

# Analysis of Argyrophilic Nucleolar Organizer Regions in Variants of Ameloblastoma

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## ABSTRACT

**Introduction:** Ameloblastomas are locally invasive, highly destructive benign tumors of odontogenic origin. There are various methods performed for evaluation of proliferative activity among which quantification of argyrophilic nucleolar organizer region is a simple method. Aim of the present study is to compare number of argyrophilic nucleolar organizer region in unicystic and multicystic variants of ameloblastoma in order to analyze their cell proliferation rates.

**Material and Methods:** This retrospective study was carried out on 10 cases each of unicystic and multicystic variants of ameloblastoma. To identify the nucleolar organizer regions, the sections were obtained and stained with silver staining technique. Argyrophilic nucleolar organizer regions were quantified by manual counting method using the image analyzer software, ProgRes CapturePro v2.8.8.

**Results:** The Argyrophilic nucleolar organizer region count showed a significant higher number of argyrophilic nucleolar organizer regions in multicystic ameloblastoma (mean=4.236) than in unicystic ameloblastoma (mean=2.684).

**Conclusion:** This study shows the expediency of argyrophilic nucleolar organizer region staining in reflecting the high proliferation rate and a more aggressive behavior of multicystic ameloblastoma in comparison to unicystic ameloblastoma.

**Keywords:** Multicystic Ameloblastoma, Unicystic Ameloblastoma, Nucleolar Organizer Regions.

were unicystic ameloblastoma and 10 were multicystic ameloblastoma.

## Procedure of AgNOR staining

The AgNOR staining solution was obtained by mixing solution A and solution B in the ratio of 2:1. Solution A was prepared by dissolving 25 grams of silver nitrate in 50 ml of de-ionized water. After dissolving silver nitrate crystals the solution was filtered and stored in dark container, well protected from light. Solution B was prepared by dissolving 500 mg of gelatin in 25 ml of de-ionized water. The solution was heated slightly to dissolve the gelatin completely. 0.25 ml of 90% formic acid was then added to the solution and mixed well.<sup>5,6</sup>

From each paraffin embedded block 4µm thick sections were cut using rotary microtome. The sections were deparaffinized, rehydrated and then washed in running de-ionized water for 10 minutes. Slides were then stained with silver colloidal solution freshly prepared by mixing solution A and solution B in the ratio of 2:1 and incubated in the dark at 30°C for 45 minutes. The sections were then washed in running de-ionized water, dehydrated, cleared and mounted in synthetic resin medium.<sup>5</sup>

## Counting criteria

AgNORs were quantified by manual counting method using the image analyzer software, ProgRes CapturePro v2.8.8. For each case, 50 nuclei of tumor cells from five microscopic fields were examined under 40X magnification (ten cells were selected randomly from each microscopic field). The images were captured in 40X magnification and classified,

## INTRODUCTION

Ameloblastoma is a locally invasive, highly destructive benign tumor of odontogenic origin. It is classified clinically into unicystic, multicystic, peripheral and desmoplastic types.<sup>1</sup>

Among which multicystic ameloblastoma has a greater recurrence rate compared to peripheral and unicystic ameloblastoma. For a better understanding of the aggressive behavior of ameloblastoma, various methods used for evaluation of proliferative activity.<sup>2</sup> Argyrophilic nucleolar organizer region (AgNOR) uses silver to stain the proteins.<sup>3</sup> Nucleolar organizer regions (NORs) are loops of DNA that transcribe to ribosomal RNA and direct ribosome formation and protein synthesis.<sup>4,5</sup>

Study aimed to compare number of AgNORs in unicystic and multicystic variants of ameloblastoma in order to analyze their cell proliferation rates.

