancerous lesions of oral cancer, and thus helping in better treatment and prognosis.

Materials & Methods

The present study was carried out, after obtaining clearance from the Institutional Ethical Committee of J. N. Medical College, on 200 individuals (100 smokers and 100 controls). These study subjects were selected from the patients attending at the Out Patient Department at the KLES Dr. Prabhakar Kore Charitable Hospital, Belagavi.

Male subjects aged between 18 and 80 years with a minimum of 3 years of tobacco smoking were included as cases in the study and male subjects having no exposure to tobacco in any form were included as controls.

The socio-demographic history was taken. The subjects having normal appearing buccal mucosa with no dentition and jaw abnormalities were included in the study. After taking an informed consent and explaining the sample collection procedure, the subjects were asked to rinse their mouth with water and the buccal scrappings were taken and smeared on a clean glass slide and were fixed with 100% ethyl alcohol. These smears were later stained by Papanicolaous staining technique using Eosin Azure, Orange Gelb and Harris Haematoxyline without Acetic Acid.

Observations

The slides thus stained and prepared were observed under the binocular microscope. Five areas of evenly spread cells were counted for the total number of normal cells, and the nuclear changes like multinucleation, binucleation, karyorrhexis, karyolysis, pyknosis and condensed chromatin were recorded. These changes were then compared with the smears of the control group. The results were tabulated and relevant graphs were prepared, and statistically analysed.

General Sample Characteristics

Distribution of subjects according to age: The present study was conducted on males ranging between 18 and 80 years. In the study group maximum number i.e. 27 out of 100 were between the age group 31 to 40 years

Table I: Nuclear abnormalities in smokers and control groups				
Nuclear Change	**Smokers**		**Controls**	
	Present	**Absent**	**Present**	**Absent**
Multinucleation	77	23	20	80
Binucleation	93	07	21	79
Karyorrhexis	28	72	00	100
Karyolysis	37	63	01	99
Pyknosis	40	60	01	99
Condensed chromatin	09	91	00	100

and minimum number i.e. 03 out of 100 were of the age of 18 to 20 years.

Distribution of subjects according to educational status: Out of the 100 subjects, the overall distribution of subjects according to their educational status varied from 1% in people who were illiterate to 46% who had post school education.

Distribution of subjects according to occupational status: In the present study, the smoking habit varied from 10% in Unemployed group to 43% in Farmers.

Distribution of subjects according to socio-economical status: In the study group maximum number i.e. 68 out of 100 belonged to lower middle income group and minimum number i.e. 02 out of 100 were belonging to high income group.

Distribution of subjects according to Total Number of Normal Cells: The total number of normal cells in the 5 areas of evenly spread cells in smokers was 0-50 in 07 cases, 51-100 in 12, 101-150 in 23, 151-200 in 24, 201-250 in 30 and 251-300 in 4 cases. While the total number of normal cells in controls were 101-150 in 3 controls, 151-200 in 13, 201-250 in 33, 251-300 in 23, 301-350 in 9, 351-400 in 12 and 401-450 in 7 controls.

Distribution of subjects according to nuclear changes: The presence was various nuclear abnormalities in smokers and control groups is shown in Table I.

Discussion

Cancer affecting the epithelium of the oral

Figure I: Multinucleation & Karyolysis
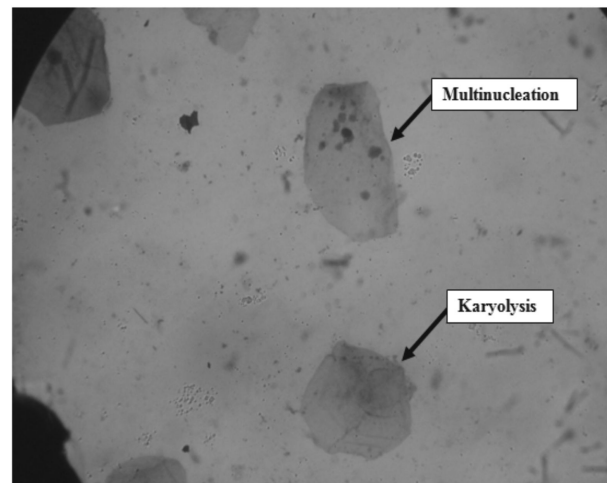
cavity is preceded by lesions that can be clinically detected, among these, leukoplakia is the most frequently occurring type. Use of biomarkers to indicate the potential of precursor lesions to evolve to the process of malignant transformation is a preventive measure that guides therapeutic management. Micronuclei (MN) are distinctively individualized structures within the cytoplasm of interphasic cells measuring between 1/5 and 1/3 of the size of the main nucleus. MN test is especially used for the identification of preclinical stages of the cancer.

