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Original Article

Quantitative analysis of Argyrophilic Nucleolar organizer regions in odontogenic cysts and tumor – A comparative study

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ABSTRACT

Background: The nucleolar organizer region (NOR) is by definition part of a chromosome, and nucleolus is a structure containing this chromosomal part and in addition the material which accumulate around the NOR, mostly rRNAs and their precursors as well as specific ribosomal proteins. Argyrophilic Nucleolar organizing region (AgNOR) are silver binding NORs often used to study cell proliferation in various types of tumors.

Aim: Quantitative assessment of Argyrophilic Nucleolar organizing region count and its comparison among dentigerous cyst, keratocystic odontogenic tumor and ameloblastoma.

Material and method: Forty-five histologically confirmed cases, 15 cases each of keratocystic odontogenic tumor, dentigerous cysts and ameloblastomas were examined for Argyrophilic Nucleolar organizing region. The sections were obtained and Argyrophilic Nucleolar organizer regions staining was done for comparing the proliferative capacity among these lesions.

Result: Post hoc analysis for inter-group comparison and one way ANOVA were done in all three groups in this study. $P < 0.001$ was considered significant. The results of AgNOR counts were higher in KCOTs as compared to ameloblastoma and least in dentigerous cysts. The mean AgNOR counts between the study groups were compared using one way ANOVA test and the differences were found to be significant ($P < 0.001$).

Conclusion: AgNOR counts were significantly higher in KCOT and ameloblastoma as compared to dentigerous cyst suggesting that these lesions have a higher proliferative capacity than dentigerous cyst. The finding of a significantly higher AgNOR counts in KCOT as compared to ameloblastoma represent a difference in proliferative activity and greater growth potential between these two lesions.

1. Introduction

Odontogenic cysts are more commonly encountered in dental practice, in contrast to odontogenic tumors.[1] Nucleolar organizing region (NORs) are loops of DNA containing ribosomal RNA (r-RNA) genes. Silver-stained NORs (AgNORs) are argyrophilic proteins of the NORs. NORs are argyrophilic due to their association with acidic proteins (C23, B23 and possibly RNA Polymerase I), which contain abundant sulfhydryl and carboxyl groups that precipitate the silver ions. They transcribe for ribosomal RNA which are located on the short arm of chromosomes 13, 14, 15, 21and 22.[2] Studies suggest usefulness of AgNOR count as maker of proliferation and may be helpful in diagnosis and prognosis of various neoplastic lesions, including oral squamous cell carcinoma and odontogenic lesions.[3,4] This study was conducted to access the role of Argyrophilic Nucleolar organizing region in the biological behavior of cysts and tumors.

The rationale of the current study was quantitative assessment of Argyrophilic Nucleolar organizing region count and its comparison among dentigerous cyst, keratocystic odontogenic tumor and ameloblastoma.

2. Material and methods

The present retrospective cross sectional study was carried out on biopsy tissues obtained from the archives of Department of Oral

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and Maxillofacial Pathology, of our college. The study sample included 45 cases of odontogenic cysts and tumors, 15 cases each of KCOTs, dentigerous cysts and ameloblastomas. The diagnoses were reviewed using routine hematoxylin and eosin stained sections. The inclusion criteria were all sub types of ameloblastomas, dentigerous cyst and KCOT were taken from both male and female patients of age between 16 and 60 years without any inflammation. Exclusion criteria were patients having any other oral lesions along with cysts and tumors were not included in the study sample. Patients having systemic diseases, pregnancy and tobacco habits were also not included in the study samples.

2.1. Equipment and armamentarium

Microscopic fields, representative of the lesion, were identified and photographed using Olympus BX51 light microscope, Live View Digital SLR Camera E-330. The photographs were analyzed using Image Pro Express 6.0, for windows (Media Cybernetics), USA.

AgNOR staining solutions: Two solutions were taken, solution A and B.

Solution A		
Silver nitrate	–	50 g
De-ionized Water	–	100 ml
Solution B		
Gelatin Powder	–	2 g
Formic acid	–	1 ml
De-ionized water	–	100 ml

Each time the final working solution was freshly prepared by mixing one volume of solution A and two volume of solution B. The staining procedure was standardized after a pilot study was carried out in the department, using internal controls such as the development of silver dots on lymphocytes and oral squamous cell carcinoma (OSCC) tissue was taken as a positive control.

2.2. Staining procedure

The slides were subjected to AgNOR (Fisher Scientific, New Hampire) staining according to the method of Ploton et al.[5] and counting was done as recommended by Crocker et al.[6] The slides were progressively rehydrated through descending grades of alcohol and dewaxed in xylene. Finally the slides were washed with de-ionized water. Excess water was shaken off from the slides and the freshly prepared working solution was poured over the slides. These were then placed in the incubator at 37 °C for 30 min. After staining the slides were washed in de-ionized water, followed by sequential dehydration in ascending grades of alcohol. The slides were cleaned in xylene and mounted in synthetic medium (DPX).

