Comparison of Exfoliative Cytology of Tongue and Buccal Mucosa among Smokers and Non-smokers using PAP Stain and AgNOR Counts

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ABSTRACT

Introduction: Oral cancer can be detected at earliest stages by Oral cytology using PAP stain and AgNOR counts. Alterations in nuclei of oral superficial epithelial cells can serve as reliable indicators of dysplastic or neoplastic changes. A study was conducted to Compare the Exfoliative cytology of tongue among smokers and non-smokers using PAP stain and AgNOR counts.

Material and Methods: Comparative study of evaluation of cellular alterations in the smoker's and non smokers oral mucosal cells was performed. Exfoliative Citology technique were used and the cytologic smears stained with AgNORs and PAP stain. Cytologic smears were collected from two anatomic sites, buccal mucosa and lateral tongue border with the purpose of relating smoking with the quantitative analyses of the AgNORs.

Result: It was found that the average number of AgNORs/ nucleus is related with smoking. These results suggest a possible relation between smoking and an increase rate of cellular proliferation in the oral mucosal cells.

Conclusion: It is concluded that the proliferative activity is enhanced in smokers compared to non smokers and hence suggestive of adverse effects of smoking on oral and general health.

Keywords: Argyrophilic Nucleolar Organizer Regions, Oral Mucosa, Prevention Oral Cytology, Smoking, Carcinoma, Papanicolaou Test.

INTRODUCTION

Due to the increasing prevalence of cancer, it has become a Public Health problem world wide, affecting at least 9 million people and kills about 5 million every year.

Oral cancer is a global health problem with rising mortality rates. Together oral and pharyngeal cancers account for the sixth most common cancer in the world. It has been documented that the new cases are arising at the rate of 19 per 100,000 in Indian population.[1] Of all cancers oral cancer represent up to 40%, leading to enormous burden of disease.[2,3]

Patients being unaware of early signs and symptoms of oral cancer, they become victim to its advanced/worse stages hence opt for the treatment at much later stages leading to more risk as well as cost burden among them. Hence the detection of the disease at its primary level would decrease the patient morbidity and mortality.

In Epidemiological and analytical studies, tobacco has been associated with the cause of malignant and precancerous lesion. Early diagnosis of oral cancer is of utmost importance and has been made possible through oral cytology using different techniques.[4,5]

Smoking is currently the most preventable cause of diseases and death worldwide and is one of the main risk factors for the development of cancer in different organs. According to Winn, smoking is the leading cause of oral cancer in 91% of men and in 59% of women.[6] Therefore, smoking patients should be carefully monitored in view of the series of alterations that tobacco can cause.

The superficial oral epithelial cells do contain nuclei, and thus, changes in these cells can serve as trustworthy and predominant marker of dysplastic or cancerous alterations. Thereby, an attempt was made to Compare the Exfoliative cytology of tongue among smokers and non-smokers using PAP stain and AgNOR counts.

Papanicolaou staining is used as a routine method for the analysis of cytological aspects and permits the identification of basic inflammatory, dysplastic or malignant alterations.[7] Histochemical AgNOR quantification consists of the nucleolar organizer regions (NORs) staining with silver (Ag) salts. NORs are proteins that are associated with the fibrillar centers and dense fibrils of the cell nucleus during interphase and are responsible for the replication of RNA. Thus, the larger the number of NORs, the higher the replication rate of ribosomes and cells. This technique has therefore been used for the quantification of cell proliferation in different tissues and lesions.[8,9] So study was conducted to Compare the Exfoliative cytology of tongue among smokers and non-smokers using PAP stain and AgNOR counts.

MATERIAL AND METHODS

A sample size selected for the study was 50 male patients which comprised of two groups; smokers (25) and non-smokers (25). A non-probability convenience sampling technique was used. The subjects ranged in the age group between 35 to 55 years of age. The participants were selected from the outpatient inflow to the Dept. of dentistry, Govt. Gousia Hospital, Srinagar. Prior to the conduct of study, permission was obtained from the Office of Medical Superintendent Govt. Gousia Hospital, Srinagar. All those patients who were willing to participate in the study and gave the informed consent were included and who were free from

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any oral lesions (benign or malignant). The subjects were divided in two groups as smokers and non-smokers. Subjects who smoked 25 cigarettes or more per day since 20 years were considered as Smokers and those who never smoked were grouped as non-smokers. The cytologic study samples were obtained from the oral mucosa and lateral borders of tongue. In order to remove debris from oral cavity, patient was instructed to wash his mouth with water. Gross debris on was removed with moist cotton swabs. A wet wooden spatula was used to collect Squamous epithelial cells from buccal mucosa and lateral borders of the tongue and a uniform thin smear in circular motion was made on a dry and clean conventional glass slide. All smears were instantly fixed with a fixative and were send for rapid Papanicolaou and AgNOR staining (Singh Path Lab, Delhi), which consumed less time and gave comparable staining characteristics as that of conventional technique. AgNORs were stained according to the method recommended by the International Committee on AgNOR Quantitation.

NORs were directly counted under a light microscope according to the parameters established by Crocker et al., i.e., well-defined black dots in the nucleus were counted, with aggregations (overlapping or fused black dots) being considered a single structure.

Statistical analysis: The data was subjected to statistical analysis using chi-square test and mean standard deviation, with the level of significance set at 5%.

RESULTS

Comparison of the mean number of AgNORs showed a significant difference between nonsmokers (2.112 ± 0.245 AgNORs/nucleus) and smokers (3.463 ± 0.247 AgNORs/nucleus) (t = 7.11, d.f. = 49, P = 0.0001).

