

How to Cite:

Dani, G., Wasti, A., Nayak, S., Wasti, J., Kumar, C. K., & Khan, N. (2022). Quantitative and qualitative analysis of AgNORS in OSMF leukoplakia and OSCC. *International Journal of Health Sciences*, 6(S1), 7266–7273. <https://doi.org/10.53730/ijhs.v6nS1.6567>

Quantitative and qualitative analysis of AgNORS in OSMF leukoplakia and OSCC

Dr Gaurav Dani

MDS, Reader, Dept. of Oral pathology and microbiology, Rungta College of Dental sciences and research centre, Bhilai

Email: drgauravdani@gmail.com

Dr. Amit Wasti

MDS, Associate professor, Dept. Of Oral Pathology and Microbiology, Government Dental College Raipur Chhattisgarh

Email: amitwasti9995@gmail.com

Dr. Sunil Nayak

MDS, Assistant professor, Department of Oral Pathology and microbiology, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India.

Email: nayaksunil09@gmail.com

Dr. Jyoti Wasti

MDS, Asst. professor, Dept. of Periodontology and Implantology, Government Dental College Raipur, Email: jyotiwasti@yahoo.in

Dr. Chokkara Kiran Kumar

MDS, Asst. Professor, Dept. of Oral Pathology and microbiology, Maitri Dental college and research center, Anjora Durg.

Dr. Naved Khan

MDS, Assistant Professor, Dept. Of oral and maxillo-facial surgery, Government Dental College, Raipur, Chhattisgarh

Email- naved1977@gmail.com

Abstract---Background: Oral cancer is one of the most significant reasons of death in India. The quantitative and qualitative assessment of AgNORS parameters has been used in oncology for both diagnostic and prognostic objectives. The number and distribution of AgNOR in the nucleus (configuration) were helpful in detecting and predicting the prognosis of various neoplasias. Aim: To assess the biologic aggressiveness of leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma and quantitative and qualitative analysis of AgNORS in OSMF, Leukoplakia and OSCC. Materials and Methods: The study samples were distributed in four different groups:-Group- I:

- Control group, 10 sample of normal mucosa. Group II: - 30 histopathologically proven samples of leukoplakia. Group III:-30 histopathologically proven samples of oral submucous fibrosis. Group IV: - 30 histopathologically proven samples of oral squamous cell carcinoma. The number of dots per nucleus was used to count AgNORs. Images were taken with a digital camera linked to a labomed572 Binocular Photo Microscope with an X100 oil immersion objective lens. The photos were categorised, copied, and saved to a computer. Results: The mean AgNOR count of the control group was found to be the lowest among all groups (1.63 ± 0.06), between premalignant group it was more in Leukoplakia group (2.93 ± 0.28), as compared to OSMF (2.53 ± 0.24) and in Oral Squamous Cell Carcinoma it was found to be the highest (3.97 ± 0.42 .) The mean AgNOR count of control group was also compared with other groups, the comparison was found to be highest between control and oral squamous cell carcinoma, and among premalignant group it was more significant in leukoplakia than OSMF. Conclusion: AgNOR can be an effective tool in assessment of OSMF LEUKOPLAKIA AND OSCC

Keywords---Leukoplakia, OSMF, OSCC, AgNOR.

Introduction

Oral cancer is one of the most significant reasons of death in India. Oral cancer cases are anticipated to rise from 10 million in 2000 to 16 million in 2020, according to the International Agency for Research on Cancer of the World Health Organization (IARC- WHO). Squamous cell carcinoma (SCC) is the most common malignant tumour in the oral cavity. Well-defined oral potentially malignant lesions, such as Leukoplakia and Oral Submucous Fibrosis, may develop in the time between the initiation of carcinogenic tobacco habits and the development of invasive oral cancers. Potentially Malignant Disorders are defined (WHO 2005) as the risk of malignancy being present in a lesion or condition at the time of initial diagnosis or at a later date.^{1,2}

In the context of leukoplakia, Gupta PC et al (1980) found a 0.3 percent annual malignant transformation rate. It is generally understood that non-homogeneous kinds, such as proliferative verrucous leukoplakia, have a substantially higher figure. In the case of OSMF, the atrophic epithelium appears to predispose to the development of squamous cell carcinoma in the presence of carcinogens, according to Murti PR, Bhonsle RB, and colleagues (1985). Similarly, the annual malignant transformation rate in OSMF was around 0.5 percent.^{3,4}

The Nucleolar Organizer Regions (NORS) are ribosomal DNA loops found in the nucleoli of cells on the acrocentric chromosomes 13, 14, 15, 21, and 22. AgNORs can be easily detected in formalin-fixed, paraffin-embedded tissues using a simple silver staining procedure (AgNOR method), allowing for quick examination of morphology and tumour cell dynamics even on tiny biopsies. The quantitative and qualitative assessment of AgNORs parameters has been used in pathology of oral cancer for both diagnostic and prognostic objectives. The number and distribution

of AgNOR in the nucleus (configuration) were helpful in detecting and predicting the prognosis of various neoplasias.^{5,6}

This study was carried out to determine the biologic aggressiveness of leukoplakia, oral submucous fibrosis, and oral squamous cell carcinoma, as well as to examine the performance of AgNORs in diagnosing the malignant potential of potentially premalignant illnesses.