Characteristics of Cellularity

Multinucleation (Figure I)

In the present study, multinucleation was seen in 77% of smokers and 20% of control subjects. On application of statistical test, a highly significant association was observed

Figure II: Binucleation & Karyorrhexis　　　**Figure III: Pyknotic cells**
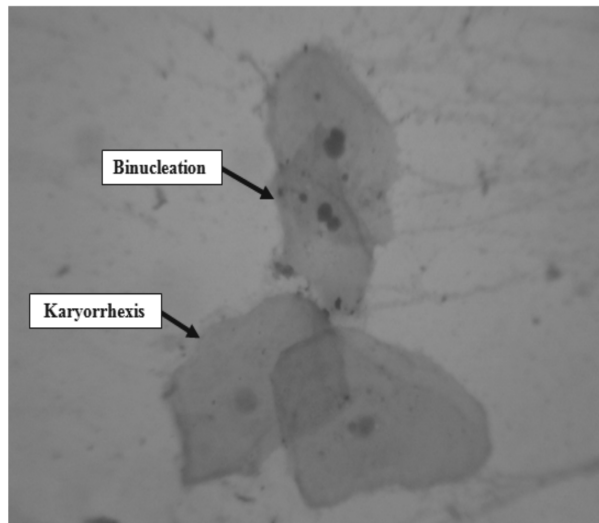
between the smoking habit and the presence of multinucleation (p<0.001). Bohrer PL et al in 2005 observed 31 controls, 49 tobacco users, and 27 tobacco and alcohol users in Brazil. Their study revealed a significant association of multinucleation with the use of tobacco and alcohol.[8] Gabriel SB *et al* in 2002 also showed a significant effect .[9]

Binucleation (Figure II)

Our study showed binucleation in 93% of smokers and in 21% of control subjects.This showed a statistically higher association between smoking and binucleation (p<0.001). Rao DN *et al* studied 713 patients at Tata Memorial Hospital from 1980 to 1984 to assess the association between chewing, smoking and alcohol habits, and frequency of binucleation, and reported in 1994 a significant correlation between the two.[10] Binucleation was significantly increased in smokers in the studies done by Tolbert PE *et al* in 1991 and 1992. [7,11]

Karyorrhexis (Figure II)

Our study showed the incidence of karyorrhexis in 28% of smokers, whereas there was no karyorrhexis observed in the control subjects. This showed a highly significant

(p<0.001) association between smoking habit and occurrence of karyorrhexis. Similar results were also obtained by the studies done by Tolbert PE *et al* in 1991 and 1992 (North Carolina) that showed incidence of karyorrhexis to be 4.5 times more common in tobacco and alcohol users than in normal cases.[7,11]

Karyolysis (Figure I)

In the present study, occurrence of karyolysis in smokers was 37% and in controls it was only 1%. It was found to have statistically highly significant (p<0.001) association between occurrence of karyolysis and smoking. The presence of karyolysis in smears from oral cavities has been well documented by Garewal HS et al in 1993.[12] Tolbert PE *et al* in 1991 reported that the incidence of karyolysis was 13 times more common in tobacco users than normal cases. [7]

Pyknosis (Figure III)

Our study showed frequency of pyknosis in 40% of smokers and in only 1% of controls. Statistical test applied on it suggested a highly significant (p<0.001) correlation between incidence of pyknosis and smoking. Chatterjee

Figure IV: Condensed chromatin

as 10-15 years prior to their malignant transformation. Exfoliative cytology is a non-invasive method which can be used for mass screening of the population for early detection of precancerous lesions of the buccal mucosa.

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S *et al* in 2009 established significant association between tobacco chewing and presence of pyknosis.[13]

Condensed chromatin (Figure IV)

Our study showed presence of condensed chromatin in only 9% of smokers. This did not occur enough to be considered in the statistical analysis.

Condensed chromatin

Resutls

A significant increase in micronucleation, binucleation, karorrhexis, karyolysis and pyknosis of cells was observed in smokers. All these data provide evidence for an increase in frequency of nuclear aberrations in the buccal smear of smokers, and suggest that oral mucosa is susceptible to cancer from tobacco smoking.

Conclusion

We conclude that tobacco smoking produces cellular alterations in the buccal mucosa. These precancerous lesions can be picked up using exfoliative cytology as early

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