

## Silver Binding of Nucleolar Organizer Regions (AgNORs) in Squamous cell carcinoma of the Head & Neck Region

D. P. Soni<sup>1</sup>, Nidhi Chitlangia<sup>1</sup>, Anupama Garg<sup>2\*</sup>

<sup>1</sup>Department of Pathology, S.P. Medical College, Bikaner, Rajasthan, India.

<sup>2</sup>Department of Anatomy, S.M.S. Medical College, Jaipur, Rajasthan, India.

**Correspondence Address:** \*Anupama Garg, Department of Anatomy, S.M.S. Medical College, Jaipur, Rajasthan, India.

### Abstract

Tumours of the head and neck region are common and are being diagnosed with greater frequency all over the world. The level of differentiation and the extent of the growth significantly modified the likelihood of metastasis and consequently the outlook of patient. As this neoplasm takes months to years to progress through the stage of marked dysplasia to carcinoma in situ to overt carcinoma, it therefore becomes imperative to establish the diagnosis with a reasonable degree of certainty, at an early stage, when the tumour has not reached the stage of incurability. The aim of present study to evaluate the role of AgNOR staining in different grades of squamous cell carcinoma.

**Keywords:** Squamous cell carcinoma, AgNORs, Histopathological, H&E

### Introduction

Tumours of the head and neck region are common and are being diagnosed with greater frequency all over the world. In the malignant neoplasms, squamous cell carcinoma is the most common and may arise from many sites in this region.<sup>1</sup> The histomorphology ranges from carcinoma in situ are poorly differentiated carcinoma with all gradation in between. In situ carcinoma or intraepithelial carcinoma implies that the malignant changes are confined exclusively to the mucosa (stratified squamous epithelium).

The grading of squamous cancer is done by modified jakobsson grading system. The level of differentiation and the extent of the growth significantly modified the likelihood

of metastasis and consequently the outlook of patient. As this neoplasm takes months to years to progress through the stage of marked dysplasia to carcinoma in situ to overt carcinoma, it therefore becomes imperative to establish the diagnosis with a reasonable degree of certainty, at an early stage, when the tumour has not reached the stage of incurability.<sup>2</sup>

The argyrophil, AgNOR technique is remarkably specific as means for detection of NORs which is demonstrates by virtue of silver binding to wide array or NOR associated proteins (NORAPs). The scoring of interphase NOR numbers using the argyrophil technique has been shown to be use of assessment of degree of malignancy like neoplasms. In all recent works on the

role of AgNORs gets dispersed throughout the nucleus to a varying extent in malignancy cases, thus enabling one to count them readily<sup>3</sup>. In the present study an attempt has been made to evaluate the role of AgNOR staining in different grades of squamous cell carcinoma.

### **Material and methods**

This study was conducted on surgical biopsies from 100 cases of squamous cell carcinoma in different region of Head & Neck, specially oral cavity, tongue, pharynx, larynx, oesophagus, paranasal sinuse and jaw. These biopsies were received in the department of pathology, S.P. Medical College, Bikaner, Rajasthan. The tissues were subjected to routine paraffin sectioning. Histopathological diagnosis was first established on these sections using the routine Haematoxylin and Eosin (H&E) stains. Based on routine histopathological diagnosis, the squamous cell carcinoma were classified according to modified Jakobsson system. Further sections were cut from the prepared paraffin blocks. The paraffin sections were subjected to AgNOR staining technique.

Following information was obtained for every case:

#### **Paraffin Sections<sup>4</sup>:**

- The tissue was fixed in 10% formalin.
- Pieces of the fixed tissue were subjected to the procedures of dehydration, clearing and embedding in an automatic tissue processor.
- The dehydrated, cleared tissue pieces were further impregnated with paraffin wax by immersion in a succession of wax bath on the automatic tissue processor.
- The treated tissue was embedded in paraffin wax. The blocks were made using L shaped metallic modules. The embedded tissue blocks allow to cool.
- The blocks were fixed on a rotator microtone and sections of 3 micron

thickness were cut and cut sections were transferred to a water bath and thereby picked on glass slides.