Materials and Method

Study Group

The study groups comprises of 10 control group, 30 histopathologically proven samples of leukoplakia, 30 histopathologically proven samples of oral sub mucous fibrosis, 30 histopathologically proven samples of oral squamous cell carcinoma.

Source and method of collection of data

The present study was conducted in Department of Oral and Maxillofacial pathology, Maitri College of Dentistry And Research Centre, Drug, Chhattisgarh, India. To accomplish the objectives, the tissue specimens were retrieved from the archives of the department.

The study samples were distributed in four different groups:

GROUP- I: - Control group, 10 sample of normal mucosa.

GROUP II: – 30 histopathologically proven samples of leukoplakia.

GROUP III:-30 histopathologically proven samples of oral submucousfibrosis.

GROUP IV: - 30 histopathologically proven samples of oral squamous cell carcinoma.

Methodology

Collection of specimen

The tissue specimens were collected from the archives of the department of Oral and Maxillofacial Pathology which consist of Control group, Oral leukoplakia, Oral Sub mucous Fibrosis, and Oral Squamous Cell Carcinoma.

Haematoxylin and eosin staining

Prior to AgNOR staining all the sections was also stained for Haematoxylin and eosin to confirm the lesions.

AgNOR staining procedure

1. The section were deparafinnized in two changes of xylene
2. Rehydration through descending grade of ethanol (all dilutions of ethanol were made in deionized water)
3. Slides were then washed in running deionized water for 8-10 minutes.
4. Slides were then stained with silver colloidal solution freshly prepared and incubated in the dark for 45 minutes at 60°C.
5. Post stained sections were washed in the running deionized water for 5-8 minutes.
6. Dehydration in ascending grades of ethanol.
7. Cleared in xylene.

8. Mounted in DPX

Observation

In the brown stained nucleus on a pale yellow backdrop of cells, the silver reaction products appeared as discrete black dots of different sizes. There were no superfluous silver deposits on the background of the slide.

The number of dots per nucleus was used to count AgNORs.

Images were taken with a digital camera linked to a labomed572 Binocular Photo Microscope with an X100 oil immersion objective lens. The photos were categorised, copied, and saved to a computer.

Ethical Issues

The study protocol was approved by the ethical committee of Maitri College of dentistry and research center, Durg, Chhattisgarh.

Statistics used

The difference in AgNOR count across groups (both quantitative and qualitative) was analysed using Student's unpaired T test, One-way ANOVA, and Tukey's Analysis for post-hoc analysis between groups. P-values less than 0.05 were deemed statistically significant.

Results and Observation

Quantitative Assessment

The mean AgNOR count of the control group was found to be the lowest among all groups (1.63 ± 0.06), between premalignant group it was more in Leukoplakia group (2.93 ± 0.28), as compared to OSMF (2.53 ± 0.24) and in Oral Squamous Cell Carcinoma it was found to be the highest (3.97 ± 0.42). The P value was <0.001 , which was highly significant. (Table 1) The mean AgNOR count of control group was also compared with other groups, the comparison was found to be highest between control and oral squamous cell carcinoma, and among premalignant group it was more significant in leukoplakia than OSMF. But the p value was found to be highly significant in all groups. (Table 2) On further analysis, we noticed that Oral submucous fibrosis and leukoplakia show significant difference from each other and the P-value was found to be significant ($p < 0.001$). (Table 3). OSCC showed statistically higher AgNOR count than leukoplakia and the P-value was found to be significant ($p < 0.001$). (Table 4). OSCC showed statistically higher AgNOR count than OSMF and the P-value was found to be significant ($p < 0.001$). (Table 5)

Qualitative Assessment

The morphological assessment of AgNORs based on their size, shape, and the pattern of distribution revealed certain differences and characteristics among the various study groups. In case of control group the AgNOR dots were of medium size, uniformly round to oval in shape which belongs to type 1 pattern of distribution. In case of OSMF and leukoplakia the AgNORs were not so uniformly

round or oval. Some of them were slightly large and irregular in shape, here they exhibit mixed type 1 and type 2 pattern of distribution. In OSCC group numerous small dots dispersed throughout the nucleoplasm of varying shape and size was found thus indicating type 3 pattern. Additionally the individual dots also showed abnormal appearances like the signet ring, spidery web, and oblong and linear patterns. Such abnormal appearances of AgNORs were seen to a greater degree in case of moderately differentiated SCC compared to well differentiated SCC.(Figure 1)

