Original Article

AgNOR as an effective diagnostic tool for determining the proliferative nature of different types of odontogenic cysts

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Abstract

Objective: To determine the proliferative nature of different types of odontogenic cysts using silver-stained nucleolar organizer region (AgNOR) staining. **Materials and Methods:** Forty histopathological sections of each of odontogenic keratocyst, dentigerous, and radicular growth were taken into study. Two sections were produced using the paraffin block of separate cases, one was stained with hematoxylin and eosin and another segment was stained with silver nitrate method. **Results:** AgNOR staining shows black dots and other parts of nuclei show yellow-brown dots. **Conclusion:** AgNOR, which stain for NOR-related proteins, corresponds to cell multiplication. Their amount regarding the number of AgNORs per cyst can be a good marker of the proliferative action of the cell in aggressive cystic lesion having malignant potential.

Keywords: Dentigerous cyst, nucleolar organizer regions (NORs), odontogenic keratocysts (OKC), silver colloid technique

Introduction

A cyst is a pathological cavity having fluid, semifluid, or gaseous content and which is not created by the accumulation of pus. Most cysts, but not all, are lined by epithelium.[1] The cyst is extensively partitioned into two gatherings that are odontogenic and nonodontogenic cysts. Most of the odontogenic cysts are derived from the tooth forming enamel organ epithelia. These are *cell rest of Serres* (rests of the *dental lamina*), the cell rests of Malassez (rests of the *root sheath of Hertwig*), and the reduced enamel epithelium (remnants of the *enamel organ* after dental crown formation). For odontogenic keratocysts (OKCs), it has additionally been recommended that they may derive from the mucosal basal cells. The etiology of the odontogenic cyst has been generally ordered into two unique gatherings: developmental (dentigerous, keratocysts, gingival cysts, etc.) and inflammatory (radicular, residual, paradental cysts). Regarding their rate of incidence, radicular cysts are the most common (mostly associated with teeth having pulp necrosis), trailed by dentigerous cysts and odontogenickeratocysts.[2]

Various kinds of odontogenic growths have trademark epithelial linings and differ in their biological behavior. Most cysts are thought to develop inactively, determined by a hydrostatic weight

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inside the lumen made by the hypertonic sore liquid substance and the semi-porous epithelial coating different growths showing dynamic cell proliferation.[3] Nucleolar organizer regions (NORs) are defined as nucleolar segments containing a lot of argyrophilic proteins, which have a high affinity for silver [silver-stained nucleolar organizer region (AgNOR) proteins]. After silver-staining, the NORs can be effectively recognized as dark specks solely restricted all through the nucleolar region and are designated "AgNORs.".[4] NORs are the loops of DNA that transcribe genes for ribosomal RNA. AgNORs are NOR-related acidic proteins which are characterized as markers of "dynamic" ribosomal genes.[5] AgNORs have been used as histochemical pointers of cell multiplication. The stained regions change fit as a fiddle as indicated by nucleolar translation and are personally identified with the cell cycle. The measure of AgNOR proteins is corresponding to the proliferative action of the cell.[6]

A few investigations have demonstrated that AgNOR counts can be used to differentiate between benign and malignant lesions, though a couple of others have expressed that it is the morphological normal for AgNOR, which is more enlightening than their outright numbers. Notwithstanding, repudiating reports are likewise accessible that prevent the potential value from claiming AgNORs, and thus equivocalness still exists over the careful job of the distinctive AgNOR parameters.[7]

Materials and Methods

The materials used for the investigation involved the example size of 40 histopathological demonstrated instances of each OKC, dentigerous, and radicular growth. An aggregate of 240 cases was incorporated into the examination. Every one of the cases was sourced from the files of the Department of Oral Pathology at D.J. School of Dental Sciences and Research, ModiNagar. Two sections were produced using the paraffin block of separate cases, one was stained with hematoxylin and eosin and another segment was stained with silver nitrate method.

