Original Article

Comparison of Argyrophilic Nucleolar Organizer Regions in Leukoplakia and Squamous Cell Carcinoma of the Oral Cavity

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ABSTRACT

Objective: To determine and compare the AgNOR staining parameters in premalignant and malignant lesions of the oral cavity. **Study Design**: Comparative Study **Setting:** Pathology Department, PGMI, Lahore. **Methodology**: Each of total 50 samples of premalignant and malignant lesions was stained with both H/E and AgNOR stain. After histological diagnosis in H/E stain, different AgNOR parameters were studied in each sample. Data was analyzed by using SPSS version 18. Student's t-test and Pearson's Chi Square test were used to compare the quantitative and qualitative AgNORs parameters respectively between both study groups. p-value ≤ 0.05 was taken as significant.

Results: Group 1(Oral Leukoplakia) consisted of 15 males and 10 females with an age ranging from 28-80 years(mean= 52.2 ± 12.5) while group2 (Oral Squamous Cell Carcinoma) consisted of 13 males and 12 females with an age ranging from 38- 82 years (mean= 62.8± 12.4). Comparison of Mean AgNOR count (mAgNOR) and AgNOR Proliferative Index (pAgNOR) between both study groups by using student's t-test showed significantly lower counts in group 1 as compared to group 2. Similarly, Comparison of variation in AgNOR size and AgNOR dispersion between both study groups by using Chi Square test showed a significant difference with p-value ≤ 0.05.

Conclusion: Premalignant lesions of the oral cavity exhibit significantly lower AgNORs counts and comparatively less AgNORs pleomorphism as compared to the malignant lesions of the oral cavity.

Key words: Leukoplakia, Oral Squamous cell carcinoma, Argyrophilic nucloelar organizer regions.

INTRODUCTION

Oral cancer is one of the major global threats to public health in many parts of the world including Pakistan.[1]It ranks the second most common malignancy in Pakistan with identical risk in both genders.[2]

It constitutes up to half of all malignancies in Indo-Pak subcontinent being attributed to the influence of various carcinogens and region-specific epidemiological factors, especially tobacco and betal quid chewing.[3]

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However, its early detection can play a vital role to reduce the incidence as well as morbidity associated with the advanced lesions.

Nearly 96.5% of all oral cancers are Squamous cell carcinoma on histological examination.[4]It is a malignant neoplasm of oral mucosal epithelium which in more than 75% of the cases, is preceded by a premalignant lesion named leukoplakia.[5]The potential for malignant transformation in oral leukoplakia varies from 3-33% over 10 years.[6]

Aberrations of proliferation kinetics of cells in a premalignant and malignant lesion are the hallmark of malignancy.[7]These include increased proliferation rate and increased nucleolar functional activity within the nucleus of epithelial cells undergoing malignant transformation.[8,9]

AgNORs a proliferation marker through study of its different quantitative and qualitative

parameters, gives information on the rapidity of cell proliferation i.e; proliferation rate. And; it is the rate of cellular proliferation rather than the proliferative activity per se that affects the neoplastic progression.[10] Nucleolar size can reliably indicate the rapidity of cell proliferation and greater degree of AgNORs dispersion in nucleoplasm has also been considered as an indicator of malignancy.[9]

The aim of this study was to compare different quantitative (mAgNORs, pAgNORs) and qualitative (Variation in size and dispersion) AgNOR parameters in premalignant and malignant lesions of the oral cavity so as to differentiate these two groups as well as to evaluate the biological aggressiveness of these lesions by using these parameters in routine histopathology.

MATERIAL & METHODS

This comparative study was carried out from July 2011 to December 2011at Pathology department PGMI, Lahore. This included total 50 cases, 25 each of clinically and histologically diagnosed Oral leukoplakia and Oral Squamous cell carcinoma of all age groups and both sexes. Cases of Squamous cell carcinoma with post operative radiotherapy and those presented as metastatic lesions in the oral cavity were excluded from the study.