Table I
Mean AgNOR count in different groups and tests of significance

Group	Range	Mean $\pm$ S.D.	P value*
Control	1.52-1.72	1.632 $\pm$ 0.06	<0.001
Leukoplakia	2.42-2.46	2.93 $\pm$ 0.28	
O.S.M.F.	2.16-2.92	2.53 $\pm$ 0.24	
O.S.C.C.	3.2-4.5	3.97 $\pm$ 0.42	

ANOVA test*

Table 2
Comparison of the mean AgNOR counts and standard deviations and test of significance between the control and different groups

Groups Compared	Mean	SD	P value*
Control	1.632	0.06	<0.001
Leukoplakia	2.93	0.28	
Control	1.632	0.06	<0.001
O.S.M.F	2.53	0.24	
Control	1.632	0.06	<0.001
O.S.C.C.	3.97	0.42	

Student's Independent t-test*

Table 3
Comparison of the mean AgNOR counts and standard deviations and test of significance between Leukoplakia and OSMF

Groups Compared	Mean	SD	P value*
Leukoplakia	2.93	0.28	<0.001
O.S.M.F	2.53	0.24	

Table 4
Comparison of the mean AgNOR counts and standard deviations and test of significance between Leukoplakia and OSCC

Groups Compared	Mean	SD	P value*
Leukoplakia	2.93	0.28	<0.001
O.S.C.C.	3.97	0.42	

Table 5
Comparison of the mean AgNOR counts and standard deviations and test of significance between OSCC and OSMF

Groups Compared	Mean	SD	<i>P value*</i>
O.S.C.C.	3.97	0.42	<0.001
O.S.M.F.	2.53	0.24	

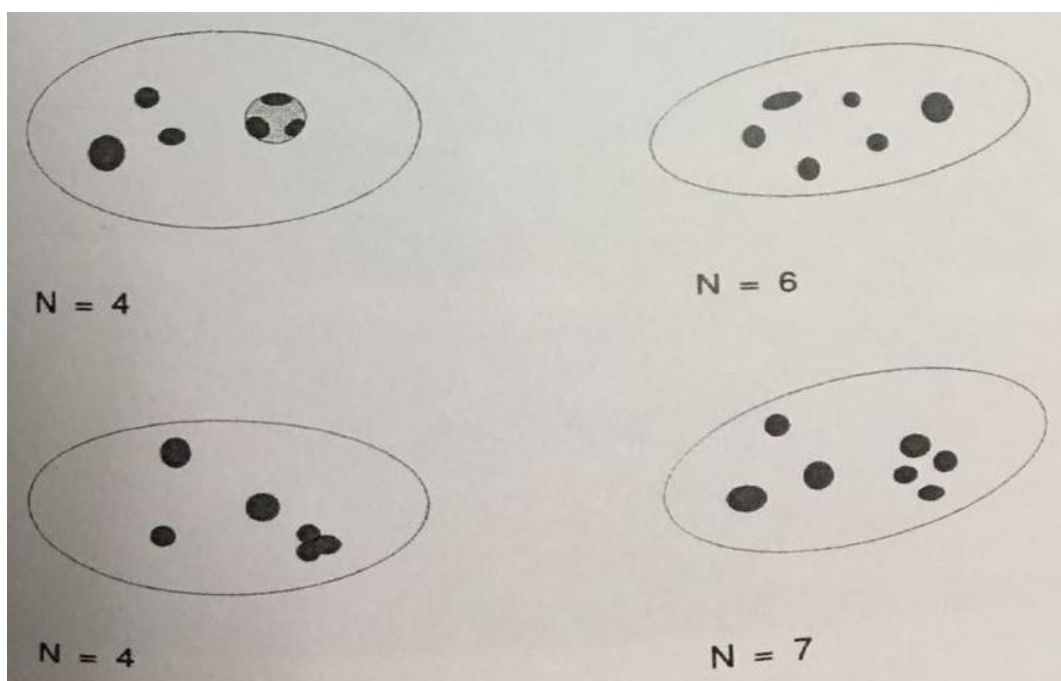


Figure 1: Method of agnor counting

Discussion

This study was carried out to determine the biologic aggressiveness of leukoplakia, oral submucous fibrosis, and oral squamous cell carcinoma, as well as to examine the effectiveness of AgNOR in diagnosing the malignant potential of potentially premalignant illnesses.

