

Quantitative assessment of silver-stained nucleolar organizer region in odontogenic cysts to correlate the growth and malignant potentiality

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Abstract

Context: The most common and important odontogenic cyst involving jaws is the odontogenic keratocyst (OKC) or primordial cyst, the dentigerous cyst and the radicular cyst. These cysts all though do not show similar behavior, they all have the potentiality to recur. Silver nitrate staining of the nucleolar organizer regions (AgNORs) of the benign and malignant lesions is becoming very useful as a diagnostic indicator. Thus, the aim of this study is to assess the diagnostic potential of AgNORs in the cystic epithelium of common odontogenic cysts.

Materials and Methods: Archived specimens of odontogenic cysts were stained with hematoxylin and eosin stain and AgNOR stain.

Results: The comparative evaluation of the AgNOR counts was done among the three varieties of odontogenic cysts, i.e., radicular cysts, dentigerous cysts and OKC and were observed that the mean for OKC was significantly higher than that of radicular cyst.

Conclusion: Therefore, AgNor could be used as an efficient tool for comparative evaluation of microscopic features such as epithelial thickness, surface keratinization and mural proliferation in dentigerous cyst to that of the AgNOR count.

Keywords: Ameloblastoma, assessment, odontogenic cyst, silver nitrate staining of the nucleolar organizer regions, squamous cell carcinoma

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INTRODUCTION

Cysts of the jaws and mouth have been recognized as clinical entities for a long time. The most common and important odontogenic cyst involving jaws is the odontogenic keratocyst (OKC) or primordial cyst, the dentigerous cyst and the radicular cyst. All these odontogenic cysts

do not show similar clinicopathological and radiological behavior though all of them have the potentiality to recur following surgical treatment. Since some cysts are more aggressive and cause severe destruction of the surrounding structures, while the others are not so aggressive in nature, the treatment modality also varies accordingly. The most

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common neoplasm originating in cyst linings is benign odontogenic tumors. Reports concerning the development of carcinoma in cysts of the jaws have suggested that about half of them originated in cysts with keratinized lining.^[1,2] The epithelial lining of OKCs appears to express their intrinsic growth potential which is absent in other types of odontogenic cysts. Its high recurrence rate, close relation to the basal cell nevus syndrome and putative increased risk of neoplastic change in comparison to other odontogenic cysts place it in a spectrum of odontogenic lesion.^[3,4] In this respect, silver nitrate staining of the nucleolar organizer regions (AgNORs) of the benign and malignant lesions is becoming very useful as a diagnostic indicator. Cellular proliferation is so active that nucleolar dissociation is present in many cells and the AgNORs are consequently dispersed throughout the nucleus. In malignancy, AgNORs become dispersed so that the numbers seen in histological sections represent a numerical index of dispersion rather than an absolute count.^[5] Increased expression of NOR sites would, therefore, be expected in actively proliferating cells and they are visualized in those cells which are functionally active in the preceding interphase.^[6] Thus, the endeavor of this study is to carry out a quantitative assessment of AgNORs in the cystic epithelium of the different jaw cysts.

Aims and objective

The aim and objective of the study were to determine the AgNOR count in radicular cyst, dentigerous cyst and OKC.

To correlate and corroborate histological findings and AgNOR counts in the respective cystic lesions, with a view to establish a diagnostic parameter, if any.

MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology, Dr. R. Ahmed Dental College and Hospital, Calcutta.

The sections used for routine hematoxylin and eosin (H and E) and AgNOR staining were obtained from the archived specimen of patients having odontogenic cysts, attending the Oral Pathology and Microbiology Department. The patients were selected after thorough clinical, radiological, hematological and general checkup followed by biopsy from the cystic lesion with prior consent. The sections were stained by H and E technique for the routine histological evaluation and confirmation of diagnosis, the other sets for localization and quantification of AgNORs in the lining epithelium of cysts by the improved one-step method of Ploton *et al.*^[7]

Staining technique

The sections were dewaxed in xylene for 5 min, rehydrated descending grades of alcohol, washed in running “deionized” water for 8–10 min. Solution A (50% silver nitrate solution) and Solution B (gelatine, pure formic acid and deionized water) were mixed in proportion of two parts to one part, respectively, to make the working solution. Slides were stained in small batches of ten slides and kept in dark for 45 min at room temperature (25–30°C). After removal from staining bath, the slides were thoroughly washed in running “deionized” water for 5-1 min. Dehydration of stained slides was done by passing through ascending grades of ethanol, cleared in xylene and mounted in dibutyl phthalate in xylene.