## MATERIAL AND METHODS

Total 20 cases of ameloblastoma were retrieved from the archives of the Department of Oral Pathology, SVS Dental College, Mahabubnagar, Telangana. Of which 10

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transferred and stored in the computer. Only well-defined and sharply stained intra-nuclear AgNOR dots were included in the counting regime. AgNORs which were located within the nuclei at the border of the microscopic field or superimposed nuclei were not included in the study. Counting was done as recommended by Crocker et al., where all silver stained structures were counted but when lying in clusters, each cluster was counted as one.<sup>6</sup>

### STATISTICAL ANALYSIS

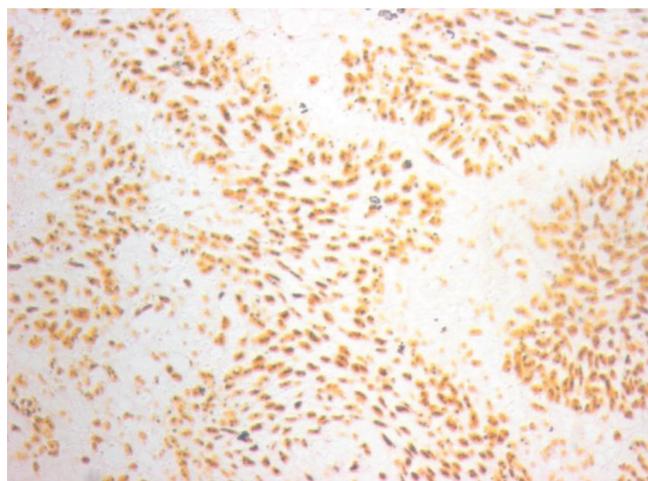
Obtained data was analysed statistically by using “Unpaired t-test” with statistical software Graph pad prism version 6.0.

### RESULTS

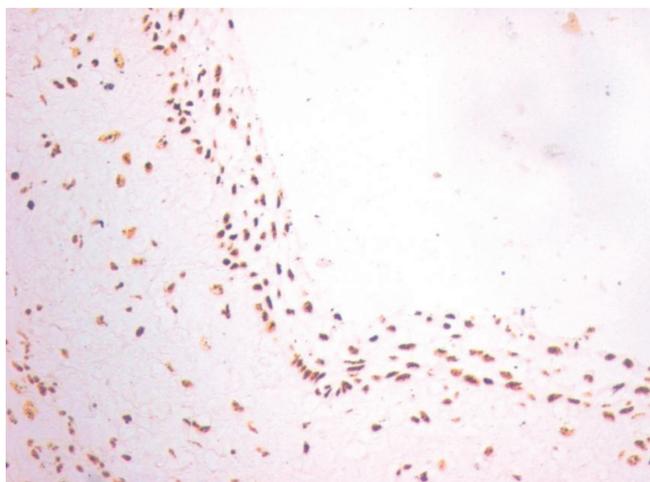
AgNORs were located within the cell nucleus and were distinctly stained in black as dots and blebs; the rest of the nucleus stained yellow-brown (Figure 1,2,3,4).

In the present study mean number of AgNOR dots per nucleus in unicystic ameloblastoma was  $2.684 \pm 0.210$

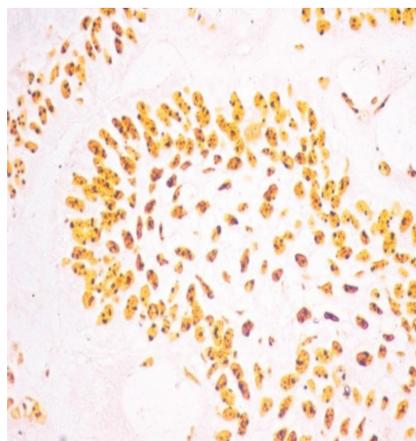
and in multicystic variant was  $4.236 \pm 0.808$  (Table1). A significantly higher number (*P*- value of 0.012) of AgNOR



