Original Article

Pathology Section

The Role of Silver Staining Nucleolar Organiser Regions (AgNORs) in Lesions of the Oral Cavity

SANDHYA PANJETA GULIA, EMANI SITARAMAM, KARRI PRASADA REDDY

ABSTRACT

Aims and Objectives:

- To establish the role of AgNORs in differentiating the benign from the pre-malignant and the malignant lesions of the oral cavity.
- To establish the degree / grade of malignancy according to the AgNOR count.
- To predict the probable prognosis of the cases on the basis of the AgNOR count, if possible.

Materials and Methods: A retrospective study was conducted on 100 cases from July 2006 – July 2007 on the biopsies which were obtained from the oral mucosa. Two slides were prepared for each case – one was stained with the haematoxylin and eosin stain and the other was subjected to silver staining. The data was analyzed by using the independent T-test and ANOVA for the intergroup comparisons.

Results: The results showed that the mean AgNOR count of the carcinomas was significantly higher than that of the normal oral epithelium, the hyperplastic lesions, papillomas and the leukoplakias ($p < 0.05$). The AgNOR dots tend to be small, homogenously stained and regular in the benign lesions and as the grade of the tumour increased, the AgNOR dots became irregular, large dots or bizarre clusters.

Conclusions: AgNOR staining can be considered as a useful adjunct to diagnostic pathology. This study was helpful in evaluating the importance of AgNORs in differentiating the benign, pre-malignant and malignant lesions of the oral cavity and it could be considered as a valuable tool along with the histopathological criteria for the evaluation of the proliferative activity of the cell.

Key Words: AgNORs, Oral lesions, Carcinoma, Grade, Silver staining

INTRODUCTION

Many problems arise microscopically in differentiating the malignant aberrations from the benign ones and the routine histopathological techniques do not reveal all the features which are of diagnostic and prognostic significance. Therefore, it is eminent to develop adjunct procedures which can diagnose malignancy at the earliest and with accuracy. Studies have revealed the correlation between nucleolar function, size and the cell doubling time in human cancer cell lines, which has stimulated a revolution of the importance of the nucleus in tumour pathology [1].

The nucleus plays an essential role in the control of proliferation and protein synthesis. AgNOR correlates with the rate of proliferation, as can be estimated by Ki-67 and the percentages of the S phase cells and the mitotic cells. Hence, 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.

The interphasic NORS can be clearly visualized at the light microscopical level by using a silver reaction which stains the acidic proteins of the NORS (RNA Polymerase 1 upstream binding factor, Topoisomerase 1, Nucleolin, Fibrillin, C23 protein and B23 protein) on routinely prepared histopathological and cytological samples [2].

After silver staining, the AgNORs can be identified as black dots throughout the nucleolar area. In quantitative terms, the number of AgNORS per nucleus suggests it to be a marker of the proliferative activity of the cell. Qualitatively (based on the shape, size and the pattern of distribution), AgNOR acts as a marker of pre-malignant or malignant change.

Thus, the advantage of the AgNOR technique in retrospective studies is that the samples can be destained and restained with silver. It can guide to a diagnosis when extra unstained slides are unavailable and also in doubtful cases with no corresponding histological specimens.

MATERIALS AND METHODS

The present study was undertaken in the Department of Pathology, Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur. A total of 100 cases were studied from June 2006-July 2007.

These were broadly classified into 8 groups according to their histopathological reports as shown below in [Table/Fig-1].

S. No.	Group	Sub Division
I	Histopathologically normal oral mucosa (Control)	Benign
II	Inflammatory lesions without dysplasia	
III	Mild and moderate dysplasia	Pre-malignant
IV	Severe dysplasia	
V	Squamous cell carcinoma (Well differentiated)	Malignant
VI	Squamous cell carcinoma (moderately differentiated)	
VII	Squamous cell carcinoma (poorly differentialted)	
VIII	Others	

[Table/Fig-1]: Distribution of the Studied Cases According to Groups
*Others include adenoid cystic carcinoma, adenocarcinoma, malignant melanoma.

The biopsy specimens which were received were subjected to routine paraffin sectioning at 4 µm thickness after proper fixation in 10% formal saline. AgNOR staining was performed as described by Ploton et al [3, 4].