The counting protocol used in this study was according to the modified method of Ploton *et al.*^[7] and used by Coleman *et al.*^[8]

RESULTS AND OBSERVATIONS

The present study comprises 41 cases of different odontogenic cysts [Table 1]. The light microscopic features of all the 19 radicular cysts were carefully evaluated, and it was observed that the thickness of the lining epithelium varied from 2 to 20 cell layers [Table 2 and Figure 1]. Among the 19 silver-stained sections obtained from radicular cysts, a minimum AgNOR count of 1.47 and a maximum count of 2.81 were found, the mean value being 2.11. Where surface keratinization was present, the mean AgNOR counts were 2.38, seven cases showing arcading pattern in the lining epithelium where the mean AgNOR

Table 1: Distribution of odontogenic cysts

Total cases: 41	
Type of odontogenic cysts	Number of cases (%)
Radicular cyst	19 (47)
Dentigerous cyst	7 (17)
Odontogenic keratocyst	15 (36)

Table 2: Comparative evaluation of silver nitrate staining of the nucleolar organizer regions with the histological findings of lining epithelium in seven dentigerous cysts

Histological features of lining epithelium	Number of cases (%)
Thickness of lining epithelium (layers)	
2-4	10 (66)
5-8	5 (34)
Keratinization	
Present	15 (100)
Absent	-
Palisaded basal layer	
Present	15 (100)
Absent	-
Microcysts	
Present	5 (33)
Absent	10 (67)

counts were 2.18 [Table 3 and Figure 2]. Evaluation of seven silver-stained sections obtained from dentigerous cysts; a minimum AgNOR count of 1.49 and a maximum count of 3.82 were observed (mean 2.64). In all the 15 cases of OKC which were stained for the purpose of counting AgNOR, it was found that in 100 lining epithelial cells, the silver positive dots varied from a minimum count of 1.71 to a maximum count of 4.33 and the mean value was 2.98 [Table 4]. It was observed that ten cases having the thickness of lining epithelium within 3–8 cell layer; the mean AgNOR counts were 2.62, the range varied from 1.71 to 3.63 (M/P24). Five cases of OKC showed the epithelial thickness of nine cell layers or more. The mean AgNOR counts were 3.68; the range varied from 3.18 to 4.33. All the 15 cases showed the presence of surface keratinization; the mean AgNOR counts were 2.98, the range varied from 1.71 to 4.33 [Table 5]. The comparative evaluation of the AgNOR counts was done among the three varieties of odontogenic cysts, i.e., radicular cysts, dentigerous cysts and OKC and were observed that the mean for OKC significantly higher than that of radicular cyst ($t = 5.32, df = 32, P < 0.001$) [Table 6]. Mean + OKC is also significantly higher than that of dentigerous ($t = 2.18, df = 24, P < 0.05$). However, the two mean values of

dentigerous and OKC groups do not differ significantly with one another ($t = 1.98, df = 20, P < 0.05$).

DISCUSSION

The qualitative and quantitative changes in AgNOR may provide useful information about nucleolar activity in hyperplastic and neoplastic conditions.^[5] Thus, variations in AgNOR size and number might be dependent 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.^[7] In the year 1993, Allison and Spencer^[4] conducted an interesting study to evaluate the NORs in odontogenic cysts have reported that the mean AgNOR counts in radicular cysts in his series were 2.02. Another similar study was conducted by Coleman *et al.*,^[8] and they observed a mean AgNOR count was 1.97. In the present study, a careful evaluation of AgNOR count in radicular cyst ($n = 19$) revealed that the mean AgNOR count was 2.11. A further in-depth, comparative evaluation of the AgNOR counts with respect to a specific microscopic feature of the lining epithelium in radicular cysts revealed a substantial increase in the mean AgNOR counts in the lesions having increased (11–20 layers) epithelial thickness was 2.36. When the cystic lining showed the presence of arcading arrangement, the mean count was 2.18, and in the presence of mural proliferation, the mean count

