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## Research Article

### ROLE OF SILVER BINDING NUCLEOLAR ORGANISER REGIONS (AGNORS) IN COMPARISON OF PREMALIGNANT AND MALIGNANT LESIONS (ORAL SQUAMOUS CELL CARCINOMA)

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#### ABSTRACT

**Background:** squamous cell carcinoma and other premalignant lesions are leading problems in many areas especially saurashtra, Gujarat, India. Tumor cell kinetics can be assessed by many ways but AGNOR counts are more reliable and statistically significant.

**Objective:** The number of AGNOR increases gradually from the normal lining to premalignant and malignant lesions of oral cavity.

**Materials and Method:** This study has been done on biopsy specimen of suspected mass from oral cavity from (2014- 2015) in pathology department of Shri. M. P. Shah. Medical College, Jamnagar. This study includes the age, gender, histological type, grading and AGNOR counting and comparison.

**Results:** On comparing mean AGNOR  $\pm$  SD of Premalignant(35.1%)  $2.53 \pm 0.32$  with malignant (64.9%)  $5.41 \pm 1.40$  are statistically significant with p value of 0.001.

**Conclusion:** As AGNOR counts goes on increasing from normal to malignant lesion; chances of malignancy increases. Being simple and cost effective; AGNOR counts can be used routinely in histopathology laboratory as they also help to decide the surgeon the treatment part and prognosis.

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#### INTRODUCTION

Cell reproduction is precisely regulated, so the production of new cells compensates exactly the loss of cells in a death. The final event ending at the beginning of first event of next cycle<sup>1</sup>. Tumor cell kinetics can be identified by measuring the growth fraction (no. of cycling cells) with the help of a number of methods like Titrated thymidine, S phase fraction, quantification of proliferation associated antigen like PCNA or Ki67. The only way to assess the cycle speed in situ on paraffin embedded material consists of quantifying AGNOR protein<sup>2</sup>.

NORs (Nucleolar Organizing Regions) are loops of DNA transcribed to ribosomal RNA which contribute to regulation of protein synthesis and therefore it follows that the number of NORs in each nucleus reflects cellular activity<sup>3</sup>.

Squamous cell carcinoma of the oral cavity is one of the commonest types of carcinoma affecting Indians. In recent decades, we have seen a dramatic switch from histopathology to molecular methods of disease diagnosis changes occur at the

molecular level before they are seen under the microscope and before clinical changes occur<sup>2,3</sup>.

AgNOR staining and scoring is simple, inexpensive and useful adjunct to routine histopathology to evaluate lesions, especially because of high sensitivity and cost effectiveness

#### MATERIAL AND METHODS

The present study consists of 168 cases of biopsy from oral cavity. The patients were admitted in our tertiary care Government Hospital affiliated to Government College. Patients presented with wide age range of 20-60 years having chief complaints of ulceration, pain, difficulty in opening mouth, foul smell from mouth, difficulty in swallowing. The histopathological material consists of Biopsy from suspected areas or mass. The material was fixed in 10% formalin and subjected to staining by Haematoxylin and Eosin and AgNOR stain. AgNOR staining was carried out by the method described by Ploton *et al*<sup>4</sup> with a few modifications. Finally AgNORs were counted in 100 nuclei under oil immersion (100x) and the mean of them calculated. The AgNOR in the nucleoli were

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counted as one dot and those dispersed throughout nucleus as separate AgNORs.

## RESULTS

Prevalence of lesions

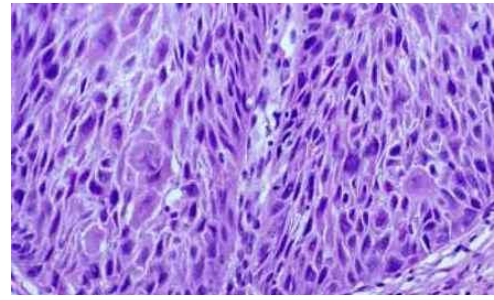
S.no	lesions	number	percentage
1	pre-malignant	59	35.1%
2	malignant	109	64.9%

mAGNOR counts of different pre-malignant lesions and different grades of malignancy

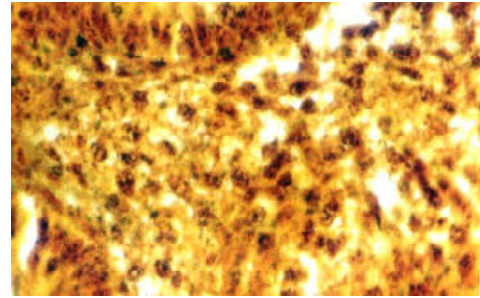
S.no	lesions	mean± sd
1	leukoplakia	2.18±0.05
2	mild dysplasia	2.37±0.07
3	moderate dysplasia	2.53±0.11
4	severe dysplasia	3.00±0.09
5	Wd-scc	4.33±0.23
6	Md-scc	5.79±0.33
7	Pd-scc	8.41±0.28

Comparison of mean AGNOR counts between pre-malignant and malignant lesion

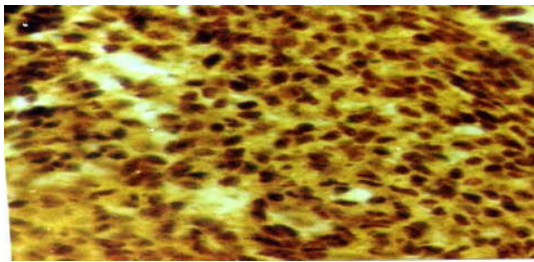
S.no	lesion	number	mean± sd	p value
1	pre-malignant	59	2.53±0.32	0.0001
2	malignant	109	5.41±1.4	