The sections were deparaffinized in xylene and hydrated through decreasing grades of ethanol to double distilled deionized water. The sections were then reacted with freshly prepared silver colloidal solution (1 part by volume of 2% gelatin in 1% formic acid and two parts by volume of 50% aqueous silver nitrate solution) in a closed coplin jar for 35 min at room temperature, while ensuring that a dark environment was maintained throughout the reaction time. The silver colloidal solution was washed with double distilled ionized water. The sections were then treated with 5% sodium thiosulphate for 5 minutes and washed in double distilled deionized water, dehydrated through increasing grades of alcohol, cleared in xylene and mounted.

AgNOR Counting Procedure

The number of AgNORs which were present in each nuclei were counted in 100 nuclei by using a 100x oil immersion lens. At this magnification, AgNORs are visible both within and outside the nuclei [3]. The mean AgNOR values were calculated for each case and group. The results which were obtained in the counting procedure were analyzed statistically by using the Student's t-test and one way analysis of variance (ANOVA) for intergroup comparisons [5].

RESULTS

In the present study, 71 patients were males and 29 patients were females, with a sex ratio of 2.3:1. As far as the site of involvement was concerned, 34 cases involved the buccal mucosa and 19 cases the tongue, while the other sites were the alveolus, lip, palate and cheek.

AgNORs were visible as black dots within the nuclei of the epithelial cells.

The mean AgNOR count of the studied groups was as follows: (as shown in [Table/Fig-2].

Groups	N	mAgNOR	SD
I	3	1.1967	0.2721
II	20	2.1505	0.8433
III	8	2.6888	0.3337
IV	11	2.9500	0.6581
V	40	4.1763	0.8157
VI	8	6.3450	0.8913
VII	9	8.7011	0.6543
VIII	1	9.9000	0

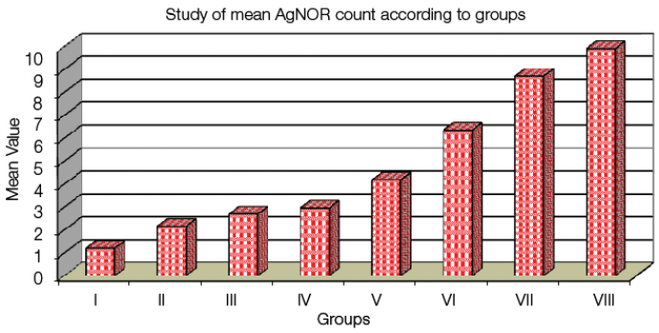
[Table/Fig 2]: Study of mean AgNOR Count According to Groups

The groups I & II (control subjects and benign lesions), could be differentiated clearly, based on the low value of the mAgNOR counts from the groups IV – VII.

Group III and group IV differed from groups V-VII, based on the value of the mAgNOR counts, which helped to demarcate between the pre-malignant and the malignant lesions of the oral mucosa and hence, helped to formulate the treatment plan for the patient, on whether a conservative approach or surgery was required.

Groups V, VI and VII observed a significantly higher mean difference for the AgNOR count with all the rest groups. This indicated that squamous cell carcinomas of the oral mucosa could be differentiated from the pre-malignant and benign lesions, based on the mAgNOR count also.

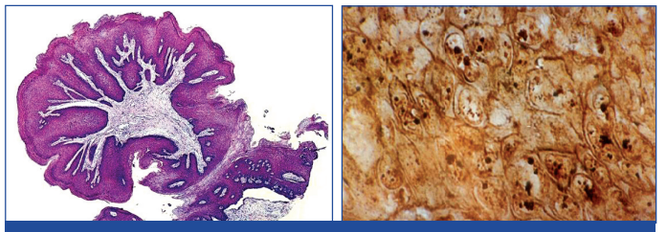
The value of the mean AgNOR count for the three grades of oral squamous cell carcinoma in the present study were found to be 4.71 ± 0.81, 6.34 ± 0.89 and 8.70 ± 0.65 respectively (as shown in [Table/Fig-2 and 3]). The mean AgNOR count showed a linear and significantly increasing trend as the histopathological grade of the tumour increased ($p<0.05$).