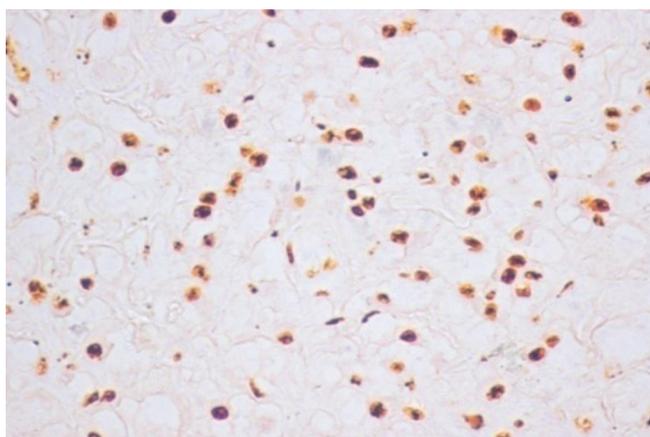
**Figure-3:** AgNOR stained section in multicystic ameloblastoma under 20X magnification.



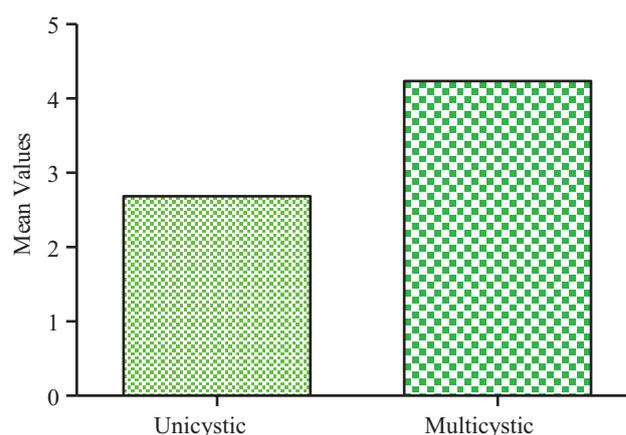
**Figure-1:** AgNOR stained section in unicystic ameloblastoma under 20X magnification.



**Figure-4:** AgNOR stained section in multicystic ameloblastoma under 40X magnification.



**Figure-2:** AgNOR stained section in unicystic ameloblastoma under 40X magnification.



**Graph-1:** The simple bar diagram for the comparison between unicystic and multicystic variants of ameloblastoma for the parameter AgNOR number.

Parameter- AgNOR number	No. of cases	Range	Mean	SD	P-value
Unicystic	10	2.58 to 3.06	2.684	0.210	0.012
Multicystic	10	3.1 to 5.02	4.236	0.808	

**Table-1:** The comparison between unicystic and multicystic variants of ameloblastoma for the parameter AgNOR number.

dots per nucleus were found in multicystic ameloblastoma than in unicystic ameloblastoma (Graph 1). Most of the unicystic variant contained one or two AgNOR dots per nucleus, while most of the multicystic variant contained two or three AgNOR dots per nucleus.

## DISCUSSION

Unicystic and multicystic variants of ameloblastoma have different recurrence rates and proliferative activity. Hence we performed analysis of AgNOR number in these two variants of ameloblastoma to compare their cell proliferation rates. On comparing the number of AgNORs between unicystic and multicystic variants of ameloblastoma significant difference was noted.

H G Coleman et al., (1996) conducted a study to determine whether nucleolar organizer regions (AgNORs) may be of value in distinguishing various odontogenic cysts from the unicystic ameloblastoma. According to their results dentigerous cysts had significantly higher AgNOR counts than the residual cysts and unicystic ameloblastomas and they concluded that AgNOR counts are not of diagnostic significance and cannot be used to differentiate the various odontogenic cysts from one another nor from the unicystic ameloblastoma.<sup>7</sup>

do Carmo MAV et al., (1998) performed a study in which ameloblastoma and adenomatoid odontogenic tumour were analyzed for proliferative activity by the AgNOR technique. The significant difference observed was only between the follicular and the plexiform variants of ameloblastoma.<sup>8</sup>