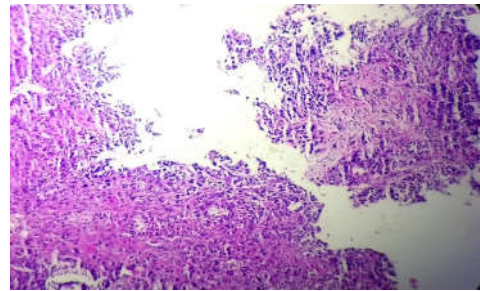
Photograph showing H & E section of oral cavity in severe dysplasia(100x)



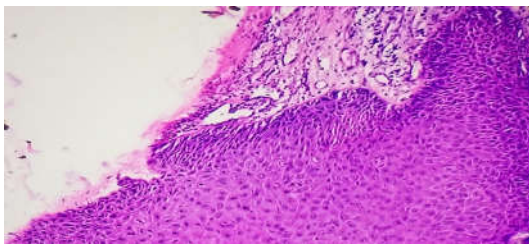
Photograph showing AGNOR staining in buccal mucosa in severe dysplasia(40x)



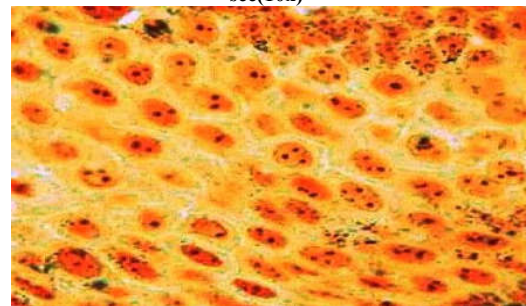
Photograph showing AGNOR staining in leukoplakia(40x)



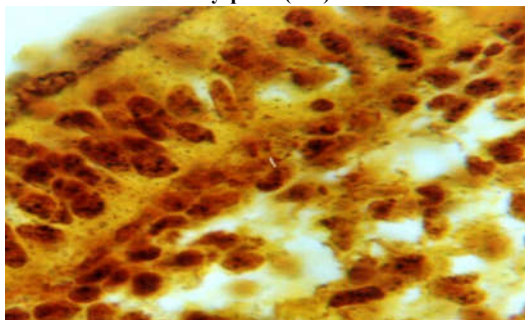
Photomicrograph showing H& E section of oral mucosa in wd-scc(10x)



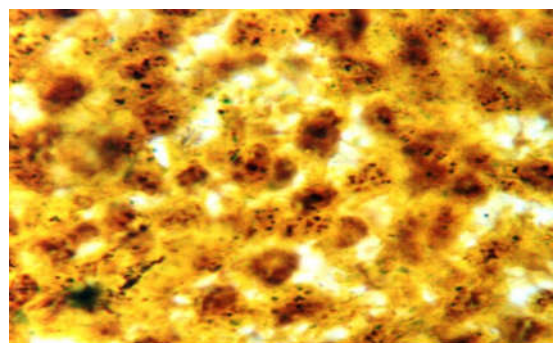
Photograph showing H & E section of oral cavity in moderate dysplasia(40x)



Photomicrograph showing AGNOR staining in wd-scc in oral mucosa(100x)



Photograph showing AGNOR staining in moderate dysplasia in oral mucosa(100x)



Photomicrograph showing AGNOR staining in md-scc (100x)

## DISCUSSION

Over the last decade, silver staining of AGNORs has become a widely used alternative method for assessing proliferative activity and grade of malignancy in tumour pathology<sup>5</sup>. The counting method of Crocker *et al*<sup>6</sup> was followed Total nucleus, extra nucleolar AGNOR dots were counted and silver stained nucleolus was counted as one dot.

SandhyaPanjeta *et al*<sup>7</sup> it was found that the lesions were divided into normal, leukoplakia, dysplasia and malignancy; the mean AGNOR count of carcinoma was significantly higher than the normal epithelium, leukoplakia and dysplasia ( $p < 0.05$ )

SushmaMehkri *et al*<sup>8</sup> the mean AGNOR count was higher in cases of oral squamous cell carcinoma with well and moderate differentiation as compared to cases of oral leukoplakia and dysplasia with a statistically significant difference ( $p < 0.0001$ ) indicating a high proliferative activity in former cases.

Elangovan *et al*<sup>9</sup> found that the maximum significance was seen in case of inflammatory and malignant lesions differ from normal mucosa with a value of ( $p < 0.0001$ ).

surgcdr v manu<sup>10</sup> *et al* the mean AGNOR counts between normal, benign and malignant lesions and between various grades of squamous cell carcinoma, was statistically significant with the p value of ( $< 0.05$ ).

Though the AGNOR technique has its own limitations as of subjective variation; still it is simple, inexpensive procedure and helps in distinguishing between normal, premalignant and malignant lesions, predicting high risk cases and can therefore serve as a useful adjunct to routine histopathological examination of upper aero digestive lesions.

## CONCLUSION

Immunohistochemistry, tumour markers which can aid the pathologist in diagnosis of the lesion but these methods are expensive. AGNOR count on the other hand is a simple cheap method which can act as an additional diagnostic tool in tumour histopathology. The number of AGNOR increases gradually from the epithelial lining to premalignant and malignant lesions of oral cavity. As the present study proves that more of AGNOR number in malignancy in comparison to severe dysplasia/premalignant lesion; it is beneficial for surgeon to choose the type of treatment and predict the prognosis. AGNOR is small, round, regular and few in normal lesions, they increase in number in premalignant lesions while they are large, increased in number and often clumped in malignant lesions.

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