After gross examination of formalin-fixed samples, these were processed in automatic processor, blocked and cut into 4-5µm thick sections. Sections were taken on albuminized slides[11] and further processed for H/E and AgNORs staining.[12,13]After making histological diagnosis in H/E stain various AgNOR parameters were studied in each case. AgNORs were visible as black dots within the nucleoli of cells. mAgNORs was determined by counting number of AgNORs dots in 100 cells of the each sample while pAgNORs was determined by counting nuclei among 100 cells having five or more AgNORs granules. [14] Variation in AgNORs size and dispersion were also recorded and graded according to Khan et al [15] as follows: **Variation in size**: Grade 0= more or less uniform in size, Grade 1+= two different sizes, Grade 2 += more than two different sizes, Grade 3+= all grades and sizes including too minute to be counted. **AgNORs dispersion**: Depending upon the dispersion area of AgNORs granules, cases were graded as: Grade 0 = limited to nucleoli, Grade 1+= occasional dispersion outside the nucleoli, Grade 2+= moderate dispersion outside the nucleoli, Grade 3+= widely dispersed throughout the nucleus

Statistical Analysis

For statistical analysis, data was analyzed by SPSS version 18. mAgNORs and pAgNORs were taken as mean± SD while variation in AgNORs size and AgNORs dispersion were taken as proportions.

To compare the AgNORs counts between premalignant and malignant lesions, student's t-test was used while Pearson's Chi square and Fisher Exact test were used to compare the variation in AgNORs size and AgNORs dispersion. p-value $\leq$ 0.05 was taken as significant.

RESULTS

Group 1 (Oral Leukoplakia) consisted of 15 males and 10 females with an age ranging from 28-80 years (mean= 52.2 ± 12.5) while group 2 (OSCC) consisted of 13 males and 12 females with an age ranging from 38- 82 years (mean= 62.8± 12.4). Duration of symptoms was 2-12 months and 3-17 months for the first and second study group respectively with maximum number of cases of both groups presented with 6-11 months duration. A statistically significant difference was observed on comparing the duration of symptoms between both study groups (p-value=
0.05).

The most common site involved by majority of cases in both study groups was buccal mucosa. After AgNOR staining, both study groups showed different expression within the nuclei of cells as shown in Figures I and II. mAgNORs and pAgNORs values of both study groups were compared by using Student's t-test and a significant difference was observed (Table I). Variation in AgNORs size and dispersion pattern between both study groups were compared by using Pearson's Chi Square test and Fisher exact test and statistically significant results were observed (Table-II).

Table- I: Comparison of mAgNORs and pAgNORs between Oral Leukoplakia and Oral Squamous cell carcinoma

Serial No.	Name of Lesion	No. of Cases	mAgNOR Mean ± SD	pAgNOR Mean ± SD
1	Oral Leukoplakia	25	3.62 ± 1.05	39.64 ± 17.07
2	Oral Squamous Cell Carcinoma	25	5.81 ± 2.48	75.68 ± 17.36

p-value= 0.001

Table-II: Comparison of variation in AgNORs size and dispersion between oral leukoplakia and oral Squamous cell carcinoma

Name of Lesion	Variation in AgNOR size		Total	AgNOR dispersion		Total
	0-1+	2 ± 3+		0-1+	2 ± 3+	
Oral Leukoplakia	17	8	25	15	10	25
Oral Squamous Cell Carcinoma	6	19	25	2	23	25
Total	23	27	50	17	33	50
p-value= 0.002				p-value= 0.001		

DISCUSSION

Oral cancer being more prevalent in developing countries, the highest incidence is observed in the Indo-Pak subcontinent accounting for 1/3 of the world burden.[16]The five year survival rate for the OSCC has not improved significantly over the past several decades and has remained at about 50-55% for a long time.[17]

The contributing factors for the high incidence include delay in diagnosis, high mortality rate as most of the cases were in advanced stages at the time of diagnosis and increased morbidity associated with aggressive treatment required for these fulminent lesions.[18,19] Thus early detection of precancerous and cancerous lesions and appropriate timely treatment are important to improve the survival index of this devastating disease.

In present study, both study groups showed a male predominance. In comparison to this, Bhurgri et al observed equal incidence of OSCC in both genders.[2] Mean duration of symptoms was 9 months for cases of OSCC and 7.3 months for cases of oral leukoplakia. On comparison of mean duration of symptoms between both study groups, a statistically significant difference was observed with p-value =0.05. This result may be taken as a positive contributing factor supporting the oral cancer control programmes with an insight view that Pakistani people have become enough conscious about their oral health and tend to consult with dental surgon without waiting for the conversion of asymptomatic white patch into a symptomatic malignant lesion.

