Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 12, pp.62534-62539, December, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PROLIFERATIVE ASSESSMENT USING AGNORS IN DENTIGEROUS CYST, KERATOCYSTIC ODONTOGENIC TUMOR, UNICYSTIC AMELOBLASTOMA AND MULTICYSTIC AMELOBLASTOMA

***[1]Gupta Nidhi, [2]Mujib Ahmed, B.R. and [2]Jadhav Kiran**

[1]Reader, Dept. of Oral Pathology & Microbiology, KM Shah Dental College, SumandeepVidyapeeth University, Vadodara, India

[2]Professor & Head, Dept. of Oral Pathology & Microbiology, Bapuji Dental Dental College, Davangere, India

ARTICLE INFO

Article History:
Received 13th September, 2017
Received in revised form
05th October, 2017
Accepted 24th November, 2017
Published online 27th December, 2017

Key words:

AgNORs, Unicystic Ameloblastoma,
Conventional ameloblastoma,
Dentigerous cyst,
Keratocysticodontogenic tumor.

ABSTRACT

Objective: To assess the usefulness of AgNORsas quantitative and qualitative criteria in assessing the proliferation potential of Dentigerous cyst, Keratocystic Odontogenic Tumor (KCOT), Conventional ameloblastoma and Unicysticameloblastoma.

Materials and Methods: Histological sections were stained usingsilver nitrate method and AgNOR dots counted at 100x magnification. Quantitative variations were assessed in terms of numbers and qualitative variations were assessedin terms of size, shape and pattern of distribution of the individual AgNOR dots. Results were expressed as Mean ± Standard Deviation, range values and coefficient of variation. One way ANOVA was applied to attain comparison between groups and within group. Post-Hoc Tukey's test was used for evaluating honestly significant difference between groups. A p-value of 0.05 or less was considered for statistical significance.

Results: Mean number of NORs in each odontogenic lesion indicated a significant difference between the four groups (P<.01) except between keratocystic odontogenic tumor (KCOT) and Unicysticameloblastoma (P<0.95). In relation to thequalitativeassessmentof AgNORs, dentigerous cysts had a single or few large dots within the nucleus, representing the nucleolus, while Unicystic ameloblastoma and keratocystic odontogenic tumor exhibited discrete small dots within the nucleolus, andconventionalameloblastomasexhibited fine black dots dispersed throughout the nucleoplasm.

Conclusion: AgNORshave a direct positive correlation with proliferative potential of odontogenic cyst and tumors.

Citation: Gupta Nidhi, Mujib Ahmed, B.R. and Jadhav Kiran, 2017. "Proliferative assessment using agnors in dentigerous cyst, keratocystic odontogenic tumor, unicystic ameloblastoma and multicystic ameloblastoma", *International Journal of Current Research*, 9, (12), 62534-62539.

INTRODUCTION

Odontogenic cysts and tumors comprise a heterogeneous group of lesions withvariable aggressiveness (Taylor, 2008). They have varying clinical behaviors, with differences in growth pattern and recurrence rate. This makes it essential to search for a reliable prognostic marker which could correlate with their proliferation potential, tendency to spread and recur (Prasanna, 2014). Dentigerous cyst is the second most common developmental odontogenic cyst, making up 20% of all jaw cysts (Eslami, 2003; Benn, 1996; Marx, 2003). Keratocysticodontogenic tumor represents 10% to 12% of developmental odontogenic cysts.It has an aggressiveclinical behavior and a significant recurrence potential (Eslami, 2003). Ameloblastoma is very common benign odontogenic tumor arising from dental epithelium, which is locally destructive and possessesa high rate of recurrence (Hume, 1990 and Stolf, 2007). Conventional ameloblastomas (solid or multicystic) exhibit a strong tendency for recurrence and local aggression due to its infiltrative growth into the surrounding bone and soft tissues (Dunsche, 2003; Kessler, 2004), 6% of ameloblastomas are of unicystic variety with a cystic growth pattern (Marx, 2003). Recognition of this growth pattern is important, asunicystic type of amelobalstoma is less aggressive, with a reduced incidence of recurrence and requires a less aggressive treatment (Marx, 2003). Odontogenic cysts possess the potential to convert to ameloblastomas.This makes it of paramount importance to assess their proliferation tendency (Eslami, 2003). The proliferation rate of these lesions furtherrelates to their clinical behavior (Aguirre, 1989). Nucleolar organizer regions (NORs) are loops of DNA on short arm of acrocentric chromosomes encoding ribosomal RNA, and are important in protein synthesis (Hume, 1990).

