

Research Article

Evaluation of AgNOR count in cytological smears of chronic bidi and hookah smokers

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Abstract: Oral Squamous cell carcinoma (OSCC) accounts for over 90% of oral cancer cases and a small percentage of these cases are thought to develop from potentially malignant disorders. Main etiological factor associated with OSCC is smoking. It's a well known fact that in chronic smokers nuclear alterations are evident. There is a correlation between nucleolar function, size and cell doubling time in human cancer cell lines, which has shown the importance of nucleus in tumor pathology. The nucleolar organizer regions (NORs) are ribosomic DNA loops which can be clearly visualized at the light microscopic level by using a silver reaction which stains the acidic, non histonic proteins present in the nucleus. AgNOR is useful in estimating nuclear proliferative activity and it acts as a marker of pre-malignant and malignant change. The aim of this study is to compare the proliferative activity by AgNOR count in cytosmears of chronic bidi and hookah smokers in a section of Haryana population.

Keywords: AgNOR, Bidi Smokers, Cytology, Hookah Smokers.

INTRODUCTION

Squamous cell carcinoma (SCC) is the most frequent malignancy of the oral cavity, accounting for more than 90% of all oral malignancies[1]. This could be even more common in India because of common use of tobacco in different forms[2]. Presently in India, it has been reported that there are about 200 million people who use tobacco, out of which about 70% smoke bidi, 10% smoke cigarettes & 20% are tobacco chewers. Social habits of "Shisha or hookah use" and "Goza" smoking also have an adverse effect on general health and may predispose to oral cancer[3]. Many cases of oral cancer are diagnosed at an advanced stage, that results in an unfavourable prognosis and high mortality[4]. In the last few years, AgNOR analysis is being used to determine the prognosis of many malignant lesions[5].

The Nucleolar Organizer Regions (NORs) are loops of ribosomal DNA which occur in the nucleoli of the cells on the short arms of the acrocentric chromosomes, 13, 14, 15, 21 and 22[6]. NORs are associated with acidic, argyrophilic, nonhistonic proteins that are visualized as black dots with the use of a silver-staining AgNOR technique[7]. AgNOR is useful in estimating nuclear proliferative activity of the cell and it acts as a marker of pre-malignant and malignant change[8]. Many histological studies have

shown the importance of AgNOR numbers in normal, reactive, dysplastic cells and various benign and malignant tumours, but there are fewer studies with cytological preparations[9-11]. The aim of present study is to compare the proliferative activity of cell by AgNOR count in cyto-smears of buccal mucosa of chronic bidi and hookah smokers in a section of Haryana population.

MATERIALS AND METHODS

The study was undertaken in the department of Oral Pathology and Microbiology, Manav Rachna Dental College, Faridabad, Haryana, India.

In this study 120 patients were chosen from a section of Haryana population. These patients were divided in the following groups:-

- **Non Smokers** - 20 cases (Control group)
- **Bidi Smokers** - 50 cases
- **Hookah Smokers** - 50 cases

The Bidi smokers group comprised those who had been smoking a minimum of 10 bidis or more per day for a minimum of atleast 10 years. The Hookah smokers group comprised those who had been using hookah atleast 4 Hrs or more per day for a minimum period of 10 years.

A detailed case history was taken for each patient and patients with any systemic disease or clinically apparent oral mucosal lesions, patients with previous history of any benign or malignant oral lesions were excluded from the study. After obtaining detailed records and taking informed consent, the exfoliative cytology was performed for each subject. Smears were prepared with the help of wooden spatula scraped firmly from the buccal mucosa cells and the cells were scattered on a dry glass slide, following which they were fixed with a 95% ethanol for 12 hours. The fixed slides were subjected to AgNOR staining according to the method of Ploton's *et al.*[12]. The AgNOR count was established in 50 cells for each cytologic smear. The cells were examined at 1000x magnification under

oil immersion lens. Statistical analysis was done using Student's t-test.