2.3. Counting

All sections were examined under 1000× magnifications in oil immersion using light microscope and AgNOR dots were counted in 100 randomly selected cells using point counting tool from the basal and parabasal layers.

AgNORs were seen as individually discernible and separate dark brown to black dots or "blebs" of varying size, observed in a light brown stained nucleus within a pale yellow cytoplasm.

2.4. Statistics

Post hoc analysis for inter-group comparison and one way ANOVA were done to compare among three groups.

$P < 0.001$ was considered significant.

3. Results

In dentigerous cyst, the AgNOR count ranged from 1.0 to 1.25 (mean 1.11) (Fig. 1). AgNOR dots/nuclei. In KCOT, AgNOR count ranged from 1.32 to 2.92 (mean 2.39) (Fig. 2). AgNOR dots/nuclei. In ameloblastoma AgNOR count ranged from 1.43 to 2.84 (mean 1.86) AgNOR dots/nuclei (Fig. 3) (Table 1 and Graph 1). The mean

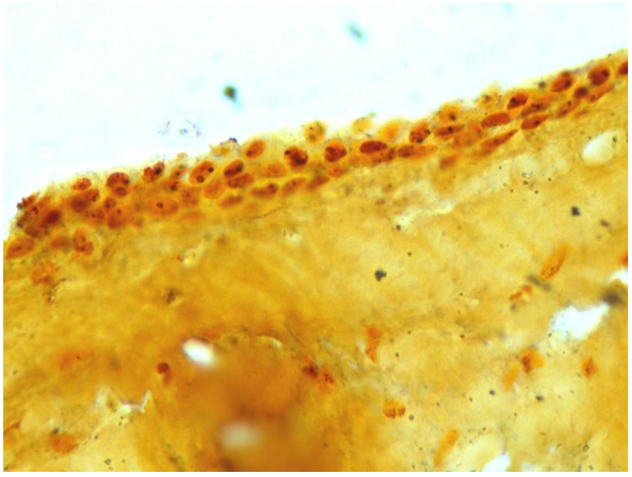

Fig. 1. Photomicrograph showing AgNOR expression in dentigerous cysts (1000×).

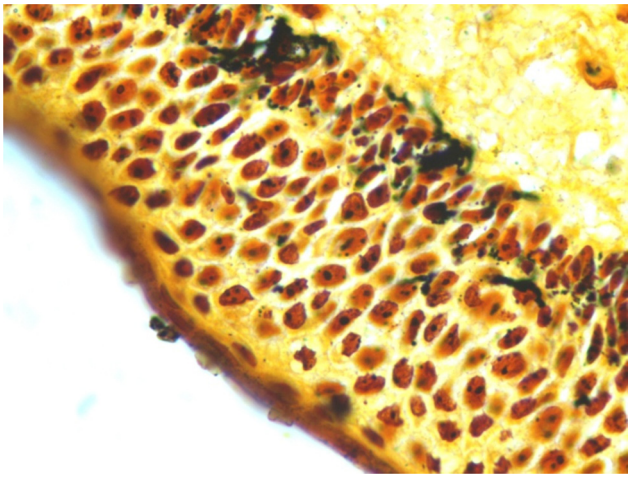

Fig. 2. Photomicrograph showing AgNOR expression in KCOT (1000×).

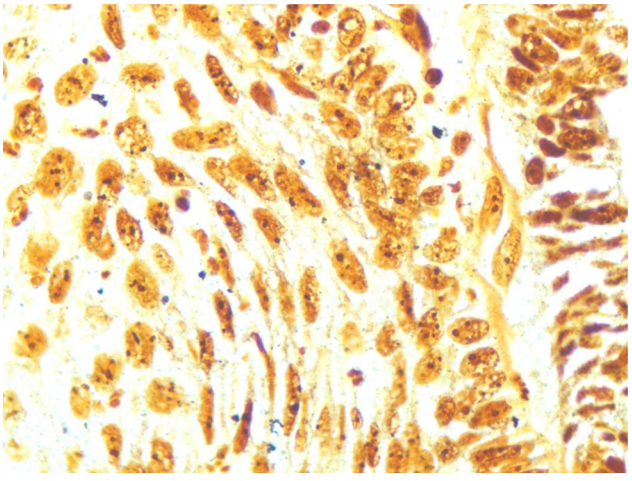

Fig. 3. Photomicrograph showing AgNOR expression in ameloblastoma (1000×).

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