Corresponding author: **Gupta Nidhi,**
Reader, Dept. of Oral Pathology & Microbiology, KM Shah Dental College, SumandeepVidyapeeth University, Vadodara, India.

NORs appear to reflect cell and nuclear activity (Aguirre, 1989 and Coleman, 1996). Their number and amount rises with proliferative activity of the cell (Hume, 1990). Variation in size and number is dependent on stage of cell cycle, transcriptional and metabolic activity of a cell. The rapidity of the cell turnover, the growth rate, and the proliferation potential of a lesion has been previously studied using AgNOR count per nucleus, but there are varying opinions about relation of AgNORs in odontogenic cysts and tumors (Marx, 2003 and Do Carmo, 1998). Assessment of proliferative potential iswarranted due to varying and often destructive clinical behavior of these odontogenic pathologies (Aguirre, 1989). As there is significant disparity in results of various studies, the current study aimed at assessing the quantitative and qualitative evaluation of Silver Nuclear organizer regions (AgNORs), and to find if there exists a relationship with proliferation and aggressiveness of these lesions.

MATERIALS AND METHODS

The study was a cross sectional retro-prospective study conducted in the Department of Oral and Maxillofacial Pathology, Bapuji Dental College and Hospital, Davangere. Ethical approval was obtained before commencement of the study. Study sample included tissue specimens embedded in paraffin wax blocks of reported cases of Conventional Ameloblastoma (Follicular / Plexiform type), Unicystic Ameloblastoma, Dentigerous cyst and KCOT. A total of sixty cases, fifteen in each group were studied. Sections of 5 micrometer thickness were made, followed by silver-nitrate staining, as per method of Plotone*t al.* (Bancroft, 2004; Howell, 1980), Further to improve the results, after the silver stain application, specimens were rinsed in distilled water and further post fixed in 5% sodium thiosulfate for 5 minutes (Chattopadhyay, 1993 and Hirsch, 1992). The stained sections were observed under binocular compound microscope. The AgNOR sappearedasblack dots / blebs with a pale yellow-brown backgrounds taining.

Quantitative assessment of AgNORswas done by counting with a graticule by using conventional light microscopy at 100x. A total of 10 fields per slide were studied. 10 cells in each field making a total of 100 cells per slide were studied. Only such 100 epithelial cells were counted for each odontogenic lesion. Both basal tall columnar cells and stellate reticulum cells were randomly selected for ameloblastomas. Cells of the lining epithelium, lining the cystic lumen were selected for KCOT and dentigerouscyst.Overall the number of stained regions in each nucleus was recorded. A mean number of these regions werecalculated for each slide.

Some of the factors which were kept in mind while counting were,

- Closely aggregated dots, but resorbable were counted and recorded as separate dots (Chattopadhyay, 1993).
- Where two or more dots were closely associated and not resorbable, they were counted as a single AgNOR dot (Payeras, 2007).
- Overlapping AgNOR dots were counted as separate dots (Payeras, 2007).
- Where there were more than two dots, so closely aggregated that there was no "halo" of nucleoplasm, the aggregate was recorded as one (Coleman, 1996).
- Where black dots were closely aggregated, but there still existed a "halo" of nucleoplasm around them, the separate (Coleman, 1996).

Qualitative assessment of AgNORs were assessed in terms of size and shape of the individual AgNOR dots, and their pattern of distribution, as defined by Warnakulasuriya and Johnson. They identified three different, patterns of AgNOR distribution. Here, Type I was considered when single or few large dots were seen within the nucleus, representing the nucleolus. Type II was when discrete small dots were noted within the nucleolus.Type III was considered when fine black dots were seen dispersed throughout the nucleoplasm (Warnakulasuriya, 1993). Results were expressed as Mean ± Standard Deviation, range values and coefficient of variation. Comparison between groups and within group was done using one way ANOVA. Post-Hoc Tukey's test was used for comparison between four types of odontogenic lesions for honestly significant difference. A p-value of 0.05 or less was considered for statistical significance.