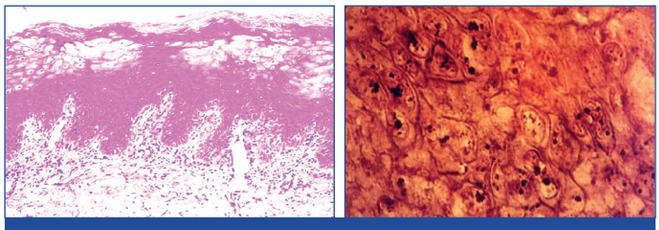
[Table/Fig-3]: Study of mean AgNOR count according to groups

In our study, it was observed that the AgNOR dots tended to be large, homogenously stained and regular in the nuclei of the normal epithelial tissues, hyperplasias and papillomas (as shown in [Table/Fig-4 and 5]), whereas significant differences (irregular, giant and bizarre clusters) were seen in the oral squamous cell carcinomas (as shown in [Table/Fig-6, 7 and 8]).

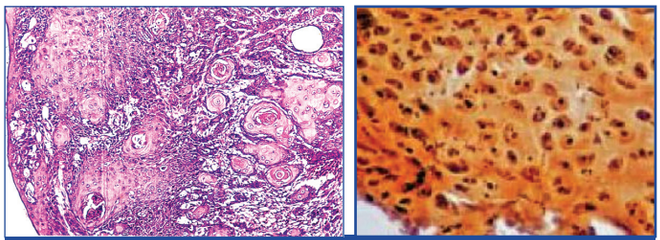
In the malignant squamous cells, they appeared to be less uniform in size and shape (as shown in [Table/Fig-6]) and some dots ap-



[Table/Fig-4]: Squamous Papilloma – AgNOR stain cells showing AgNOR dots in the nuclei (× 100 oil immersion)



[Table/Fig-5]: Leukoplakia – AgNOR stain cells showing AgNOR dots in the nuclei (× 100 oil immersion)

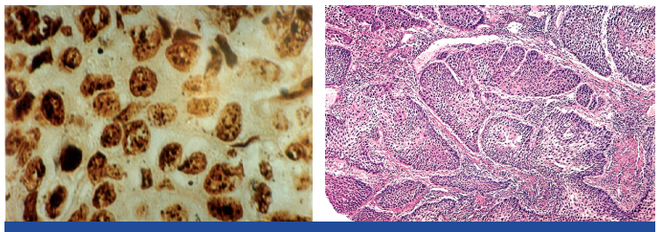


[Table/Fig-6]: Squamous cell carcinoma Grade I – Nuclei contain 2-4 dense regular AgNOR dots

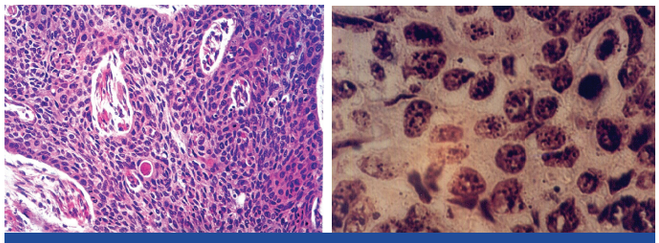
peared to be clumped together to form irregular bizarre shaped clusters (as shown in [Table/Fig-7 and 8]), which were more obvious in the moderately and poorly differentiated squamous cell carcinomas. Hence, by seeing the number and morphology of the AgNOR dots, squamous cell carcinomas of the oral mucosa could be graded into well differentiated (as shown in [Table/Fig-6]), moderately differentiated (as shown in [Table/Fig-7]) and poorly differentiated (as shown in [Table/Fig-8]) grades, which would further help in the assessment of the prognosis of the lesion and hence, the outcome [5-7].

As shown in the [Table/Fig-9] below, the intergroup comparison was done among seven (the 8th group was not taken into account for the statistical analysis because of the small sample size) groups, which were divided by using the ANOVA test. The value was found to be statistically significant among the various comparative groups ($p < 0.05$).

As shown in the [Table/Fig-10 and 11], the mAgNOR count showed a linear and significantly increasing trend as the histopathological grade [4] of the tumour increased ($p<0.05$). The total 57 cases of squamous cell carcinoma in our study were further graded into three grades according to the increasing number of the AgNOR dots (as shown in [Table/Fig-10]) and according to their morphology ([Table/Fig-6, 7 and 8]) into well, moderately and poorly differentiated carcinomas.