Hematoxylin and eosin staining technique

The section was deparaffinized in 5% xylene and then dehydrated in alcohol. It was then washed with distilled water and stained with Harris hematoxylin for 5 min and washed under running water for 1 min. Following staining, the section was differentiated with 1% acid alcohol, kept in running water for 15 min, and counterstained with Eosin for 1 min. After that, it was washed with water, dehydrated with ascending grades of alcohol, cleaned with xylene, and mounted in DPX and dried. After that, in each case, another section was subjected to AgNOR staining.[8,9]

Preparation of AgNOR stain

The shelf-life of the AgNOR stain is hardly 4 days and it would, in general, crumble after this period. Henceforth, in our examination, AgNOR stain was freshly prepared each time, when staining was done to maintain the staining quality. Two solutions were prepared, solution A and solution B. Silver colloid solution was prepared by mixing one part of solution A (2 g gelatin in 100 mL of 1% formic acid) and two parts of solution B (50% silver nitrate in reverse osmosis water).[5,10]

AgNOR staining (silver colloid technique)

AgNOR recoloring was done by the modified technique of Smith *et al.*[10] Segments of 4-μm thickness were cut from the routinely prepared paraffin squares. These were dewaxed in three changes of xylene and after that were hydrated through diving evaluations of liquor to refined water. The fresh AgNOR arrangement was readied and quickly poured over the tissue segments and left for 40 min at 370°C temperature in the incubator. After staining was done, the slides were washed in deionized water. The slides were then submerged in 5% sodium thiosulfate for 2–3 min, and this was trailed by consecutive dehydration in evaluations of alcohols (half, 70%, 80%, and 90%). After that, the slides were cleared in xylene and mounted in synthetic medium DPX. The slides were then put away in an opaque holder in a clean and dry condition.[10]

Evaluation for AgNOR staining and counting protocol

AgNOR counts were done according to the method of Crocker *et al.*[11] In each case, the AgNORs (discrete black dots) were counted randomly in the nuclei of 100 basal and parabasal cells in the cyst lining using conventional light microscopy with 100× with 100× oil immersion objective and cedarwood oil.[11] Figures [1-3].

Results

AgNOR staining shows black dots and other parts of nuclei show yellow-brown dots.

As shown in Table 1, standard deviation, range, and standard error mean of AgNOR counts among different types of cysts were maximum in OKC, whereas they were least in the dentigerous cyst.

So it shows maximum AgNOR count in the odontogenic cyst, then in radicular and minimum in the dentigerous cyst. One-way analysis of variance test was performed to obtain the *P* value by calculating the sum of square, degree of freedom, mean sum of

Table 1: Comparative mean, standard deviation and standard error of mean of AgNOR dots among different types of cysts

Cyst type	AgNOR counts (Mean±SD)	Range	SEM
Odontogenic keratocyst	184.15±37.710	131-287	5.962
Dentigerous cyst	122.62±15.011	92-151	2.373
Radicular cyst	134.35±12.503	100-156	1.977

AgNOR: silver-stained nucleolar organizer region; SD: standard deviation; SEM: standard error of mean

Table 2: Statistical analysis of AGNOR counts for odontogenic keratocyst, dentigerous, and radicular cyst using probability of unpaired *t*-test

Groups	Cysts types	Probability of unpaired *t*-test in AgNOR counts B/W different types of cysts	*P*/ significance
1.	Odontogenic keratocyst and dentigerous cysts	0.0001*	*P*<0.05 (significant)
2.	Dentigerous cysts and radicular cysts	0.0002*	*P*<0.05 (significant)
3.	Odontogenic keratocysts and radicular cysts	0.0001*	*P*<0.05 (significant)

AgNOR: silver-stained nucleolar organizer region. *Significant difference at 0.05 level of significance

Table 3: One way ANOVA F table for comparing the significant difference in AgNOR counts among different types of cysts

Source of variance	SS	Df	MS	F	*P*	F-crit
Between groups	85,380.984	112	42,690.492	71.005	0.001	3.07376290
Within groups	70,344.270	117	601.233		*P*<0.05 (significant)	
Total	155,725.254	119				

ANOVA: analysis of variance; AgNOR: silver-stained nucleolar organizer region; SS: sum of square; Df: degree of freedom; MS: mean of square

square, Fisher's (F) value, and Fisher's crit (F-crit) value. If "F" is greater than the F-crit value, then the results are thought to be significant. In our study, a significant difference was observed as shown in Table 2.