Table 1. Quantitative assessment of AgNORs

Groups	AgNOR counts			
	Range	Mean	Standard deviation	Coefficient of Variation
Group 1 - DC	1.37 – 2.09	1.64	0.25	15
Group 2 - OKC	1.93 – 3.22	2.53	0.36	14
Group 3 - CA	2.22 – 4.22	2.96	0.65	22
Group 4 - UA	2.01 – 3.54	2.44	0.38	16

Table 2. Comparison of Mean AgNOR counts between different groups

Groups	Mean counts ± Standard deviation	Difference between groups		
		Groups compared	Mean difference	P value
		1 vs 2	- 0.88	< .01, s
		1 vs 3	1.32	< .01, s
Group 1 - DC	1.64 ± 0.25	1 vs 4	- 0.80	< .01, s
Group 2 - OKC	2.53 ± 0.36	2 vs 3	0.43	< .05, s
Group 3 - CA	2.96 ± 0.65	2 vs 4	0.09	0.95, ns
Group 4 - UA	2.44 ± 0.38	3 vs 4	0.52	< .05, s

RESULTS

Age distribution of the subjects taken for the group has been tabulated in the Table I. AgNORs were strictly located within the nucleus and were distinctly stained in black, being visible as dots or blobs that were either round or oblong. The rest of the nucleus stained yellow-brown. The mean number of NORs was found to be most for conventional ameloblastoma (2.96 ±

0.65), and least for dentigerous cyst (1.64 ± 0.25), Table I &II: Fig 1-4. The graphical representation of mean values has been shown in Fig 5. There was a significant difference between the groups with a p<0.01 indicating significance, except between KCOT group and Unicysticameloblastoma (Table II).

three groups (p<0.01%); Table IV. The AgNORs in dentigerous cysts were medium sized, uniformly round or oval. In KCOTs, AgNORs were found to be relatively smaller in size as compared to Unicystic ameloblastoma, though both lesions had a predominance of Type II.

Table 3. Qualitative assessment (Morphological assessment of AgNORs)

Groups	No. of Subjects	Pattern of AgNOR distribution (Types)					
		I		II		III	
		n	%	n	%	N	%
Group 1- DC	15	12	80.0	1	6.7	2	13.3
Group 2 - OKC	15	1	6.7	10	66.7	4	26.6
Group 3 - CA	15	-	-	8	53.3	7	46.7
Group 4 - UA	15	-	-	12	80.0	3	20.0
Total	60	13	21.7	31	51.7	16	26.6