[Table/Fig-7]: Squamous cell carcinoma Grade II – Nuclei show 3-4 AgNOR dots with clustering and irregularities in size of AgNOR dots



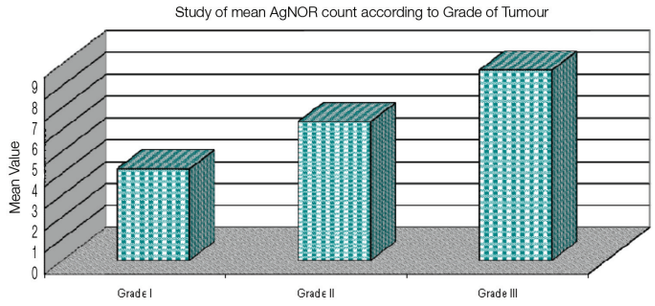
[Table/Fig-8]: Squamous cell carcinoma Grade III – Nuclei show 6-10 AgNOR dots which appear clumped together forming irregular bizarre clusters

Groups	Statistically significant difference with group	p value (less than)*	Not significant difference with group	p value (more than)
I	IV, V, VI, VII	<0.0001	II, III	>0.0001
II	V, VI, VII	<0.0001	I, III, IV	>0.0001
III	V, VI, VII	<0.0001	I, II, IV	>0.0001
IV	V, VI, VII	<0.0001	I, II, III	>0.0001
V	I, II, III, IV, VI, VII	<0.0001	–	>0.0001
VI	I, II, III, IV, V, VII	<0.0001	–	>0.0001
VII	I, II, III, IV, V, VI	<0.0001	–	>0.0001

[Table/Fig-9]: Comparison of mAgNOR count between various groups using ANOVA
*Significant *p*-value

Grade	N	Mean	SD
I	40	4.1763	0.8157
II	8	6.3540	0.8913
III	9	8.7011	0.6543

[Table/Fig-10]: Study of mAgNOR Count According to the Grade of Tumor



[Table/Fig-11]: Study of mean AgNOR count according to grade of tumor (Squamous cell carcinoma

DISCUSSION

The present study was conducted to evaluate the importance of AgNORs in differentiating benign, pre-malignant and malignant lesions of the oral cavity. The neoplastic cells generally exhibit a rise in the synthesis of normal and abnormal products and thus frequently feature a significant rise in the AgNOR material [8]. The AgNOR counts increase with increased cell ploidy and with increased transcriptional activity in the stages of active cell proliferation [6]. Variations in the size and/or number of the AgNOR dots may depend on the stage of the cell cycle, the transcriptional and metabolic activity of the cell or the number of NOR-bearing chromosomes in the karyotype. In a rapidly proliferating cell, the chromosomal and AgNOR distribution remains disorganized with the resultant formation of multiple, small and dispersed nucleoli. Actively proliferating cells have impaired nucleolar association and therefore they exhibit a higher AgNOR count, regardless of the ploidy state of the cell.

Of the various newer techniques which are used for assessing the tumour tissue based on nuclear studies, the staining of AgNORS by a silver compound has become popular for its

- Simplicity
- Ease of use
- Low Cost
- Good Correlation with other proliferative markers

Several studies have shown variations in the number and shape of the AgNORs of the normal mucosal cells and the malignant cells. In our study, it was observed that the AgNOR dots tended to be large, homogenously stained and regular in the nucleus of the normal epithelial tissues, hyperplasias and papillomas, whereas significant differences (irregular, giant and bizarre clusters) were seen in oral squamous cell carcinomas.

The mean AgNOR count of the normal epithelia in the present study was 1.19 ± 0.27. This value was comparable with that of the previous studies which were done by Kobayashi [26] 1.83 ± 0.66; Xin Xie [22] 2.3 ± 0.4; Yue et al (1999) [8] 1.67 ± 0.19; Chattopadhyay [10] 1.47 ± 0.39; Abbas et al (2002) [6] 1.98 ± 0.34 and Manu Rai et al (2006) [7] 1.56 ± 0.42.

The values of the mean AgNOR count for the hyperplasias and papillomas showed comparison with those of the other studies

which were conducted by Braz Dent [9], with the mean AgNOR count for papillomas being 3.15 ± 0.58 and for hyperplasias being 1.98 ± 0.24. The value of the mean AgNOR count which was obtained in the present study (2007) for the papillomas was 2.15 ± 0.84 and for hyperplasias, it was 2.68 ± 0.33.