Unpaired *t*-test was applied to find the significant difference in the AgNOR count between all possible groups as shown in Table 3. It shows that a significant difference was present in the AgNOR count between OKC and dentigerous cyst (group 1) and dentigerous cyst and radicular cyst (group 2), respectively, at 0.5% level of significance. However, no significant difference was present between OKC and radicular cyst (group 3).

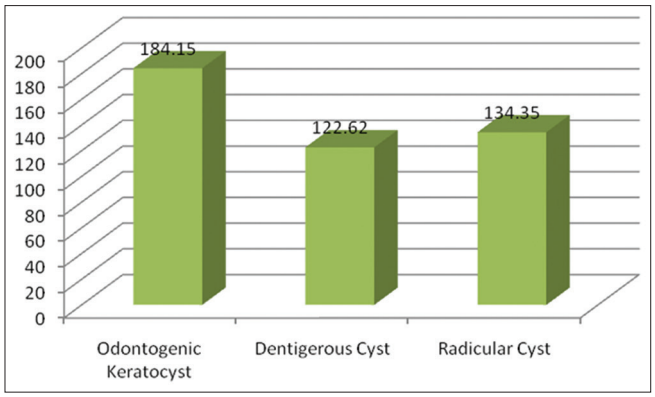
Discussion

The complex pattern of dental lamina begins to fragment or crumble due to ectomesenchymal intrusion soon after the foundation of tooth germs. A portion of these cell rests or waste results of ordinary human odontogenesis appears to be activated by the heretofore obscure system to expansion and a subsequent generation sometime down the road of surely understood pathologic entities.[12,13] The evaluation of cell multiplication in histological material is a profitable segment of customary histopathological examination and different techniques can be used to measure this; in a perfect world such strategies ought to be basic, reproducible, and pertinent to expectedly handled histological and cytological arrangement. This technique ought to be moderately inexpensive and direct in understanding; the AgNOR amount is identified with the quickness of cell expansion. AgNORs were first revealed by Goodpature and Sprout in 1975. Howell and Dark (1980) presented a one-stage silver recoloring technique in which impregnation and improvement were performed at the same time. Silver nucleolar sorting out district is a circle of DNA containing ribosomal RNA quality. They are situated on the short arm of five acrocentric chromosomes, that is, numbers 12, 14, 15, 21, and 22. AgNOR is silver-restricting NORs. The NOR-related protein goes about as a replicator of R-DNA.

Late histopathologic investigations of AgNORs have brought about effective analysis, classification, and anticipation of different benign and malignant lesions. Counting is the most broadly used strategy for assessing AgNOR tally. This procedure has been broadly used because this system is basic and reproducible.[14] In our investigation, AgNORs included in OKCs were various, small in size, and noticeable even at a low-power view. In any case, it was noticed that AgNORs in dentigerous and radicular cysts were less, bigger, and relatively less unmistakable. Egan and Crocker[15] and Smith *et al.*[10] found that for threatening cysts, the AgNOR in OKCs were various, small in size, and noticeable even at low power. The after-effects of the accompanying investigation have corresponded with our examination.

The present investigation indicates statistically significant worth ($P = 0.001$) among various kinds of odontogenic cysts. The higher mean score of AgNOR was seen in OKC (184.15), followed by dentigerous cyst (122.62) and radicular cysts (134.35) [Graph 1]. Along these lines, proposing a potential for a higher biologic animosity and clinical conduct for OKC. These findings are per an investigation done by Prabhu[16] and Ajayi.[17] They additionally revealed a higher mean score in OKC among all odontogenic growths. In our examination, a variety in the AgNOR score was seen in contrast to the development example and conduct of different sorts of odontogenic scores with higher AgNOR included in OKC pursued by dentigerous and radicular growths.