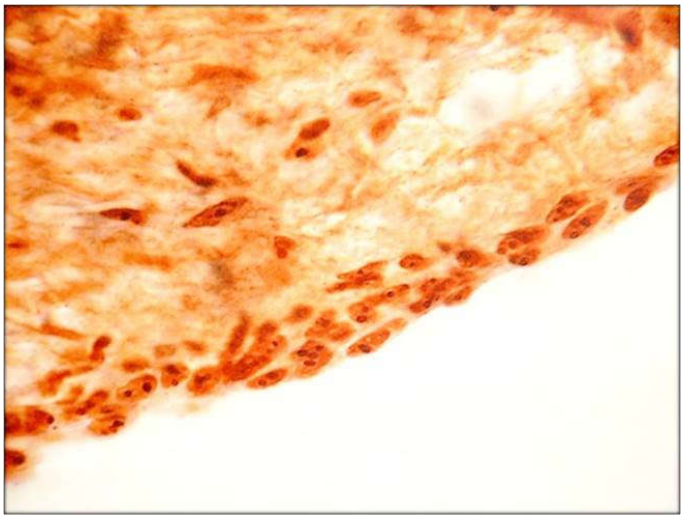

Fig. 1. Photomicrograph showing AgNORs in Dentigerous cyst at 100 X

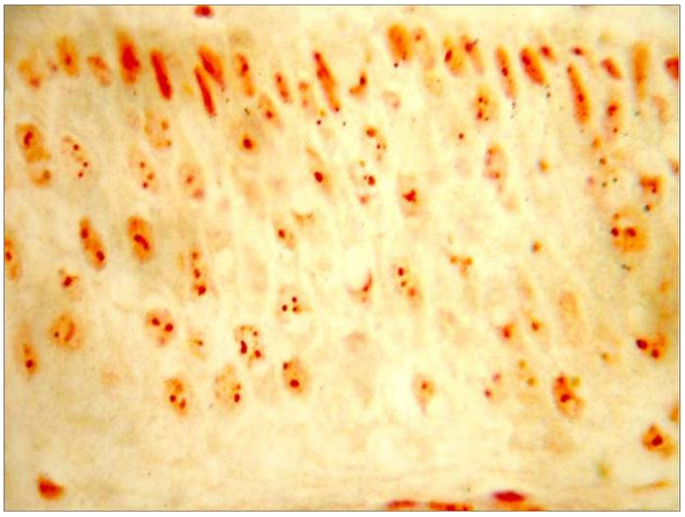

Fig. 2. Photomicrograph showing AgNORs in Keratocystic Odontogenic Tumor at 100 X

When morphological variations of AgNORs were assessed in terms of size and shape, Type I morphology was predominant in dentigerous cysts (80%), Type II in keratocystic odontogenic tumors (66.7 %), Type II and III in conventional ameloblastoma (53.3% and 46.7% respectively) and Type III in Unicysticameloblostoma (80.0%); (Table III, Fig 6). Only dentigerous cyst exhibited a significant difference in the morphology of AgNORs when compared to the remaining

Table 4. Group wise comparison of types of AgNORs

Groups compared	X^2 Value	p value
1 & 2	17.3	<.01, s
1 & 3	20.2	<.01, s
1 & 4	21.5	<.01, s
2 & 3	2.04	ns
2 & 4	1.32	ns
3 & 4	2.40	ns

Fig. 3. Photomicrograph showing AgNORs in Multicystic Ameloblastoma at 100 X

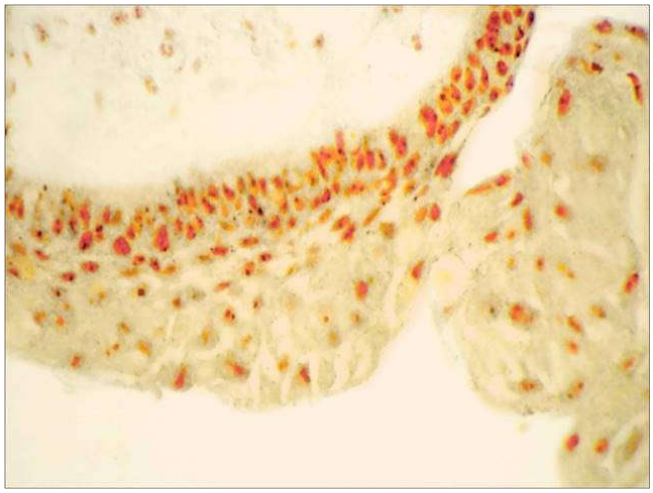

Fig. 4. Photomicrograph showing AgNORs in Unicystic Ameloblastoma at 100 X

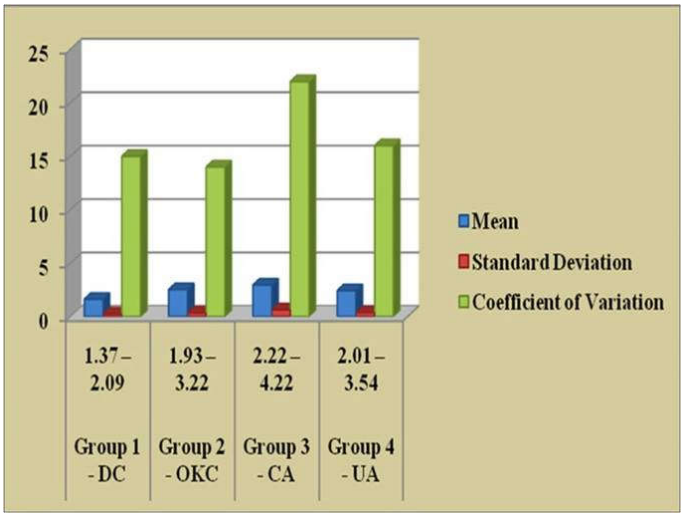

Fig. 5. Graphical representation of Quantitative assessment of AgNORs in all four groups