The studies by Chattopadhyay [10] (mAgNOR – 2.37 ± 0.12) on leukoplakia of the oral mucosa showed comparable results with those of the present study (mAgNOR - 2.95 ± 0.65).

The values of the mean AgNOR count for the three grades of oral squamous cell carcinoma in the present study were found to be 4.71 ± 0.81, 6.34 ± 0.89 and 8.70 ± 0.65 respectively. The mean AgNOR count showed a linear and significantly increasing trend as the histopathological grade of the tumour increased ($p<0.05$). These findings were consistent with those of the studies which were done by Yue et al 1999 [8], Abbas et al 2002 [6] and Rai in 2006 [7], as shown in [Table/Fig-12].

Study	Year	Grade I	Grade II	Grade III
Yue et al	1999	3.1 ± 0.9	3.8 ± 1.1	5.1 ± 1.3
Braz Dent J	2000	6.56 ± 1.25	–	7.07 ± 1.60
Abbas et al	2002	3.2 ± 1.53	6.01 ± 4.61	6.94 ±5.83
Oliveira	2005	1.59 ± 0.22	2.15 ± 0.41	2.58 ± 0.60
Manu Rajaram	2006	3.29 ± 0.63	4.29 ± 0.78	5.21 ± 0.16
Present study	2007	4.17 ± 0.81	6.34 ±0.89	8.70 ± 0.63

[Table/Fig-12]: Mean AgNOR Count according to the Grades of Squamous Cell Carcinoma

All efforts were made to standardize the tissue processing and the staining techniques.

However, it must be mentioned that several technical difficulties were encountered during this study and it was felt that the staining method must be meticulously established with regards to the duration of the staining, the temperature, the purity of water, the reagents, etc. (all these parameters had a great influence on the final result) before it was adopted as a routine procedure. The use of the plastic slide containers for staining gave adequate and uniform staining at a low cost as compared to the use coplin jars or the inverted incubation technique.

Particular attention was paid to the cleanliness of the glassware and the purity of water in order to avoid background staining and non-specific granular deposits on the tissue sections [32]. The silver incubation time which rendered the most distinct diagnostic difference in the AgNOR content of the benign and malignant tissues varied considerably. Accordingly, the staining time had to be adjusted for the individual argyrophilia of each tissue block or tissue section, for which the use of internal staining standards such as lymphocytes or connective tissue was found to be mandatory. For routine purposes, the appropriate silver incubation time was achieved if the AgNORs are visible as black dots, mainly within the nucleoli of the proliferating cells [33]. The use of digital image analysis provided information about the size and distribution of the AgNORs. Evidently, these data were more important than the AgNOR number. In the hands of experienced pathologists and under standardized conditions, AgNOR staining and AgNOR quantification are a valuable completion of established methods [34].

However, there are certain limitations in our study like:

- Resolution of individual AgNORs within relatively small nucleolus
- Affinity of the nucleolus for silver stain which obscures the individual AgNORs in cases of intense staining
- A variable degree of overlap between high and low grade tumours.

A correlation between the AgNOR count and prognosis was too found in pre-malignant and malignant lesions of the cervix [12], colorectal cancer[18], benign and malignant effusions [17], adenoid cystic carcinoma [24] and breast carcinoma [14] It is of prognostic value also in ovarian cancer [15], transitional cell carcinaoma of the bladder [21] and glottic cancer[16].

Though the staining procedure is simple and cost effective, it needs a lot of dedication, standardization and meticulous bench work to achieve good results. Thus, we feel that the AgNOR technique can definitely be used as a supportive tool to the routinely performed haematoxylin and eosin staining and that it will help in the prognosis and the therapeutic decision making in squamous cell carcinomas of the upper aerodigestive tract.

CONCLUSION

Based on the present study, it appeared that the AgNORS which stained for the NOR associated proteins, acted as the markers for both cell proliferation and malignancy.

Hence, the AgNOR count can be a used as a supplementary factor for the difficult histoprognostic evaluation of various malignancies.

Further studies on larger numbers of samples are required to confirm the association of AgNOR with malignancy. Thus, the AgNOR numbers are of clinical importance in malignancies of the oral cavity and their estimation should be regarded as a valuable adjunct, in addition to the histopathological criteria for the evaluation of proliferative activity. It has been emphasized that patients with higher AgNOR counts in their tumours should be subjected to a careful follow up and more intensive radiotherapy.

