Pleomorphism of argyrophilic nucleolar organizer regions in oral submucous fibrosis and oral squamous cell carcinoma

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Abstract

Background: Argyrophilic nucleolar organizer regions (AgNORs) is demonstrated to be useful in diagnostic pathology, mainly to distinguish benign lesions from their malignant counterparts. We aimed to correlate AgNORs pleomorphism with the severity of lesion in oral submucous fibrosis (OSMF) and oral squamous cell carcinoma (OSCC) using a retrospective study on 45 archival tissues. **Materials and Methods:** Silver nitrate staining was performed on archival tissues consisting of 20 OSMF and 20 OSCC. Five biopsies from normal oral mucosa acted as a control. One hundred cells per slide were observed for AgNORs dots, which were classified as typical (spherical) and atypical (large, kidney-shaped and clustered). **Results:** A positive and significant correlation was found between increased atypical shapes and increasing grades of OSMF and OSCC. **Conclusions:** AgNORs pleomorphism can be a reliable criterion to assess disease severity and progression in OSMF and OSCC.

Key words: Malignant, nuclear organizer regions, pleomorphism of argyrophilic nucleolar organizer regions, premalignant

INTRODUCTION

The argyrophilic staining of nucleolar organizer regions (AgNORs) is demonstrated to be useful in diagnostic pathology, mainly to distinguish benign lesions from their malignant counterparts. It detects a class of acidic proteins associated with ribosomal DNA in some chromosomal segments that codify ribosomal RNA. Their size and number reflect the nucleolar and cell proliferative activity of tumors.[1] Previous studies have reported that epithelial hyperplasia showed a definite increase in the AgNOR count, intermediate between that in the normal mucosa and oral squamous cell carcinoma (OSCC). Suggesting that AgNOR quantity is strictly proportional to the proliferative activity of the epithelium and is not a reliable tool to assess the premalignant/malignant status of any lesion in the absence of epithelial proliferation. Contrarily, various studies have shown that a qualitative assessment of AgNORs based on their shape, size, and distribution pattern is a more reliable parameter to determine the premalignant/malignant status of lesions.[2-4] Hence, we analyzed the pleomorphism of AgNORs in various histological grades of oral submucous fibrosis (OSMF) and OSCC to determine if the AgNOR qualitative analysis could be used as a marker for disease severity and progression of the lesion.

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MATERIALS AND METHODS

The study consisted of 40 archival tissues, 20 each of OSMF and OSCC. Five biopsies from the normal oral mucosa (NM) acted as a control.

Oral submucous fibrosis tissues were histologically graded as early, moderately advanced (Mod-A) and advanced (Adv). Out of 20 OSMF tissues, 6 were of early OSMF and 7 were of Mod-A and Adv OSMF each. OSCC tissues were graded as early invasive (EI), well differentiated (Well-D), moderately differentiated (Mod-D) and poorly differentiated (Poor-D). EI OSCC were cases that were clinically diagnosed as OSMF but histologically showed severe epithelial dysplasia and invasion into the connective tissue. Five tissues each of all four grades of OSCC were taken. Silver staining was performed according to the method proposed by Ploton *et al*.[5] One hundred cells per slide were studied for AgNORs dots using an ×100 objective. These dots were classified as typical (spherical) and atypical (large, kidney-shaped and clustered) shapes as quantified by Cortés-Gutiérrez *et al*.[6] Mean value of AgNORs pleomorphism was then calculated for each group.

Statistical analysis was performed using Kruskal–Wallis test to assess the differences between median values for AgNORs pleomorphism. The difference between the groups was measured using ANOVA test.

RESULTS

The mean of atypical AgNORs or pleomorphism increased progressively with the histological grades of OSMF [Graph 1] and OSCC [Graph 2]. The mean of atypical dots increased from 1 in early OSMF to 9.7 in advanced OSMF and 16 in EI OSCC to 92.4 in Poor-D OSCC. On

comparing Mod-A and Adv OSMF a significant $P = 0.001$ was obtained [Table 1]. The intergroup comparison of different grades of OSCC showed a significant $P = 0.009$ except between EI and Well-D OSCC, where the P value was 0.016 [Table 2].