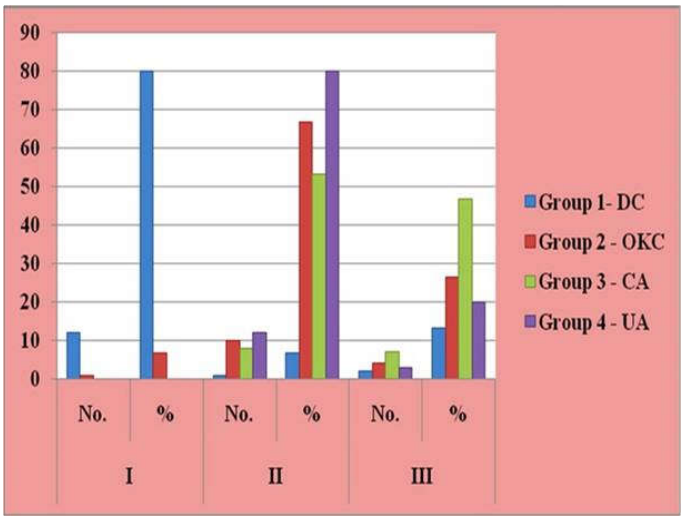

Fig. 6. Graphical representation of Qualitative assessment of AgNORs in all four groups

In most of the cases of conventional ameloblastoma, AgNORs were distributed throughout the nucleus and appeared as fine, minute, numerous dots giving it a granular appearance.

DISCUSSION

KCOTshave an aggressive nature with rare cases of transformation into squamous cell carcinomas. Conventionalameloblostoma (solid /multicystic) is a benign epithelial odontogenic tumor showing a strong tendency to recurrence and local aggression (Dunsche, 2003). Unicystic ameloblostomas are the second and far less frequent growth pattern among the intraosseous ameloblastomas. Recognition of the growth pattern is important, as the proliferative tendency and biological behavior of these lesions correlates withan overall prognosis and recurrences. A nucleolar organizer region,on the five acrocentric chromosomes participates in formation of nucleolus in the interphase. Therefore there can be more than one nucleolus in one nucleus. However multiple nucleoli generally fuse into 1 or 2 larger structures as a result of their association. Silver staining demonstrates these proteins, which are called asArgyrophilic Nucleolar Organizing Regions (AgNORs). (Hernandez-Verdun, 1980). The AgNOR counting reflects the rate in which the cell follows the cell cycle. The rapidity of the cell turnover is evaluated by speed of the cell cycle. This correlates with the growth rate of the lesion, and can be assessed by AgNOR count per nucleus, measurement of area of AgNOR dots, analysis of their distribution pattern (Sano, 1991). Actively proliferating cells have impaired nucleolar association and, therefore, exhibit a higher AgNOR count, regardless of the ploidy state of the cell (Eslami, 2003; Matsumura, 1989 and Selvi, 2012). Chatopadhya*etal*suggested that an AgNOR count of 2.3 can be used as a cut-off point for distinguishing between mild and moderate dysplasia (Chattopadhyay, 2008 and Santos, 2009). In 2003, Eslami*et al* found AgNORsas an effective quantitative criterionfor diagnosis odontogenic cysts and tumors (Eslami, 2003). They found minimum mean AgNOR counts in dentigerous cysts and the maximum in conventional form of ameloblastoma like in the present study (Eslami, 2003). This implies that, as the number of AgNOR nuclei increases,the epithelial cells undergo neoplastic transformation. In contrast to the present study they observed a significantly higher (P<.05) number of AgNORs in conventional and Unicysticameloblostoma as compared to KCOTs and dentigerous cysts.