ACKNOWLEDGEMENT

We are indebted to Surg Lt Commander, (Dr) Manish Gulia, for his valuable contribution in formatting and editing the above article. We wish to thank Mr Anand Kavishwar for his help in the statistical analysis.

REFERENCES

[1] Trere D, Pession A, Derenzini M. The silver stained proteins of the interphasic nucleolar organizer regions as a parameter of the cell duplication rate. *Experimental Cell Research* 1989;184 :131-37.
[2] Derenzini M , Farabegoli F, Trere D. Relationship between interphase AgNOR distribution and nucleolar size in cancer cells. *Histochemical Journal* 1992 ; 24:951-56.
[3] Orell JM, Evans AT, Grant A. A critical evaluation of AgNOR counting in benign naevi and malignant melanoma. *Journal of Pathology* 1991; 163: 239-44.
[4] Crocker J, David A, Boldy R, Egan MJ. How Should We Count AgNORs? Proposals for a standardized approach. *Journal of Pathology* 1989; 158: 185-88.
[5] Elangovan T, Mani NJ, Malathi N. Argyrophilic nucleolar organizer regions (AgNORs) in inflammatory, pre-malignant and malignant oral lesions: a quantitative and qualitative assessment. *Indian J Dent Res* 2008; 19(2):141-46.
[6] Abbas NF, Abbas EA, Eabdel Aal W. Image cytometric analysis of mean nuclear area and nucleolar organizer regions (AgNORs) in oral squamous cell carcinoma. *Egypt. Med. J. NRC* 2002; 1(1): 141-57.
[7] Manu V, Rajaram T, Rai R: Value of silver binding nucleolar organizer regions (AgNOR) in squamous cell carcinomas of upper aerodigestive tract. *MJAFI* 2006; 62:123-28.
[8] Yue L, Iwai M, Furuta I. Evaluation of argyrophilic nucleolar organizer regions in tongue squamous cell carcinoma. *Oral Oncology* 1999; 35: 70-76.