RESULTS

AgNOR dots were seen as dark brown to black dots inside a brownish nucleus within a yellow cytoplasm. The mean range of AgNOR dots per nucleus in each group is shown in (Table 1). The mean range and Standard Deviation (SD) of AgNOR dots per nucleus in hookah smokers was statistically higher than the bidi smokers and the non smokers group. Range of AgNOR dots per nucleus was 1- 3 in non smokers (fig 1) , 2- 5 in bidi smokers (fig 2) and 2-7 in hookah smokers (fig 3). The bar diagram depicting the mean of AgNOR count is shown in (Fig-4).



Fig-1: Two AgNOR dots in the squamous cell of normal buccal mucosa in non smokers (AgNOR staining, 1000X)

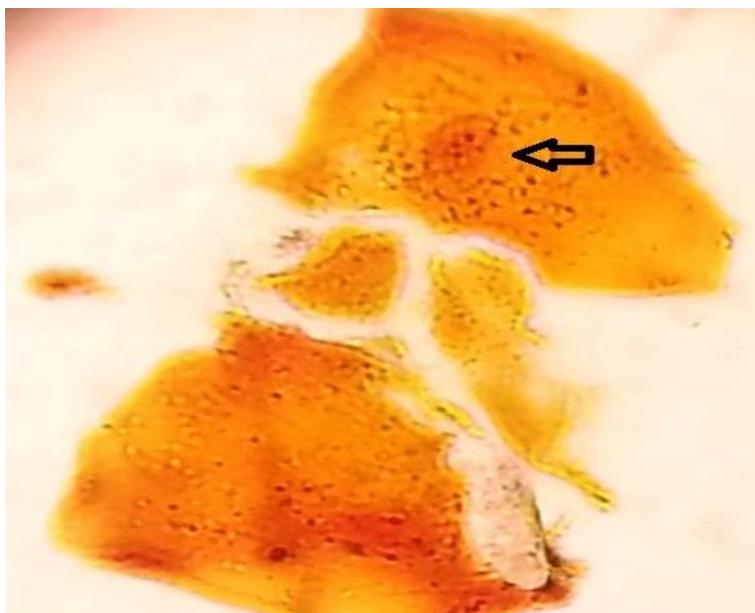


Fig-2: Five AgNOR dots in the squamous cell of normal buccal mucosa in bidi smokers (AgNOR staining, 1000X)

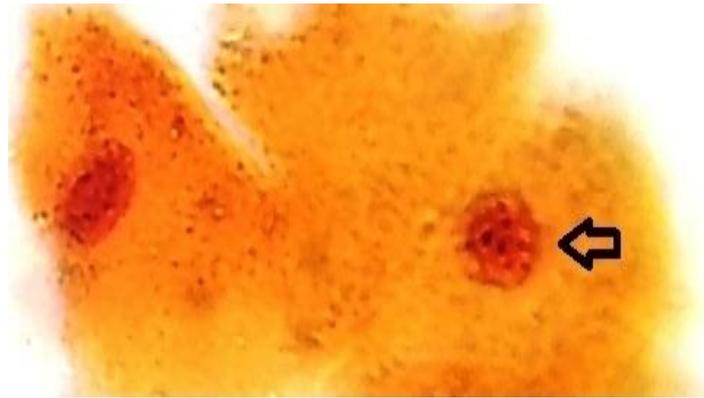


Fig-3: Seven AgNOR dots in the squamous cell of normal buccal mucosa in hookah smokers (AgNOR staining, 1000X)