They observed a significantly higher (P<.005) count in the nuclei of basal cells compared to parabasal cells of the epithelial lining of KCOTs. In the present study not any differentiation was made between basal and parabasal layers in OKC and the selection of cells was kept random. Also that the AgNORs in conventional and unicysticameloblastomas were smaller, but more broadly distributed correlating with their proliferative activity (Eslami, 2003). In 1993, Allison and Spencer reported significant differences in AgNORs between Dentigerous cyst and KCOTs ($P < .01$). AgNORs have been used to diagnose between different odontogenic lesions by many authors, with varying results (Santos, 2009 and Alliso, 1993). The diversity in the methods of counting NORs could be the reason for varying results. The authorsof this study believe as most of the odontogenic lesions can be readily diagnosed with hematoxylin and eosin staining, use of AgNORs should be restricted to assess the proliferative potential of these lesions. In 1996, Coleman *et al* reported a significant difference (P < 0.05) in the number of NORs between four odontogenic lesions: dentigerous (2.25+/- 0.09) and residual (1.97+/-0.09) cysts, unicystic ameloblastoma with clear ameloblastoma characteristics (1.66+/-0.1) and Unicysticameloblostoma without clear ameloblastomatous characteristics (1.93+/- 0.09). The disagreement related to a higher number of AgNORs in dentigerous cyst could be due to assessment of basal cell layer rather than a random selection of cells and also because they considered adjacent small NOR dots as one instead of counting them separately (Coleman, 1996). In 1997, Rosa *et al*observedNORs in ameloblastoma in range of 1.652 +/- 0.032. (Rosa, 1997).

In 1998, do Carmo and Silva observeda significant difference only between the follicular and the plexiform patterns (P<0.05) with follicular exhibiting a higher proliferative rate.In the present study, the authors did notclassify the conventional ameloblastoma into its histological subtypes. This couldbe a reason for variation in results. In 2007, Payeras*et al* evaluated the proliferation activity by quantification assessment of AgNORs and expression of epidermal pattern of growth factor receptor (EGFR). They observed that smaller islands were associated with a higher proliferative activity and therefore could be responsible for tumor proliferation (Payeras, 2017).

SSeifi in 2011 observed a significantly higher value of AgNORsinparabasal layer cells in multicysticameloblastoma and unicystic ameloblastoma as compared to KCOT, and follicular cyst. They concludedthat the higher values in KCOT and ameloblastomascorrelate with a higher proliferative activity in these lesions as compared to dentigerous cysts (Seifi, 2011). Do carmo*et al*., observedaggressive behavior of ameloblastoma in comparison to adenomatoid odontogenic tumors (Do Carmo, 1998). In astudy conducted in 2010 by Carnelio*et al*., the number of NOR dots were noted to be higher in ameloblastoma compared with ameloblastic fibroma and proposed it to bedue to more aggressive behavior of ameloblastomas (Carnelio, 2010). The results are similar to that of Santos et al (Santos, 2009). In contrast to the above studies A Ananthaneni*et al* observed the AgNOR count to be more in KCOT when compared to multicysticameloblastoma and unicystic ameloblastoma (Ananthaneni, 2014).

F Selvi*et al* investigated on the possible role and correlation of Ki-67 and argyrophilic nucleolar organizing regions (AgNOR) between the recurrent and non-recurrent KCOTs. The Ki-67 and AgNOR counts were found significantly higher in the recurrent lesions comparing to the non-recurrent lesions (Selvi, 2012). MD Prasanna*etal* found a mean AgNOR count of 1.905 for KCOTs, 1.565 for Dentigerous cyst and 2.14 for Conventional ameloblastoma (Prasanna, 2004). The variation in the AgNOR counts between our study and other studies cited above may be attributed to many factors, such asfixation of tissues, section thickness, staining protocols followed, counting criteria ofAgNORs and method of analysis in odontogenic lesions (some merely studied the basal layer and some others studied all the epithelial layers). The different observed patterns of NORs in odontogenic lesions depend on the stage of the cell cycle (Hirsch, 1992 and Payeras, 2007). The relationship between the AgNOR count and cellular proliferation has also been studied using immunohistochemistry with markers such as Ki67, Proliferating cell nuclear antigen (PCNA) and bromodeoxyuridine. In all cases it was demonstrated that the greater the proliferative activity, the higher the AgNOR counts (Coleman, 1996 and Li, 1995).