M. R. Payeras et al., (2007) conducted a study to evaluate the proliferation activity by means of the quantification of the argyrophilic nucleolar organizer regions (AgNORs) and the epidermal growth factor receptor (EGFR) expressed in different histologic variants of ameloblastomas. Their results did not show a significant statistical difference regarding the quantification of the AgNORs.<sup>9</sup>

Anna Corrêa Santos et al., (2008) investigated the proliferative activity present in luminal and mural areas of unicystic ameloblastomas, in conventional ameloblastomas, and in dentigerous cyst, comparing them according to their biological behavior. Unicystic ameloblastomas showed significant lower number of NORs / nucleus in the luminal proliferation area than in the area of mural proliferation and in conventional ameloblastomas.<sup>10</sup> Results are in correlation with the present study with increased number of AgNORs in multicystic / conventional ameloblastomas.

Jain et al., (2012) conducted a study on follicular and plexiform ameloblastoma and observed increased morphometric area measurements of AgNOR for plexiform and significantly higher number of AgNOR for follicular ameloblastoma and they concluded that follicular ameloblastoma was more aggressive than plexiform ameloblastoma, as it showed smaller AgNOR area and higher AgNOR number compared to plexiform ameloblastoma which showed higher AgNOR area and lower AgNOR number.<sup>5</sup>

In a study done by Rizvi et al., (2013) mean AgNOR values were studied among 4 study groups (desmoplastic,

plexiform, acanthomatous, follicular). Among these variants mean AgNOR was highest for acanthomatous with a mean of 3.15 and lowest for follicular variant with a mean of 1.39. Our study showed similar results as we noted more AgNOR number in multicystic ameloblastoma with a mean of 4.236.<sup>11</sup> Anuradha et al., (2014) conducted a study using AgNOR in keratocystic odontogenic tumor, multicystic ameloblastoma, unicystic ameloblastoma where they noted AgNOR count was more in keratocystic odontogenic tumor when compared to multicystic, unicystic ameloblastoma and the pattern of distribution of AgNORs more in basal than in the parabasal layer in keratocystic odontogenic tumor. The qualitative analysis showed small to large oval AgNOR's in keratocystic odontogenic tumor and few clusters in multicystic ameloblastoma whereas in unicystic ameloblastoma irregular clusters were seen and the results are correlating with present study.<sup>12</sup>

Navjeevraj et al., (2016) and Alessandra Dutra da Silva et al., (2016) assessed and compared the role of AgNORs in cell proliferation between ameloblastic carcinoma, solid and cystic variants of ameloblastoma. They found higher mean AgNOR in ameloblastic carcinoma than in solid ameloblastoma and unicystic ameloblastoma. We found similar results as we also noted less number of Ag NOR in unicystic ameloblastoma compared to multicystic ameloblastoma.<sup>13,14</sup>

Gupta Nidhi et al., (2017) investigated the usefulness of AgNORs as quantitative and qualitative criteria in assessing the proliferation potential of dentigerous cyst, keratocystic odontogenic tumor, conventional ameloblastoma and unicystic ameloblastoma. The mean number of AgNORs was found to be most for conventional ameloblastoma ( $2.96 \pm 0.65$ ) and least for dentigerous cyst ( $1.64 \pm 0.25$ ) where as unicystic ameloblastoma showed value of ( $2.44 \pm 0.38$ ).<sup>15</sup> The present study showed similar results.

Rise in AgNOR material denotes a rise in protein synthesis.<sup>16</sup> Therefore, we may infer that almost half of the ameloblastoma cells would be synthesizing protein and this protein might participate in a mechanism that would contribute in part to the biologic behavior of this neoplasm. The aggressive behavior of the lesion can be assessed with this simple stain without involving the procedures such as immunohistochemistry and other molecular methods.

## CONCLUSION

Cells with an active proliferation have an impaired nucleolar activity and hence exhibit an increase in AgNOR count regardless of the ploidy state of the cell. This shows the expediency of AgNOR staining in reflecting the high proliferation rate and a more aggressive behavior of multicystic ameloblastoma in comparison to unicystic ameloblastoma.

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