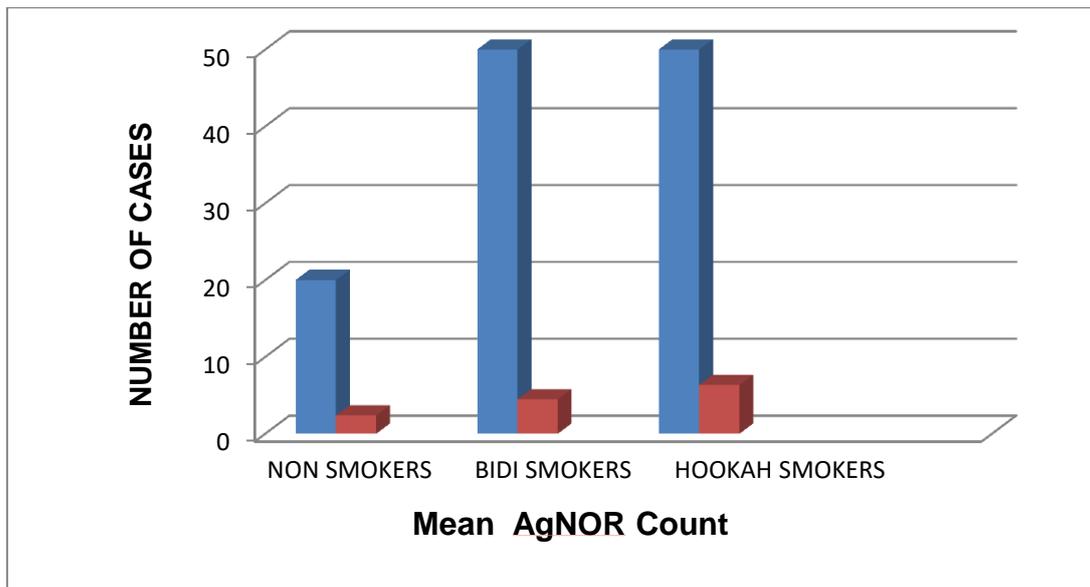


Fig-4: Bar diagram depicting mean of AgNOR counts

Table 1. Independent student’s t-test is used in statistical analysis of AgNOR dots in bidi & hookah smokers and non smokers

STUDY GROUP	NUMBER OF CASES	MEAN & SD OF AGNOR	P-VALUE
Non Smokers	20	2.36+ 0.30	0.01
Bidi Smokers	50	4.47+ 0.93	0.02
Hookah Smokers	50	6.34+ 0.46	0.04

The comparison of mean and p-value among three groups is shown in (Table 1) which demonstrates that the p-value<0.05 for bidi and hookah smokers which is statistically significant.

DISCUSSION

“Exfoliative cytology” is an easy, painless, non invasive & out-patient technique which aids in early diagnosis and mass screening of premalignant and malignant change[13]. The malignant transformation at the beginning of carcinogenesis affects only few cells long before small parts of tissues are involved. Thus, cytologic evaluation is a suitable method to elucidate the dignity of suspicious oral lesions earlier than histology, especially when used with sensitive markers like AgNORs[5].

The silver-staining method for identification of NORs has been introduced into pathology and is frequently utilized in formalin-fixed, paraffin embedded specimens[14]. Of the various newer techniques which are used for assessing the tumor tissue based on the nuclear studies, the staining of AgNORs by a silver compound has become popular for cytologic smears due to its simplicity, ease of use, low cost and good correlation with other proliferative markers[8].

The advantage of using exfoliated cells for AgNOR counting is that the whole cell can be

examined, reducing the possibility of underestimating the AgNOR counts per nucleus. The risk of obscuring some AgNORs by superimposition and coalescence is minimal[15]. The method is applicable with simple light microscopes without additional and expensive technical options and has demonstrated a correlation between the number of AgNORs per nucleus in the proliferative cell. Thus, it acts as a specific marker for proliferative activity of cell when used in cytologic smears[5].

Sampio et al used AgNOR count in exfoliative cytology of normal buccal mucosa of smokers and non smokers and concluded that cigarette smoking influences proliferative activity in the cells of normal buccal mucosa[16]. Sharma A and Saxena S used AgNOR count in exfoliative cytology and found out that percentage of AgNOR in OSCC patients, smokers and tobacco chewers was higher as compared to normal individuals[2].

In our study, AgNOR count in smears of hookah smokers is found to be higher than those of bidi smokers. It indicates increased proliferative activity in cells of hookah smokers than bidi smokers. The results of this study are in accordance with the studies done by Sowmya GV et al [17] and Salehinejad J et al [14] which showed that the mean values of AgNOR for chronic bidi and hookah smokers were significantly higher than those of non smokers. Higher AgNOR count in hookah smokers could be attributed to the higher content of tobacco and direct contact of carcinogens for longer duration. Hence, hookah smoking has more potential for malignancy as compared to bidi smoking.

CONCLUSION

AgNOR plays a major role in screening and early detection of pre-malignancies and malignancies. Ease of demonstration and high specificity to cellular proliferation makes it the best available cytopathological marker in the arsenal of the oral pathologist.

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