Eslami *et al.*[18] showed comparative discoveries with mean AgNOR score for OKC (mean = 1.68) trailed by dentigerous cyst (mean = 1.49). Also, Allison *et al.*[19] assessed the indicative and prognostic estimation of AgNORs in OKCs, radicular growth, and dentigerous cyst. Their outcomes uncovered that mean AgNOR means and OKC (2.65) trailed by dentigerous cyst (2.43) and radicular cyst (2.02), individually, demonstrating that OKC and dentigerous cyst contrasted fundamentally from a radicular cyst, which is as per our examination. In the present examination, the mean AgNOR esteem for OKCs (184.15 ± 37.710), dentigerous cyst (122.62 ± 15.01), and



Graph 1: BAR chart of average value showing AgNOR counts for different types of cyst

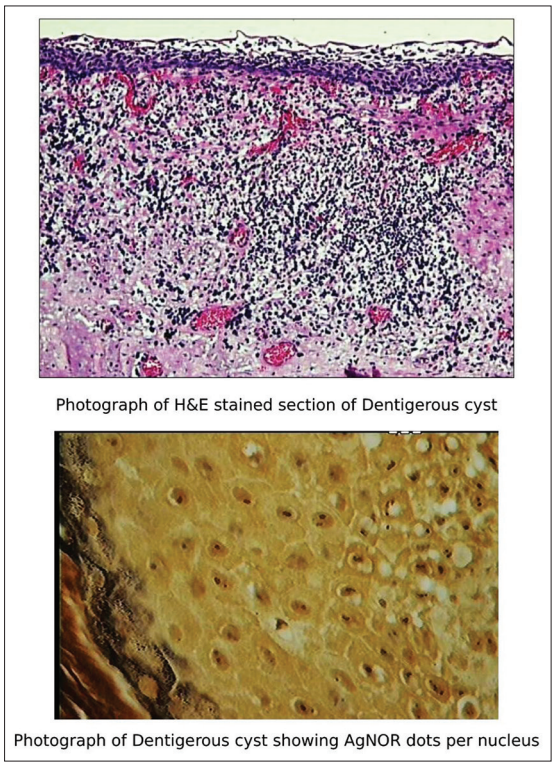


Figure 2: Evaluation of H&E stained section and AgNOR dots per nucleus of Dentigerous cyst

radicular cyst (134.35 ± 12.503) indicated noteworthy contrast in *P* value between group 1 ($P = 0.0001$) and group 2 ($P = 0.0002$), and group 3 ($P = 0.0002$) demonstrating comparable discoveries as per concentrate done by Allison *et al.*[19] and Eslami *et al.*[18] In our examination, AgNOR dabs were seen in a more noteworthy number in OKC when compared with dentigerous cyst and radicular cysts.

Giri[20] suggested that in the multiplying cells, the chromosomal and AgNOR circulation stays scattered with the results of numerous, little scattered nucleoli. These effectively multiplying cells have weakened nucleolar affiliation and in this way show a higher AgNOR tally, regardless of the ploidy condition of the cell. During mitosis, nucleolar discontinuity happens, bringing

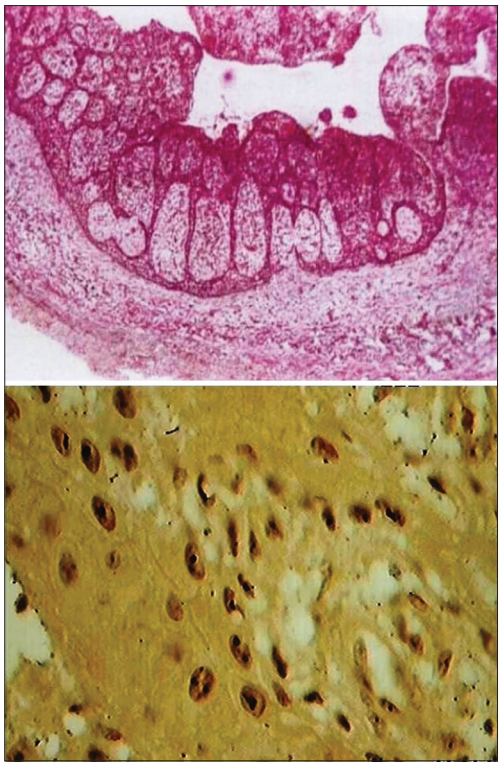


Figure 1: Evaluation of H&E stained section and AgNOR dots per nucleus of Radicular cyst