Evaluation of the biological behavior, in terms of proliferation kinetics at cellular level, of a clinically suspicious premalignant lesion and a malignant lesion can be helping to assess the stages of tumor progression.[20] With this in mind, AgNOR was used in the present study, its various parameters were determined and compared in premalignant and malignant lesions of the oral cavity.

Nucleolar organizer regions (NORs) are the focal aggregates of intra-nuclear non-histone proteins associated with potential sites of ribosomal DNA transcription. These proteins are visualized by virtue of their argyrophilic nature, as brown or black dots in nuclei of the cells, hence the acronym AgNOR (argyrophilic nucleolar organizer regions).[21]AgNORs are considered to reflect the biosynthetic and nucleolar functional activity of a cell and serve as an indicator of the rapidity of cell cycle.[8]

In present study, on comparing the mean AgNORs of both study groups, a statistically significant difference was observed reflecting different growth rates of these lesions. However, Elangovan et al and Kulkarni et al conducted similar studies on same and some other oral epithelial lesions with the conclusion that AgNORs quantity is strictly proportional to the proliferative activity of the epithelium but it is not a reliable tool to assess the premalignant status of any lesion.[20,21]

Based on the results of the present study, where some of the leukoplakic lesions were having

mAgNORs count equal to or more than well differentiated OSCC, mAgNOR can be helpful to asses the biological aggressiveness of a clinically suspicious premalignant lesion and early cancer.

Evaluation of pAgNORs as suggested by Xie et al is more easier and less cumbersome to perform as compare to conventional method used for mAgNORs.[23] In present study, premalignant group has shown significantly lower pAgNORs as compared to malignant group, indicating the lesser number of rapidly proliferating cells in first study group.

Progressive enlargement of proliferative compartments may be a sign of the development of a malignancy.[24] In present study, a few of the premalignant lesions were having pAgNORs equal to the well-differentiated OSCC, pointing the fact that a premalignant lesion can have a biological aggressiveness comparable to a malignant group thus demanding an aggressive treatment and strict follow ups.

Qualitative analysis of AgNORs based on their size, shape and dispersion pattern seem to give more information than quantitative analysis about the malignant status of a lesion. Elangovan and colleagues showed that it is the qualitative characteristics of AgNORs that act as a marker of premalignant and malignant change and also help in distinguishing them from normal. They found that occurrence of AgNORs pleomorphism is a true reflection of the underlying tissue changes in a premalignant and malignant oral lesion.[20]

In the present study, variation in AgNORs size was predominantly of grade 0 to 1+ in premalignant group while of grade 2+ to 3+ in majority of malignant group. AgNORs size has been shown to indicate the transcriptional activity of nucleolus and a clear relationship exists between the cell proliferation rate and the size of nucleoli.[9] Based on the results of present study, malignant lesions have more rapid proliferative activity as demanded by progressively enlarging cell population as compared to premalignant group.

Dispersion of AgNORs in nuclei of premalignant group was of grade 0 to 1+ while of grade 2+ to 3+ in malignant group. Greater degree of AgNORs dispersion in nucleoplasm is considered as an indicator of malignancy as more dispersion is observed in carcinomatous lesions as compared to normal and premalignant lesions.[25]

CONCLUSION

In this study, a proliferation marker AgNOR was used on premalignant and malignant lesions of the oral cavity. It showed that premalignant lesions exhibit significantly lower values of mAgNOR and pAgNOR as compared to the malignant lesions of the oral cavity. Moreover, premalignant lesions show significantly less AgNORs pleomorphism in the form of variation in size and dispersion pattern as compared to the malignant lesions of the oral cavity.

Based on the results of this study, proliferation marker AgNOR can be used as an adjunct to routine histopathology to evaluate the neoplastic cellular alterations in a small sized biopsy of the premalignant lesions which may remain unexplored otherwise. This study has been performed on small sample size. Further studies on large sample size are required to better understand and associate the role of AgNORs in controlling the progression of this devastating disease of oral cancer in Pakistan.

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