To evaluate the proliferative activity, the percentage of cells with more than three AgNORs per nucleus was evaluated in the 25 smokers and 25 nonsmokers. The mean percentage of cells with more than three AgNORs per nucleus was higher in smokers (44.5 ± 12.2%) than in nonsmokers (17.4 ± 7.0%), with significant difference (t = 8.60, d.f. = 49, P = 0.0001. (Figure 1)

The influence of the duration of smoking on proliferative activity was evaluated by dividing smoking patients into four groups: group I: 21–30 years; group II: 31– 40 years; group III: 41–50 years; group IV: 51 years or more. The mean number of AgNORs per nucleus and the respective standard deviation were determined for each group and the results are depicted in Table 1. No significant difference in mean AgNOR number per nucleus was observed between groups II and III. No comparisons of groups II and III with groups I and IV were performed because of the small sample size of the latter groups (n = 2).

In the smoking group, 22 (88%) slides were classified as Papanicolaou class II, whereas the three remaining slides were class I. In the nonsmoking group, 5 (20%) slides were classified as class II and 20 (80%) as class I. The number of AgNORs was also determined in smokers and nonsmokers divided according to cytologic evaluation and the results are shown in Table 2. Figure-2.

Groups	Duration of smoking (years)	Patients (n = 25)	AgNORs/ nucleus
I	21-30	3(12%)	3.01±0.567
II	31-40	16(64%)	3.52±0.265
III	41-50	4(16%)	3.56±0.422
IV	≥ 51	2(8%)	3.03±0.208

Table-1: Mean of AgNORs per nucleus among smokers classified according to the duration of smoking (in years).

Patients	Cytological aspects	
	Class I	Class II
Smokers	3.101 ±0.215	3.376 ±0.319
Non smokers	2.540 ±0.311	2.711 ±0.301

Table-2: Number of AgNORs per nucleus in smears from smokers and non-smokers classified according to the cytologic evaluation.

Figure-1: AgNOR quantification. (a) Smear obtained from a nonsmoking patient (b) Smear obtained from a smoking patient.

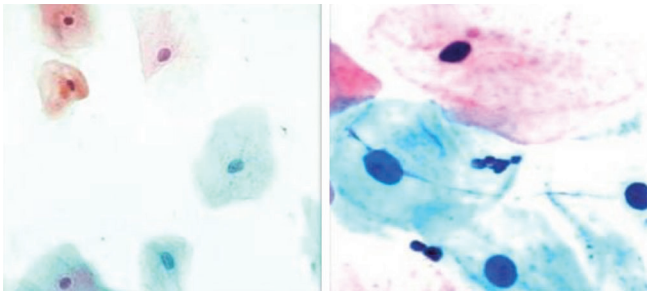

Figure-2: PAP staining. Ist Slide classified as class I and 2nd Slide classified as class II.

DISCUSSION

There is a need for studies that direct towards oral cancer prevention and control measures and to the development of strategies that increase the population participation.[10] Monitoring the appearance of lesions in specific groups that may be classified as risk groups is an effective method of preventing cancer.[11] Dysplasia and early carcinomas are asymptomatic and commonly misinterpreted as benign lesions or innocuous oral problems. The inconspicuous nature of these lesions or misleading perception of practitioners may primarily be responsible for the advanced stages of these tumors at the time of discovery.[12] So, a reasonable approach is required for the prompt detection at early stages of the fatal disease so as to prevent and control it well in time.

In the present study, tongue and buccal mucosa was used

for collection of the sample as in previous studies the buccal mucosa and tongue were the most frequently involved sites while the palate was the least commonly involved site.[13] In united states posterior lateral border and ventral surface of the tongue were mostly effected, while in India anterior two third of the tongue was the commonly involved sites.[14]

In the present study, the samples taken from lateral border of tongue were analysed by both AgNOR and Papanicolaou staining. The histochemical AgNOR quantification showed a significantly higher proliferative activity in smokers compared to nonsmokers.

Different markers of cell proliferation have been used as an supporting tool in the diagnosis of oral cancer in previous studies as these cell proliferation markers are of great significance for the understanding of cellular alterations. However, studies which analyze the cell proliferation markers by exfoliative cytology are limited.[15-17]

AgNORs were observed as well distinct black dots inside the nucleoli in the present study.[16,17] Higher proliferative activity was found in smoking patients by AgNOR quantification. These results showed the significance of exfoliative cytology; however the technical accuracy of staining of the smears by the AgNOR method should be improved to allow its regular application.

As far as the mean number of AgNORs per nucleus is concerned, a significance difference was found when compared between smokers and nonsmokers. These findings were in accordance to the results found by Sethi and Shah and Orellana-Bustos et al.[18,19]

On comparing the mean number of AgNORs per nucleus as per the duration of smoking, no significant difference was found. These results were similar to the previous study done by Cancado et al.[8]

In the present study a significantly higher mean percentage of cells with more than three AgNORs per nucleus was seen in smokers than nonsmokers showing the increased proliferative activity among smokers.[9]

The results of the present study suggest that cigarette smoking produces alterations in the mechanisms of cell growth control. In previous studies,tobacco has been considered to be an initiating factor in the process of oral carcinogenesis, which is frequently associated with alcohol as a promoting factor.[21] In response to the smoking the oral mucosal cells show higher proliferation hence being susceptible to the hazards of tobacco use. The results of the present study show that there are alterations produced in the process of cell growth control among cigarette smokers. In the process of carcinogenesis, the use of tobacco has been regarded as an initiative factor which is aggravated by alcohol consumption.

CONCLUSION

It is concluded that the proliferative activity is enhanced in smokers compared to non smokers and hence suggestive of adverse effects of smoking on oral and general health. Thus PAP and AgNORs quantification is of utmost significance in cancer screening at much earlier stage and hence useful in prevention and treatment of oral cancer.

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