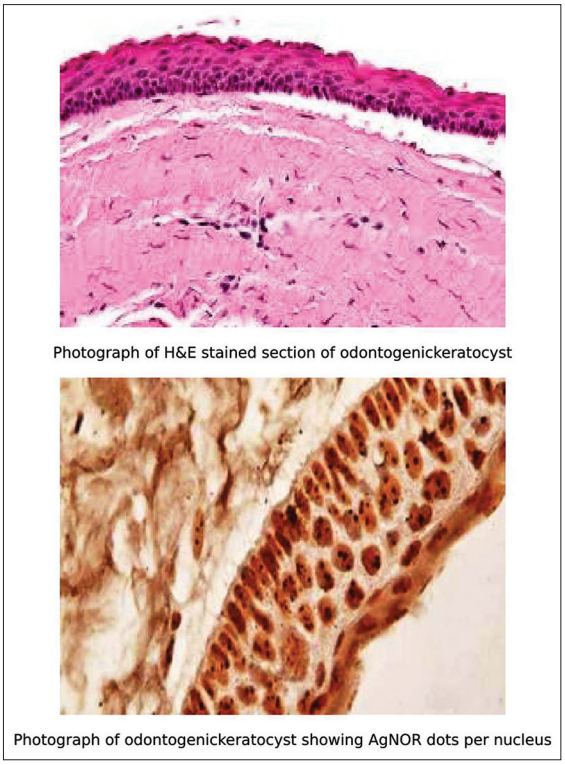


Figure 3: Evaluation of H&E stained section and AgNOR dots per nucleus of Odontogenic cyst

about the scattering of NORs all through the core. In this way, AgNOR spots are seen in a more noteworthy number on account of OKCs when compared with dentigerous cysts and radicular

cysts. Coleman *et al.*[21] likewise observed an enormous number of AgNOR specks in OKCs when compared with dentigerous cysts. The after-effects of our investigation recommend the potential convenience of AgNORs in separating the proliferative conduct of OKCs, radicular cyst, and dentigerous cyst.

Eslami *et al.*[18] additionally discovered smaller, increasingly various, and progressively boundless AgNORs in OKCs, suggesting a higher cell expansion rate. In 1993, Warnakulasuriya *et al.*[22] gave three distinct examples of AgNOR dispersion. In Type I, single or a couple of huge spots inside the core, representing the nucleolus, Type II discrete little dabs inside the nucleus, and Type III fine dark dabs scattered all through the nucleoplasm.[19] In our examination, OKC demonstrated the AgNOR dabs which were medium measured, consistently round or oval, and have a place with the Type I example of conveyance, related to the examples portrayed by Warnakulasuriya *et al.*[22] In dentigerous cyst, the individual specks looked like those in OKC; however, they were smaller in size and furthermore displayed a Type II example of appropriation associated with the examples depicted by Warnakulasuriya *et al.*[22] In radicular cyst, the AgNORs were not all that consistently round or oval. Some of them were marginally bigger in size, sporadic fit as a fiddle, and showed blended examples of conveyance.

AgNOR can also be used as a diagnostic and prognostic tool for many periodontal lesions which are proliferative in nature and maybe a quantitative marker of incipient cellular alterations before the histologic hallmarks appear.[23] The result of the study suggests that AgNOR is a good quantitative marker of initial changes in various cystic lesions before histological confirmative diagnosis. This can be easily practiced at the primary care center level for the screening of enlarging cystic lesions and early referral of the patient to higher centers for definite treatment to avoid morbidity and mortality.

Conclusion

Based on the present examination, it creates the impression that AgNORs – which stain for NOR-related proteins – go about as markers of cell multiplication. Their amount regarding the number of AgNORs per cyst is carefully a marker of the proliferative action of the cell. Then again, their subjective profile, in light of their size, shape, and example of circulation, goes about as a marker of proliferative change. Increment in the unusual size and state of AgNORs is seen as lesion advance. More than the quantitative, it is the subjective qualities that are increasingly significant in surveying cell changes happening in the cysts and in recognizing them from the typical. The present discoveries, particularly on the AgNOR pleomorphism, might be viewed as a primer. Further studies on larger samples are required to confirm this association of AgNOR quality with cellular proliferation.