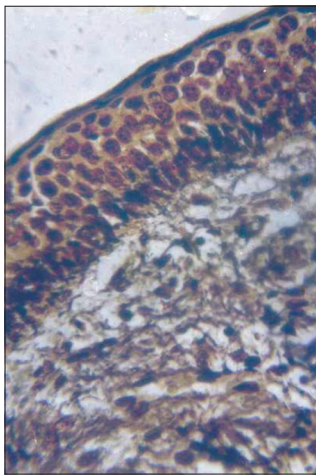


Figure 1: Silver nitrate staining of the nucleolar organizer regions staining in odontogenic keratocyst

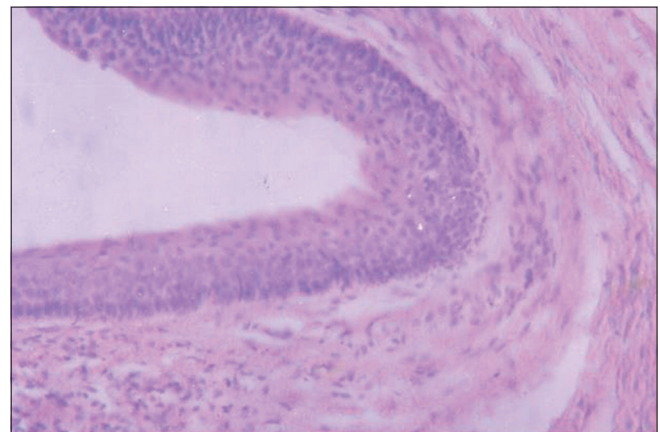


Figure 2: Hematoxylin and eosin staining of odontogenic keratocyst

Table 3: Light microscopic features of lining epithelium in nineteen radicular cysts

Histological features of cases	Number of cases	Range of AgNOR	Average AgNOR count	Total average AgNOR count
Thickness of lining epithelium (layers)				
2-4	4	1.49-3.74	2.15	2.64
5-8	3	2.14-3.82	3.21	
Surface keratinization				
Present	2	3.67-3.82	3.74	
Absent	5	1.49-3.74	2.15	
Mural proliferation				
Present	3	3.67-3.82	3.74	
Absent	4	1.49-3.74	1.75	

AgNOR: Silver nitrate staining of the nucleolar organizer regions

Table 4: Comparative evaluation of silver nitrate staining of the nucleolar organizer regions with the histological findings of lining epithelium in 15 odontogenic keratocyst

Types of cases	Number of cases	Range of AgNOR count	Mean AgNOR count	Total average
Radicular	19	1.47-2.81	2.11	2.51
Dentigerous	7	1.49-3.82	2.64	
Odontogenic keratocyst	15	1.71-4.33	2.98	

AgNOR: Silver nitrate staining of the nucleolar organizer regions

Table 5: Comparative evaluation of silver nitrate staining of the nucleolar organizer regions counts among the nineteen radicular cysts, seven dentigerous cysts and fifteen odontogenic keratocyst with total mean of 41 cases

Histological features	Number of cases	Range of AgNOR	Average of AgNOR	Total average AgNOR count
Thickness of lining epithelium (layers)				2.98
3-8	10	1.71-3.63	2.62	
>9 layers	5	3.18-4.33	3.68	
Surface keratinization				
Present	15	1.71-4.33	2.98	
Well-polarized basal cell layer				
Present	15	1.71-4.33	2.98	
Microcyst/daughter cyst				
Present	5	3.45-4.33	3.78	
Absent	10	1.71-3.39	2.57	

AgNOR: Silver nitrate staining of the nucleolar organizer regions

Table 6: Comparative evaluation of silver nitrate staining of the nucleolar organizer regions counts among the nineteen radicular cysts, variation of argyrophilic nucleolar organizer regions count in different odontogenic cysts

Specimens	Sample size	Range	Mean	SD	Coefficient of variation
Radicular	19	1.47-2.81	2.11	0.32	15.13
Dentigerous	7	1.45-3.87	2.64	0.47	19.00
Odontogenic keratocyst	15	1.71-4.33	2.98	0.62	20.84