DISCUSSION

Oral submucous fibrosis is the most common lesion representing the group of conditions/lesions categorized as potentially malignant. OSCC is the most common malignancy of oral cavity. The malignant transformation rate of OSMF is 3-19%.[7]

Nuclear organizer regions are loops of DNA that encode ribosomal RNA and are considered important in the

### Table 1: *P* values of intergroup comparison in OSMF

OSMF		*P*
Early OSMF	Mod-A OSMF	0.78
	Adv OSMF	0.12
Mod-Adv OSMF	Adv OSMF	0.001*

*Significant $P < 0.05$ levels, OSMF: Oral submucous fibrosis, Mod-A: Moderately advanced, Adv: Advanced

Table 2: *P* values of intergroup comparison in OSCC

OSCC		*P*
EI OSCC	Well-D OSCC	0.016*
	Mod-D OSCC	0.009*
	Poor-D OSCC	0.009*
Well-D OSCC	Mod-D OSCC	0.009*
	Poor-D OSCC	0.009*
Mod-D OSCC	Poor-D OSCC	0.009*

*Significant $P < 0.05$ levels, OSCC: Oral squamous cell carcinoma, EI: Early invasive, Well-D: Well differentiated, Mod-D: Moderately differentiated, Poor-D: Poorly differentiated
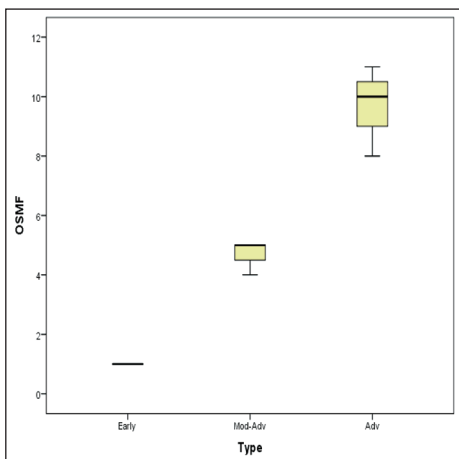
Graph 1: Mean pleomorphism of argyrophilic nucleolar organizer regionss in different grades of oral submucous fibrosis
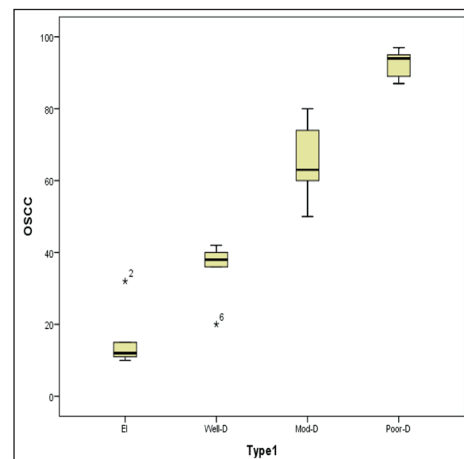
Graph 2: Mean pleomorphism of argyrophilic nucleolar organizer regions in different grades of oral squamous cell carcinoma

synthesis of protein. They are located on the short arms of acrocentric chromosomes-13, 14, 15, 21 and 22. It has been suggested that the number of AgNORs in a nucleus may reflect the proliferation activity of the cells and degree of malignant transformation of certain tissues. The silver staining technique (AgNOR) neither identifies rRNA nor rDNA but the acidic proteins associated with these sites of rRNA transcription. AgNOR dots are seen as dark brown to black dots inside a brownish nucleus within a yellow cytoplasm.[1,5]

Argyrophilic of nucleolar organizer regions have recently been studied in malignant lymphoma, in nevocellular nevi and melanomas, in the cutaneous tumor, in the cervical epithelium with and without intraepithelial neoplasia and with human papillomavirus infection. These studies demonstrated that the number and more importantly the size and shape of AgNORs might reproduce the histologic grading in malignant tumors and are useful in discriminating between benign and malignant tumors.[3,4,6,8]

In oral pathology, AgNORs have been used in differentiating OSCC from benign and reactive lesions, and also in detecting incipient cellular alterations. AgNORs have been shown to be useful as a marker of tumor progression. It also helps to predict the response of tumor to treatment and to detect residual viable tumor.