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Conflict of interest

There is no conflict of interest.

References

1. Speight P, Mervyn S. Cysts of the oral regions. Edition. 4th ed. Published. Oxford: Blackwell Munksgaard, 2007.
2. Rajendran A, Sivapathasundharam B. Shafer's Textbook of Oral Pathology. 7th ed. New Delhi: Elsevier; 2012.
3. Landini G. Quantitative analysis of the epithelial lining architecture in radicular cysts and odontogenic keratocysts. Head Face Med 2006;2:2-4.
4. Trere D. AgNOR staining and quantification. Micron 2000;31:121-6.
5. Savithri V, Sudha S, Shameena PM, Varghese I. A clinicopathologic study of odontogenic keratocyst (OKC) and the role of AgNORS in cell proliferation. Oral Maxillofac Pathol J 2010;1.
6. Batista de Paula AM, Carvlhais JN, Domingues MG, Barreto DC, Mesquite RA. Cell proliferation markers in the odontogenic keratocyst: Effect of inflammation. J Oral Pathol Med 2000;29:477-82.
7. Elangovan T, Mani NJ, Malathi N. Argyrophilic nucleolar organizer regions in inflammatory, premalignant and malignant oral lesions: A quantitative and qualitative assessment. Indian J Dent Res 2008;19:141-6.
8. Culling CFA. Handbook of Histopathological and Histochemical Techniques. 3rd edition, London: Butterworth; 1974.
9. Bancroft JD. Theories and Practice of Histological Technique. 6th ed. Churchill Livingstone, Elsevier: China;2008.
10. Smith PJ, Skilbeck N, Harrison A, Crocker J. The effect of a series of fixatives on the AgNOR technique. J Pathology 1988;155:109-12.
11. Crocker J, Boldy DA, Egan MJ. How should we count AgNORs? Proposal for a standardized approach. J. Pathol. (1989): 158: 185-188
12. Reichart PA, Philipsen HP. Eds. Odontogenic Tumor and Allied Lesions. Quintessence Publishing Co Ltd; 2004. p. 2930.
13. Schwarzacher GH. Nucleolus organizer regions and nucleoli. Hum Genet 1983;63:89-99.
14. Cabrini RL. Morphometric study of nucleolar organizer regions in human oral normal mucosa, papilloma, and squamous cell carcinoma. J Oral Pathol Med 1992;21:275-9.
15. Egan JM, Crocker J. Nucleolar organizer regions in cutaneous tumors. J Pathol 1988;154:247-53.
16. Prabhu VD. Quantitative and qualitative evaluation of silver staining Nucleolar Organiser Regions in the normal odontogenic epithelium and odontogenic cysts – A comparative study. J Oral Health Res 2010;1.
17. Ajayi OF. Argyrophilic nucleolar organiser region (AgNOR) study of some odontogenic cyst epithelium. Niger Postgrad Med J 2010;17.
18. Eslami B, Rahimi H, Rahimi F, Khiavi MM, Ebadifar A. Diagnostic value of silver nitrate staining for nucleolar organizer regions in selected head and neck tumors. J Cancer Res Ther 2006;2:129-31.
19. Allison RT, Spencer S. Nucleolar organizer regions in odontogenic cysts and ameloblastomas. Br J Biomed Sci 1993;50:309-12.

20. Giri DD. Silver-binding nucleolar organizer regions (AgNORs) in benign and malignant breast lesions: correlation with ploidy and growth phase by DNA flow cytometry. J Pathol 1989;157:307-13.
21. Coleman HG, Altini M, Groeneveld HT. Nucleolar organizer regions (AgNORs) in odontogenic cysts and ameloblastomas. J Oral Pathol Med 1996;25:436-40.
22. Warnakulasuriya S, Mak V, Möller H. Oral cancer survival in young people in southeast England. Oral Oncol 2007;43:982-6.
23. Laxman VK, Madhushankari GS, Saluja M, Singh S. Periodontal implications of argyrophilic nucleolar organizer region: A mini-review. J Oral Res Rev 2019;11:48-51.