[9] Linaena Mericy da Silva F, Maaria Auxiliadara Vieira C. AgNORs in hyperplasia, papilloma and oral squamous cell carcinoma. *Braz Dent J* 2000; 11(2): 105-10.
[10] Chattopadhyay A, Ray JG, Caplan DJ. AgNOR count as an objective marker for the dysplastic features in oral leukoplakia. *J Oral Pathol Med* 2002; 31: 512-17.
[11] Chiu K Y, Loke S K, Wong K K. Improved silver technique for showing nucleolar organizer regions in paraffin wax sections. *J Clin Pathol* 1989; 42: 992-94.
[12] Pratibha D, Kuruvilla S. Value of AgNORs in pre-malignant and malignant lesions of the cervix. *Indian J Pathol* 1995; 38:11-16.
[13] Leek R D, Alison M R, Sarraf C E. Variations in the occurence of silver-staining nucleolar organizer regions (AgNOR) in non-proliferating and proliferating tissues. *Journal of Pathology* 1991; 165: 43-51.
[14] Ghazizadeh M, Sasaki Y, Araki T, Konishi H, Aihara K. Prognostic value of the proliferative activity of ovarian carcinoma as revealed by PCNA and AgNOR analyses. *Am J Clin Pathol* 1997; 107: 451-58.
[15] Sivridis E, Sims B. Nucleolar organizer regions: new prognostic variable in breast carcinomas. *J Clin Pathol* 1990; 43: 393-96.
[16] Yamamoto Y, Itoh T, Saka T, Sakakura A , Takahashi H. Nucleolar organizer regions in glottic carcinoma: comparison of DNA cytoflurometry and clinicopathological analysis: *Euro Arch Otorhinolaryngol* 1995; 252: 499-503.
[17] Akhtar G, Chaudhary N A, Tayyab M, Khan S A. AgNOR staining in malignant and benign effusions. *Pak J Med Sci* 2004; 20(1): 29-32.
[18] Jin W, Gao M Q, Lin Z W, Yang D X . Multiple biomarkers of colorectal tumour in a differential diagnosis model: a quantitative study. *World J Gastroenterol* 2004;10(3): 439-42.
[19] Underwood JCE, Giri DD. Nucleolar organizer regions as diagnostic discriminants for malignancy [editorial]. *J Pathol* 1988;155: 95-96
[20] Derenzini M. The AgNORs. *Micron* 2000; 31(2):117-20.
[21] Lipponen P. Image analysis of the AgNOR proteins in transitional cell bladder cancer. *Journal of Pathology* 1993; 171: 279-83.
[22] XinXie, Clausen OPF, Sudbo J, Boysen M. Diagnostic and prognostic value of the nucleolar organiser regions in the normal epithelium, dysplasia and squamous cell carcinoma of the oral cavity. *Cancer* 1997; 79:2200-228.
[23] Crocker J, David A, Boldy R, Egan MJ. How Should We Count AgNORs? Proposals for a Standardized Approach. *Journal of Pathology* 1989; 158: 185-88.
[24] Fonseca I, Sceares J. Adenoid cystic carcinoma: A study of the nucleolar organizer regions (AgNORs) counts and their relation to prognosis. *Journal of Pathology* 1993; 169: 255-58.
[25] Caldeira PC, Cassia M, Aguiar F, Mesquita RA, de Carmo MAV. Oral leukoplakias with different degrees of dysplasia. *Journal of Oral Pathology and Medicine* April 2011; 40(4): 305-11.
[26] Kobayashi I, Matsuo K, Ozekis Ohishi M. The proliferative activity in oral epithelial dysplasia which was analysed by proliferating cell nuclear antigen immunostaining and argyrophilic nucleolar organizer region staining. *Mol Pathol* 1995; 48M : 239-40.
[27] Biswal BM, Othman NH. Correlation of nuclear morphology and AgNOR score with radiation response in squamous cell cancers of the head and neck: A preliminary study. *Malaysian Journal of Medical Sciences* 2010: 17(3); 19-26.
[28] Dinesh R, Jagdish J. Early detection of oral cancer: PAP and AgNOR staining in brush biopsies. *Journal of Oral and Maxillofacial Pathology* 2010: 14(4): 52.
[29] Oliveira MG, Isabel da Silva Lauxen I, Neto MM, Rados PV. Tongue squamous cell carcinoma: Relationship between argyrophilic nucleolar organizer regions (AGNORs) and histopathologic grading. *Applied Cancer Research* 2005; 25(1): 20-24
[30] Charles HCM, Osinga BJ. Abundance of protein bound sulfhydryl and disulphide groups at chromosomal nucleolus organizing regions. *Chromosoma* 1980; 77: 1-11.
[31] Sudbo J, Bryne M, Johannessen AC, Kildal W, Danielsen HE, Reith A, et al. Comparison of histological grading and large scale genomic status (DNA ploidy) as the prognostic tools in oral dysplasia. *Journal of Pathology* 2001;194: 303-10.
[32] Lakra S. AgNOR expression in the central nervous system. *J of Medical and Biological Sciences* 2011;4(1).
[33] Ruschoff J, Plate KH, Contractor H , Schalte B, Thomas C. AgNOR cytometry by means of automatic image analysis–a contribution to standardization. *Verh Dtsch Ges Pathol* 1990; 74: 248-52.
[34] Martin H. Importance of AgNOR analysis in malignant tumours: *Zentralbal Pathol* 1994; 140(1): 15-22.

AUTHOR(S):
1. Dr. Sandhya Panjeta Gulia
2. Dr. Emani Sitaramam
3. Dr. Karri Prasada Reddy

PARTICULARS OF CONTRIBUTORS:
1. MD Pathology, Assistant Professor, Sri Venkateshwaraa Medical College Hospital & Research Centre, Pondicherry, India.
2. MD Pathology, Associate Professor, Maharajahs Institute of Medical Sciences, Vizianagram, India.
3. MD Pathology, Professor, Maharajahs Institute of Medical Sciences, Vizianagram, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sandhya Panjeta Gulia
H.no 40/4, Inderpuri, Ramlila ground,
Jacobpura, Gurgaon, Haryana
Phone: 09394134207
Facsimile: 09394537104
E-mail: sandhya_path@yahoo.com

DECLARATION ON COMPETING INTERESTS:
No competing Interests.

Date of Submission: **Jun 15, 2011**
Date of peer review: **Jul 28, 2011**
Date of acceptance: **Aug 19, 2011**
Online first: **Sep 05, 2011**
Date of Publishing: **Oct 05, 2011**