The results of the present study indicate that a qualitative assessment of AgNORs gives more information about the proliferative activity. Quantification of total AgNORs in sections may be prone to an unacceptable degree of observer variation. The authors conclude that the AgNOR counts definitely supplements the information obtained from other prognostic indices in clinical use.This proliferative potential indicated by AgNORs further relates with cellular activity, transformation to malignancy and predicting survival.Astandardized staining and computer-aided analysis of AgNORs are prerequisites for making a more objective and reproducible AgNOR.

Acknowledgement: Nil

REFERENCES

Aguirre, A., Takai, Y., Meenaghan, M., Neiders, M.E., Natiella, J.R. 1989. Lectin histochemistry of ameloblastomas and odontogenic keratocysts. *J Oral Pathol Med*., 18:68-73.

Allison, R.T., Spencer, S. 1993. Nucleolar organizer regions in odontogenic cysts and ameloblastomas. *Br J Biomed Sci.*, 50:309–12.

Ananthaneni, A., Udayashankar, U., Guduru, V.S., Ramprasad, V.V., Ramisetty, S.D., Namala, S., Badavath, K.K. 2014. A Qualitative and Quantitative Analysis of AgNORs in Keratocystic Odontogenic Tumor, Unicystic Ameloblastoma and Multicystic Ameloblastoma. *Journal of Clinical and Diagnostic Research : J ClinDiagn Res.* 2014;8(9):FC14-FC15.

Bancroft, J.D. 2004. Cytoplasmic Granules, Organelles and Neuroendocrine. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques.5th ed. China: Elsevier Ltd; 350-351.

Benn A, Altini M. Dentigerous cysts of inflammatory origin: A clinicopathologic study. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 1996 Feb; 81(2):203-9.

Carnelio, S., Vij, H. 2010. Expression of tenascin and nucleolar organizer region in ameloblastoma and ameloblastic fibroma. *J Oral Pathol Med.,* 39:223-9.

Chattopadhyay, A. 1993. AgNORs in tumoral pathology. Review of literature and observations on the technic and reaction in normal oral epithelium. *Indian J Dent Res.,* Apr-June; 4(2):47-53.

Chattopadhyay, A., Ray, J.G. 2008. AgNOR cut-point to distinguish mild and moderate epithelial dysplasia. *J Oral Pathol Med.,* 37:78–82.

Coleman, H.G., Altini, M., Groeneveld, H.T. 1996. Nucleolar organizer regions (AgNORs) in odontogenic cysts and ameloblastomas. *J Oral Pathol Med* 25:436-40.

Do Carmo, M.A., Silva, E.C. 1998. Argyrophilic nucleolar regions (AgNORs) in ameloblastomas and adenomatoid odontogenic tumours (AOTs). *J Oral Pathol Med.,* 27:153-6.

Dunsche, A., Babendererde, O., Luttges, J., Springer, I.N. 2003. Dentigerous cyst versus unicysticameloblastoma – differential diagnosis in routine histology. *J Oral Pathol Med.,* Sep;32(8):486-91.

Elangovan, T., Mani, N.J., Malathi, N. 2008. Argyrophilic nucleolar organizer regions in inflammatory, premalignant, and malignant oral lesions: a quantitative and qualitative assessment. *Indian J Dent Res*., Apr-Jun; 19(2):141-6.

Eslami, B., Yaghmaei, M., Firoozi, M., Saffar, A.S. 2003. Nucleolar organizer regions in selected odontogenic lesions. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod*., Feb; 95:187-92.

Hernandez-Verdun, D., Hubert, J., Bourgeois, C.A., Bouteille, M. 1980. Ultrastructural localization of Ag-NOR stained proteins in the nucleolus during the cell cycle and in other nucleolar structures. Chromosoma., (3):349–362.