- The sections were fixed to the slides by keeping them in a incubator at 37° c overnight.
- The sections were then subjected to the H&E and AgNOR stains.

#### **Haematoxylin and Eosin stain (H&E stain)<sup>4</sup>:**

- The paraffin sections were dewaxed in xylene, hydrated through various grades of alcohol.
- Sections were rinsed in running water for 1 minute and then briefly in distilled water and then stained in Harris's Alum haematoxylin for 8 minutes.
- Differentiation was done by dipping the stained sections in 1% acid alcohol Then rinsed well in water.
- Sections were dehydrated through different grades of alcohol and then passed through two baths of xylene.
- Sections were dried and mounted with Distrene 80 Dibutylphthalate xylene (DPX) mountant.

#### **AgNOR staining Technique<sup>4</sup>:**

- Paraffin sections were incubated at 37°c overnight, further dewaxed in xylene, hydrated through various grades of ethanol and washed well with triple distilled water. The sections were dried thoroughly and subjected to AgNOR stains.
- The AgNOR stain prepared was poured over the tissue sections and left for 60 minutes at room temperature.
- The silver colloid was washed off with triple distilled water thoroughly and sections were counter stained with 0.5% saffranine.
- Stained sections were mounted with DPX mountant.

**Counting Procedure<sup>4</sup>:**

- AgNOR were counted as black dots in the nuclei of cells using of 100x oil immersion objective.
- 100 cells were studied in each case and the mean AgNOR per nucleus was calculated.
- The final score will be compared in various grades of squamous cell carcinoma of Head & Neck region.
- The data obtained is subsequently correlated with histopathological diagnosis.

**Results**

The study was conducted on surgical biopsies from hundred cases of squamous cell carcinoma of the head & neck regions-received in the Department of Pathology, S.P. Medical college, Bikaner, Rajasthan and total number of cases distributed ,various grades of squamous cell carcinoma in different organs (table 1) and total no. of cases statistical analysed by unpaired t- test and observed that mean AgNOR score in grade I had a statistically significant difference ( $p<0.001$ ) in comparison to grade II, Grade II compared with grade III, Grade III comparison between grade IV are statistically significant ( $p<0.001$ ) respectively (table 2).

**Table 1: Mean AgNOR counts in squamous cell carcinoma of Head & Neck Region.**

Organs	No. of cases	Mean AgNOR count/cell	SD
Larynx	23	7.26	2.82
Buccal Mucosa	21	7.27	2.60
Pharynx	18	7.84	2.95
Oesophagus	13	7.66	2.92
Tongue	10	5.26	2.25
Jaw	6	4.70	1.83
Nose	04	5.38	1.93
Neck Nodes	05	10.14	1.58

**Table 2: Statistical analysis of mean AgNOR counts in different grades of squamous cell carcinoma of Head & Neck region.**

Grade	No. Of cases	Mean AgNOR per cell	SD	Grades compared	Difference of mean	S.E	P value	Remark
Grade I	41	4.42	0.80	I & II	2.81	0.33	<0.001	**
Grade II	26	7.25	1.54	II & III	2.03	0.36	<0.001	**
Grade III	16	9.22	0.79	III & IV	1.97	0.36	<0.001	**
Grade IV	17	11.19	1.22	I & IV	6.77	0.32	<0.001	**

**Discussion**

The present study conducted surgical biopsies from 100 cases of squamous cell carcinoma in different region of Head & Neck. Tumours of head & neck region are

common and are being diagnosed with greater frequency all over the world. Incidence of squamous cell carcinoma of head & neck region at this centre was 7.6% of all malignant tumours.

The present study show the mean AgNOR counts in different grades of squamous cell carcinoma of the head & Neck region. Grade I Squamous cell carcinoma had lowest mean AgNOR count is  $4.42 \pm 0.80$ , Grade II had  $7.25 \pm 1.54$ , Grade III had mean value is  $9.22 \pm 0.79$  and Grade IV had highest mean value is  $11.19 \pm 1.22$ . The AgNOR count in Grade I had a statistically significant difference in comparison to Grade-II ( $p < 0.001$ ), there was a statistically significant difference between grade-II and Grade-III squamous cell carcinoma ( $p < 0.001$ ), Grade-III had a statistically significant in comparison to Grade-IV ( $p < 0.001$ ), and Grade-I had a statistically significant in comparison to Grade-IV squamous cell carcinoma of head & neck region. According to Sano K et al (1991)<sup>5</sup> highest mean AgNOR count in Grade-II squamous cell carcinoma as compared to Grade-I & III but present study the highest mean value in Grade-IV.