SD: Standard deviation

was 2.29. While the lesions with relatively less epithelial thickness (2–10 layers), absence of arcading arrangement and absence of mural proliferation had average AgNOR counts compatible to that of the counts noted by Allison and Spencer^[4] and Coleman *et al.*^[8]

It has been reported by earlier workers such as Smith and Crocker^[9] that raised AgNOR count reflect the rate of cellular proliferation and biologic behavior of non-Hodgkin's lymphoma and breast malignancies. Accordingly, it can be predicted that the raised AgNOR counts in the present study involving radicular cyst with increased lining epithelial thickness, arcading arrangements and mural proliferation reflect an active biologic behavior of those odontogenic cysts. However, further in-depth studies are required before arriving any final conclusion. The AgNOR count and its biological importance in dentigerous cysts were also evaluated by

Allison and Spencer^[4] ($n = 19$) and Coleman *et al.*^[8] ($n = 15$). They have observed that the mean AgNOR count was 2.43 (range 1.73–3.1) and 2.25 (range: 1.58–2.98), respectively. In the present study, a thorough evaluation of the AgNOR counts in dentigerous cysts ($n = 7$) revealed that the mean count was 2.64 and the range being 1.49–3.82. Thus, the present findings are compatible with the observation made by Allison and Spencer^[4] and Coleman *et al.*^[8]

On comparative evaluation of the important light microscopic features such as epithelial thickness, surface keratinization and mural proliferation in dentigerous cyst to that of the AgNOR count, it was observed that the cystic lesions having thickened epithelial lining presence of surface keratinization and presence of mural proliferation revealed much higher AgNOR count, 3.21, 3.74 and 3.74, respectively, than the average count 2.64. This higher AgNOR count reflects the same active biological behavior as observed by Smith and Crocker^[9] in other neoplastic lesion. While in the present study, the mean AgNOR count in OKC ($n = 15$) was 2.98 and the range being 1.71–4.33. Thus, the findings were compatible with the findings of the previous workers.^[5,8] However, comparative evaluation of the important histological features such as, epithelial thickness, surface keratinization, well-polarized hyperplastic basal layer and presence of microcyst/daughter cysts in OKC to that of the AgNOR count, it was observed that the lesions having thickened epithelial lining and microcyst formation revealed higher AgNOR counts 3.68 and 3.78, respectively, than the average count 2.98. These increased AgNOR counts as observed in OKC is much higher than that of the average AgNOR counts as noted in ameloblastomas and adenomatoid odontogenic tumors do by do Carmo and Silva.^[10] Accordingly, this increased AgNOR activity in OKC as noted in the present study can be considered as a prognostic marker and indicator of biologic behavior of OKC. This observation has been further supported by the views as expressed by Toller^[1] who considered OKC as a neoplastic cyst. However, further detailed studies are necessary involving more number of OKC lesions. Interestingly, in the present study, the comparative interrelationship between different odontogenic cysts such as radicular, dentigerous and OKC, it was observed that OKC had the highest mean AgNOR count (2.98) followed by dentigerous cyst (2.64) and followed by lowest in the radicular cyst (2.11). Moreover, the statistical evaluation and assessment of interrelationship between these cystic lesions revealed that the mean for OKCs was significantly higher than that of the radicular cyst ($t = 5.32$, $df = 32$, $P < 0.001$) and the mean value of the dentigerous cysts also higher than that of radicular cyst ($t = 2.18$, $df = 24$, $P < 0.05$) and all these findings were statistically significant.

CONCLUSION

Interestingly, higher AgNOR counts were recorded in all these odontogenic cysts showing proliferation of lining odontogenic epithelial cells, mural proliferations, arcading arrangements and daughter cysts formations than the overall average counts, especially in OKC. Thus, the present study indicated that the AgNOR counts are not of diagnostic significance and cannot be used to distinguish between various odontogenic cysts but definitely reflects the prognostic and biologic behavior of these odontogenic cysts, especially that of the OKCs.

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Conflicts of interest

There are no conflicts of interest.

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