Hirsch, S.M., DuCanto, J., Caldarelli, D.D., Hutchinson, J.C., Coon, J.S. 1992. Nucleolar Organiser Regions in Squamous cell Carcinoma of the head and neck. *Laryngoscope.* 102:39–44.

Howell, W.M., Black, D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia., Aug; 15;36(8):1014-5.

Hume, W.J., Moore, J.K., Main, D.M. 1990. Differences in in vitro growth of epithelium from inflammatory and developmental odontogenic cysts. *Br J of Oral MaxillofacSurg.,* Apr; 28(2):85-8.

Kessler, H.P. 2004. Intraosseous ameloblastoma. Oral *Maxillofac Surg Clin N Am.,* 16:309-322.

Li, T.J., Browne, R.M., Matthews, J.B. 1995. Epithelial cell proliferation in odontogenic keratocysts: a comparative immunocytochemical study of Ki67 in simple, recurrent and basal cell naevus syndrome (BCNS)-associated lesions. *J Oral Pathol Med*., May;24(5):221-6.

Marx, R.E., Stern, D. 2003. Odontogenic cysts, Odontogenic tumors. In: Lisa C, Bywaters, editors. Oral and Maxillofacial Pathology. Illinois: Quintessence Publishing Co; 573-685.

Matsumura, K., Sasaki, K., Tsuji, T., Shinozaki, F. 1989. The nucleolar organizer regions associated proteins (AgNORs) in salivary gland tumors. *Int J Oral Maxillofac Surg.,* 18:76–78.

Payeras, M.R., Sant'Ana Filho, M., Lauxen, I.S., Barbachan, J.J.D. 2007. Quantitative analysis of argyrophilic nucleolar organizer regions and epidermal growth factor receptor in ameloblastomas. *J Oral Pathol Med.,* Feb; 36(2):99-104.

Prasanna, M., Charan, C., Reddy, Ealla, K.K., Surekha, V., Kulkarni, G., Gokavarapu, S. 2014. Analysis of silver stained nucleolar organizing regions in odontogenic cysts and tumors. *J Oral Maxillofac Pathol.* 2014;18(Suppl 1):S45–8.

Rosa, L.E., Jaeger, M.M., Jaeger, R.G. 1997. Morphometric study of nucleolar organizer regions in ameloblastoma and basal cell carcinoma. *Oral Oncol.* 33:209–14.

Sano, K., Takahashi, H., Fujita, S., Inokuchi, T., Pe, M.B., Okabe, H., Tsuda, N. 1991. Prognostic implication of silver-binding nucleolar organizer regions (AgNORs) in oral squamous cell carcinoma. *J of Oral Pathol Med*., Feb; 20 (2):53-56.

Santos, A.C., Tarquinio, S.B., Rivero, E.R., Araujo, L.M., Krause, C.I. 2009. Quantitative AgNORs study in ameloblastomas. *Rev Odontol Sci.*, 24:10–4.

Seifi, S., Shafigh, E., Allaie, A. 2011. Quantitative and qualitative analysis of argyrophilicnuclearorganizerregions in follicular cysts, keratocysticOdontogenic Tumor and ameloblastoma. *J Cancer Res Ther*., July-Sep;7(3):280-5.

Selvi, F., Tekkesin, M.S., Cakarer, S., Isler, S.C., Keskin, C. 2012. Keratocystic Odontogenic Tumors: Predictive Factors of Recurrence by Ki-67 and AgNOR Labelling. *International Journal of Medical Sciences.* 9(4):262-268.

StolfD, P., Karim, A.C., Banerjee, A.G. 2007. Genetic aspects of ameloblastoma: a brief review. *Biotechnology and Molecular Biology Review.,* Dec; 2(5):116-122.

Taylor, A.M. 2008. New findings and controversies in odontogenic tumours. *Med Oral Patol Oral Cir Bucal.,* 13(9)E555-58.

Warnakulasuriya, K.A., Johnson, N.W. 1993. Nucleolar organizer region (NOR) distribution as a diagnostic marker in oral keratosis, dysplasia and squamous cell carcinoma. *J Oral Pathol Med.,* Feb;22(2):77-81

****