According to Warnakulasuriya et al (1993)<sup>6</sup> reported mean AgNOR count is  $8.37 \pm 6.11$  in Squamous cell carcinoma but did not studied the different grades of squamous cell carcinoma.

In the present study statistically significant difference mean value of AgNOR counts in Grade I & Grade II ( $p < 0.001$ ). This observation supported by Epivatio S et al (1994)<sup>7</sup> who reported the mean AgNOR count is  $4.73 \pm 1.04$  and  $7.04 \pm 0.95$  for Grade I and Grade II respectively.

According to Chattopdhyay et al (1994)<sup>8</sup> reported over all mean AgNOR count  $3.48 \pm 0.42$  (range 2.39 to 4.07) irrespective of grades of squamous cell carcinoma of oral mucosa. In contrast, in present study over all mean AgNOR count is  $7.27 \pm 2.6$  (range 3.12 to 11.39) irrespective of grades of squamous cell carcinoma of oral mucosa is comparatively slightly high.

Some problems faced in this study were due to the number of cases because old sections which were older than one year did not stain properly so the study done on fresh section

only. Small nucleus such as in benign lesions the intense affinity of the nucleus for silver staining obscure individual AgNOR.

The above findings denotes that AgNOR count can be used as diagnostic tool together with routine H&E staining to differentiate benign and malignant lesions and is of value in borderline cases. It also helps to assess the various types of malignancy in the increasing trends for the purpose of prognostic aspect of cancer. This method being simple, reproducible and cost effective adds to its benefits and must be used in adjunct to other method, thus rendering earlier diagnosed and better prognosis.

### Conclusion

The findings of this study indicate that the AgNOR staining is an important diagnostic tool in this branch of Medical science. So this technique can be used as adjunct to be used along with conventional Haematoxylin and Eosin stain to reach a diagnosis in problematic cases of squamous cell carcinoma of head & neck region.

### References

1. Jussawala DJ, Cancer incidence pattern in subcontinent of India, *proc. R. Med.* 1973;66: 308-312.
2. Anderson's Text book of Pathology, 10<sup>th</sup> edition.
3. Crocker J, Skilbeck N: Nucleolar organization region associated proteins in cutaneous melanotic lesions. A quantitative study: *J Clin Pathol.* 1987;40: 885-889.
4. Trede D.; Technical and methodological aspects of silver staining and measurements of nucleolar organizer regions, *Zentrcebl Pathol/1994 Mar.* 140(1): 11-14. Trede D.; Technical and methodological aspects of silver staining and measurements of nucleolar organizer regions, *Zentrcebl Pathol/1994 Mar.* 140(1): 11-14.
5. Sano K. Takahashi H, Fujita S. Inoduchi T, Pe Mb, Odabe H. Tsuda N: Prognostic

- implication of silver binding nucleolar organizer regions (AgNORs) in oral squamous cell carcinoma; J. Oral Pathol. Med; 1991;20:53-56.
6. Warnakulasuriya KA, Johnson NW: Nucleolar organizer region distribution as a diagnostic marker in oral keratosis, dysplasia and squamous cell carcinoma. J. Oral Pathol. Med; 1993 Feb; 22(2): 77-81.
  7. Epivatianos J: Evaluation of the nucleolar organizer region associated proteins in oral squamous cell carcinoma; Ann. Dent. 1994 Summer; 53(1): 33-36.
  8. Chattopadhyay A. Chawda JG, Doshi JJ: Silver binding nucleolar organizing regions a study of oral leukoplakia and squamous cell carcinoma: Int. J. Oral Maxillofac. Surg.: 1994: 23; 374-377.