

# SILVER STAINING NUCLEOLAR ORGANIZER REGION (AgNOR) TYPING IN BUCCAL MUCOSAL CYTOLOGY OF SMOKERS AND NON-SMOKERS

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**Background:** Argyrophilic nucleolar organizer region (AgNOR) size and distribution are related to malignant potential in a tissue or cell. Typing of AgNOR has been attempted in cytological specimens & found useful in grading dysplasia from mild to severe in oral lesions. This study was conducted in an attempt to find any difference between smokers and non-smokers oral mucosal cells as regards AgNOR size and distribution.

**Methods:** The cytological smears of buccal mucosa in 75 smokers and 75 nonsmokers were stained for AgNOR. AgNOR typing based on size and distribution was performed in both smokers and nonsmokers using Ahsan's criteria.

**Results:** No statistically significant difference was found between smokers and nonsmokers. AgNORs were round and had clustered distribution in both group.

**Conclusion:** It is not possible on the basis of AgNOR typing to differentiate between smoker's buccal mucosal cells and that of nonsmoker's. This study also indicates that AgNOR technique is simple, inexpensive and easily reproducible. Moreover Ahsan's criteria for AgNOR typing are simplest and don't depend on the availability of expensive precision instruments.

**Keywords:** Buccal Mucosa, Cytology, Argyrophilic Nucleolar Organizer Region (AgNOR)

## Introduction

The simple one stage argyrophil technique for demonstration of NORs (nucleolar organizer regions) has proved to be useful in tumor pathology<sup>1</sup> and cytological specimens.<sup>2,3</sup>

The morphometric analysis of AgNOR, reflected cell's proliferative activity and histological differentiation of tumor rather than malignant transformation in different nasopharyngeal epithelia and nasopharyngeal carcinoma. AgNOR area, AgNOR area and count ratio are the most valuable features for differential diagnosis of normal, preneoplastic and cancer cells.<sup>4</sup>

AgNOR size and distribution are related to malignant potential in a tissue or cell. AgNOR area, AgNOR/nuclear area ratio and AgNOR area/count ratio of malignant tumors were statistically significantly higher than those of normal epithelium<sup>5</sup>. Typing of AgNOR has been attempted in buccal mucosal cytology<sup>2</sup>, in benign and malignant prostatic lesions<sup>1</sup>. In our study we used Ahsan's criteria<sup>6</sup> for AgNOR typing in order to find any difference in buccal mucosal cells of smokers and non-smokers.

## Materials and Methods

Seventy five smokers and seventy five non-smokers

were selected for the study. Cytological smears were made in all cases from buccal mucosa. These were stained with silver stain according to method used by the reaction mixture was prepared as under.

Gelatin was dissolved in 1% formic acid to make a 2% solution. This was mixed with 50% aqueous silver nitrate solution in a proportion of 1:2 to obtain final working solution. The smears were postfixed in 3:1 ethanol:acetic acid mixture. They were brought to water through graded alcohols, covered with filter papers and soaked drop wise by working solution. The smears were placed in dark for 30 minutes, in a humid chamber, washed with deionized water, dehydrated, taken to xylene and mounted.

All the smears were viewed for AgNOR size and distribution using criteria<sup>6</sup>.

## Size Variation:

- 0 = More or less uniform.
- 1+ = Two different sizes.
- 2+ = More than two different sizes (but not those of 3+).
- 3+ = All grades and sizes including too minute to be counted.

## Distribution:

- 0 = Limited to nucleoli.
- 1+ = Occasional dispersion outside

nucleoli.  
 2+ = Moderate dispersion outside nucleoli.  
 3+ = widely dispersed through the nucleolus.

The results were analyzed statistically by Chi Square test.

## Results

Comparison of AgNOR sizes in buccal mucosa of smokers and non-smokers (Table-1) showed that AgNOR sizes upto 1+ were viewed in 68 cases of smokers and 72 cases of non-smokers. Whereas 07 cases of smokers and 03 cases of non-smokers exhibited sizes upto 2+. On comparison no significant difference was found between two groups ( $p>0.05$ ).

**Table-1:** Comparison of Agnors Size Variation in Buccal Mucosa of Smokers and Non-Smokers

AgNORs Sizes	Smokers	Non Smokers	Total
0 and +1	68	72	140
2+	07	03	10
Total	75	15	150

$P>0.05$

Comparison of AgNOR distribution (Table-2) in buccal mucosa of smokers and non-smokers revealed that 74 cases of smokers and 75 cases of non-smokers had AgNOR distribution upto 1+. Only one case among smokers showed 2+ distribution and no case with 2+ distribution was found in non-smokers. On comparison between groups difference was not significant statistically ( $p>0.05$ ).

**Table-2:** Comparison of agnors distribution in Buccal mucosa of smokers and non-smokers

AgNORs Sizes	Smokers	Non-Smokers	Total
0 and +1	74	75	149
2+	01	Nil	01
Total	75	15	150

$P>0.05$

## Discussion

Subjective examinations of prominent nucleoli in various cells have aroused interest about the role of nucleoli in malignancy. The visualization of nucleoli in the haematoxylin and eosin stained sections have limitations because of effects of sectioning and relative hyperchromasia of the nucleoli<sup>7</sup>. The AgNOR technique can be easily applied to paraffin sections and cytological specimens. AgNOR typing based on size and distribution distinct between benign and malignant lesions<sup>1</sup>. Higher counts, wider scatter and smaller AgNOR size are useful in grading dysplasia from mild to severe in oral lesions<sup>8</sup>.

In our study we performed AgNOR typing using Ahsan's creteia<sup>6</sup> and found no difference between smokers and non-smokers. The AgNORs were round and had clustered distribution in both smokers and non-smokers. Our results are similar to Sampaio who observed similar findings by using same stain<sup>2</sup>. So, we concluded that silver staining of nucleolar organizer region simple, inexpensive and reproducible method for studying AgNOR size and distribution.

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