Pleomorphism is a term used in histology and cytopathology to describe variability in the size and shape of cell or nucleus. Parameters such as cellular and nuclear areas, nuclear-cytoplasmic, and nucleolar-nuclear ratios have been utilized to characterize dysplasia. A correlation between nuclear size and the type of carcinoma as well as its prognosis in has been reported. Nuclear polymorphism in hepatocytes is more sensitive than nuclear size as a parameter for evaluation of dysplastic changes and changed significantly even in those cases when nuclear size was not changed relative to controls as studied by Zusman *et al.*[9]

Similarly AgNORs quality, that actually are the proteins associated with NOR in rRNA transcription sites, reflects the cellular differentiation degree. Studing the pleomorphism or quality of AgNORs that is its size, shape and distribution pattern allows determining differentiation indexes of the transformed cells. It can be safely concluded that AgNOR pleomorphism is the product of cellular alterations that are clearly related to the progression of the lesion to malignancy.

The result of the present study showed progressive and significant increase in mean atypical AgNORs among the different groups of OSMF and OSCC when compared to NM [Graph 3].

On comparing the normal mucosa with the lesional tissues, there were noticeable differences in the appearance of the individual dots. AgNOR in NM appeared uniformly as spherical medium size black dots located within the nucleoli [Figure 1]. Not much difference was noted in AgNORs shape between NM and early OSMF. In Mod-A and Adv OSMF, AgNORs predominantly varied from small dots present in the nucleolus [Figure 2] to fine dots present
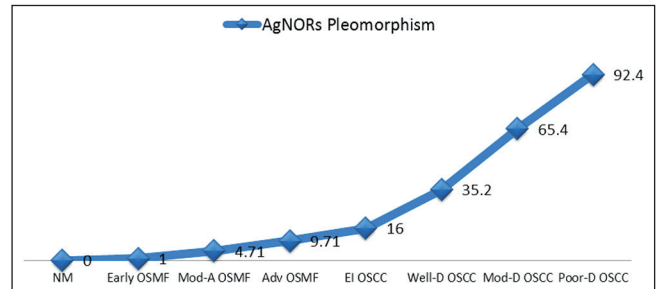
Graph 3: Increase in mean atypical argyrophilic nucleolar organizer regions among the different groups of oral submucous fibrosis and oral squamous cell carcinoma when compared to normal oral mucosa (NM)
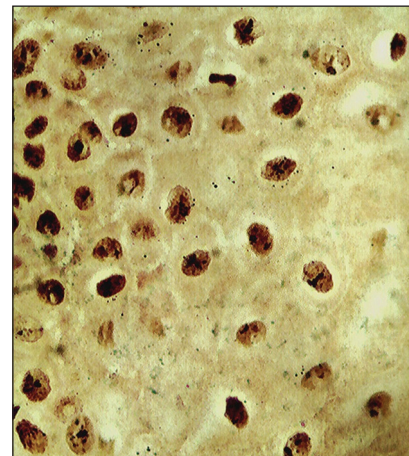
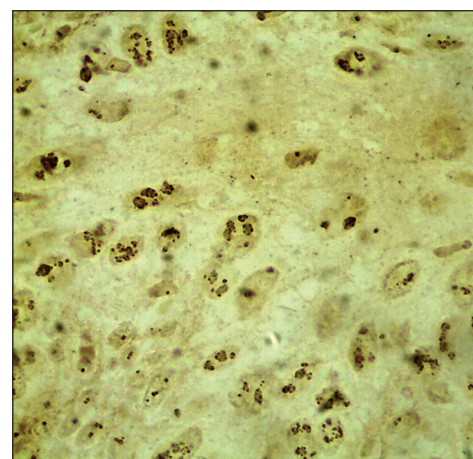