In our study the mean AgNOR count of the control group was found to be the lowest among all groups (1.63 ± 0.06), between premalignant group it was more in Leukoplakia group (2.93 ± 0.28), as compared to OSMF (2.53 ± 0.24) and in Oral Squamous Cell Carcinoma it was found to be the highest (3.97 ± 0.42 .) The mean AgNOR count of control group was also compared with other groups, the comparison was found to be highest between control and oral squamous cell carcinoma, and among premalignant group it was more significant in leukoplakia than OSMF. On further analysis, we noticed that Oral submucous fibrosis and leukoplakia show significant difference from each other OSCC showed statistically

higher AgNOR count than leukoplakia OSCC showed statistically higher AgNOR count than OSMF

In a 2007 study, Elangovan T, et al found that OSMF had the lowest AgNOR count when compared to leukoplakia and malignancy. Similarly, the current investigation found that the OSMF group had a higher AgNOR count than normal mucosa and a lower count than leukoplakia. Chattopadhyay et al. (1994) looked at AgNOR counts in the epithelia of oral buccal mucosa, oral leukoplakia, and oral squamous cell carcinoma (SCC), and found a statistically significant difference between normal epithelium and leukoplakia, normal epithelium and squamous cell carcinoma, and leukoplakia and squamous cell carcinoma. Patients with squamous cell carcinoma have the highest AgNOR levels.⁷ Aggressive and nonaggressive lesions, as well as other benign and malignant lesions, yielded the same results. However, AgNOR counts were found to be non-contributory to the identification of dysplastic lesions in one research by Jay G. Ray, Amit Chattopadhyay. Another study by Sethi et al. (2003) looked at the percentage of AgNORs present in the nucleus and concluded that smoking increases proliferative activity in cells of smokers who have no clinical lesion, and that oral cancer has the highest proliferative activity. This data backs up our findings, since oral squamous cell carcinoma patients have higher proliferative activity, as compared to normal healthy people.⁸

The mean value of AgNOR in oral squamous cell carcinoma was much higher in the invasive tumour front than in the central portions of the tumour, according to J Piffko et al. They also observed a link between clinical staging (TNM Staging) and histological grading, which they found to be significant. The dynamism of the cell cycle, as measured by AgNOR concentration, is most likely one of the biological processes that underpin the prognostic importance of OSCC invasive zone histomorphological traits. However, according to Dantas et al, there was no link between TNM classification and malignancy histology scores.

Tumors that are well differentiated are more likely to invade tissues in a pattern with well-defined margins, whereas tumours that are less well differentiated infiltrate organs in small cell groups or even as solitary cells, which may explain the link between prognosis and differentiation.^{9,10} In a study published in 2016, Khiavi et al found that nucleolar NORs in normal and dysplastic epithelium were lighter than nucleolar NORs in SCC. AgNORs in SCC were larger than those found in precancerous lesions, and precancerous lesions AgNORs were larger than those found in normal epithelium. AgNOR dots appeared to be more homogeneous in well-differentiated SCCs and grew more irregular in size and shape as tumour grade increased, according to our findings.

Conclusion

AgNOR can be an effective tool in assessment of OSMF LEUKOPLAKIA AND OSCC

References

1. Newell w. Johnson, prasannajayasekara& a. A. Hemalatha k. Amarasinghe, squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology, oral cancer epidemiology/aetiology
2. Neville, Damm, Allen, Bouquot , Oral and Maxillofacial Pathology. 3rd edition
3. Brad W. Neville *et al* Oral Cancer and Precancerous Lesions, CA Cancer J Clin 2002, 52:195-215
4. Isaãc van der Waal, Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management, Oral Oncology,2008
5. Sandhya Panjeta Gulia *et al.*, Role of Silver Staining Nucleolar Organizer Regions (AgNORs) in Lesions of the Oral Cavity, Journal of Clinical and Diagnostic Research. 2011 October, Vol-5.
6. Vishnudas Dinesh Prabhu *et al*, Quantitative and qualitative evaluation of silver staining Nucleolar Organizer Regions in normal odontogenic epithelium and odontogenic cysts – a comparative studyjournal of Oral Health Research, Volume 1, Issue 4, December 2010
7. Carvalho de M. Thiele *et al.*, Nucleolar Organizer Regions of Oral Epithelial Cells in Crack Cocaine Users, *Iranian Biomedical Journal April 2013*
8. M. H. Bukhari *et al*, Modified method of agnor staining for tissue and interpretation in histopathology, International journal of experimental pathology, 2007, 88, 47–53
9. D. W. Morgan *et al*, salivary gland tumours studied by means of the agnor technique
10. Donia Sadri *et al*, Quantitative analysis of argyrophilic nuclear organizer regions in giant cell lesions of jaws, Journal of Oral Pathology and Medicine, 2010 39: 431–434.