Figure 1: Single or few spherical dots in nucleolus

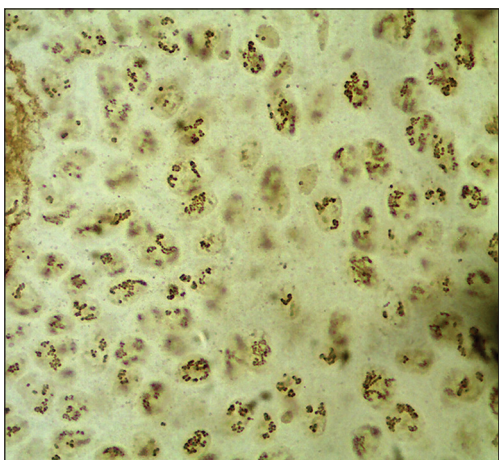
Figure 2: Small dots in the nucleolus

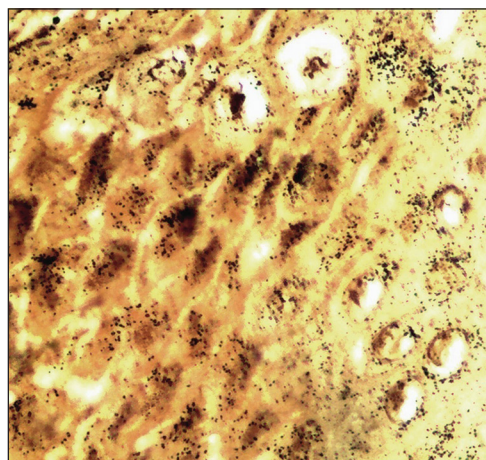**Figure 3:** Numerous fine dots in nucleoplasm

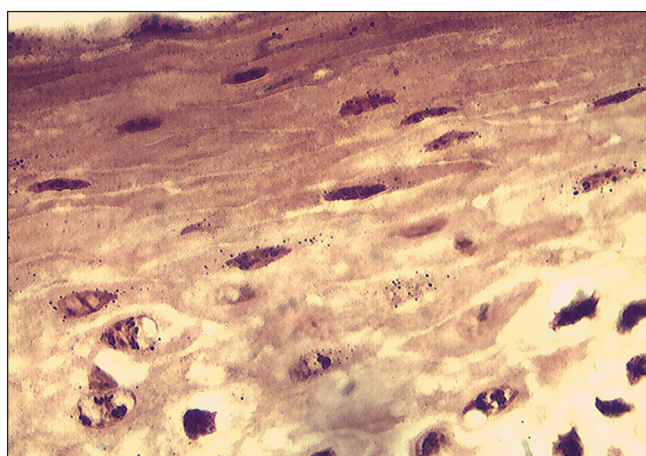**Figure 4:** Cluster of dots
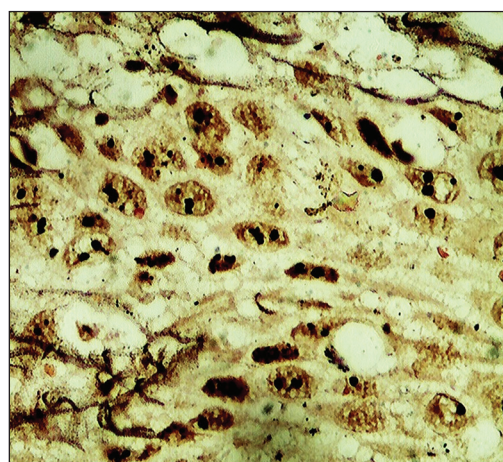

Figure 5: Irregular dots

Figure 6: Large kidney shaped dots

throughout the nucleoplasm giving granular appearance [Figure 3]. In some cases, dots in OSMF appeared in cluster [Figure 4] and did not have uniform round shape. In Well-D OSCC, the dots were predominantly small and had irregular and bizarre shapes [Figure 5]. In Mod-D and Poor-D OSCC irregular shapes increased and few of them were slightly larger in size and kidney shaped [Figure 6]. Our result was in accordance with studies carried out by Alarcón-Romero *et al.*[3] on cervical lesions and by Elangovan T *et al.*[2] on oral lesions.

It is evident from this study that pleomorphism of AgNORs increases with the severity of the lesion. Not many studies have been carried out in oral lesions and so this study may be considered preliminary. Larger sample size is required to confirm the association of AgNORs quality to malignancy. Furthermore, standardization and grading of pleomorphism of AgNORs needs to be determined by further studies in order to fully explore the tumor marker potential of AgNOR dots. We conclude that AgNORs pleomorphism can be a reliable criterion to assess disease severity and progression in OSMF and OSCC. It appears that the occurrence of AgNOR pleomorphism is a true reflection of the underlying tissue changes and hence can be useful in tumor detection and prognosis.

Conflict of interest

There are no conflicts of interest.

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