

Role of AgNORs to Analyse Cell Proliferation in Histologic Variants of Ameloblastoma (Original Research)

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Abstract: Ameloblastomas are the most clinically significant benign odontogenic neoplasm. This study was conducted to find the role of AgNORs in cell proliferation in Ameloblastoma. The study population consisted of 34 histologically diagnosed cases of Ameloblastoma. AgNOR counting was done on silver stained sections to analyse the proliferative activity. A statistically significant difference in AgNOR counts was found between conventional and malignant types, unicystic and malignant types.

1. Introduction

Ameloblastoma described by Robinson are usually unicentric, non-functional, intermittent in growth, anatomically benign and clinically persistent. AgNOR's are NOR- associated acidic proteins selectively stained by silver methods¹. The number of nuclear organizer regions (NOR) can be used to reveal the degree of cell activity or metabolism in histopathologic specimens². The NOR's loops of DNA that transcribe genes for ribosomal RNA in nucleolus and are intimately related to the cell cycle. The amount of AgNOR proteins is strictly proportional to the proliferative activity of the cell. Here study was conducted regarding proliferative activity of 34 cases of ameloblastomas by AgNOR staining.

2. Aims and Objectives

- 1) To quantitatively assess the AgNOR in ameloblastomas.
- 2) To evaluate whether AgNOR can be of value in determining the proliferative capacity of histological forms of ameloblastomas

3. Materials and Methods

The paraffin blocks of the cases were cut with the help of a rotary microtome and two sections of 4-6 µm thickness and 2 µm thickness were taken for the haematoxylin and eosin staining and the silver nitrate staining respectively. The slides for the two staining procedures were marked and kept separately.

Silver staining:

The nucleolar organizer regions were stained by the silver nitrate method described by Ploton et al³. The staining was done with the working solution which was prepared as follows:

Solutions:

50% silver nitrate solution
Silver nitrate 50g

Distilled water 100 ml

Gelatin solution:

Gelatin 2g
Formic acid 1 ml
Distilled water 100 ml

Working solution:

Silver nitrate solution 2 parts
Gelatin solution 1 part

The two solutions were mixed immediately before use.

Staining method:

- 1) Dewax sections in xylene, hydrate through alcohols to water
- 2) Rinse sections in distilled water
- 3) Incubate in freshly prepared working solution for 45 minutes at room temperature
- 4) Wash in distilled water for 1 minute
- 5) Dehydrate, clear and mount in DPX

Results

AgNOR sites → intranuclear black dots.

Background → pale yellow

The silver reaction product is seen as discrete black dots at the light microscope level and these can be enumerated using a x100 oil immersion lens. Counts in 100 cells of epithelium were made. The results were expressed as the mean number of NOR's per nucleus.



Figure 1: Materials used for AgNOR staining

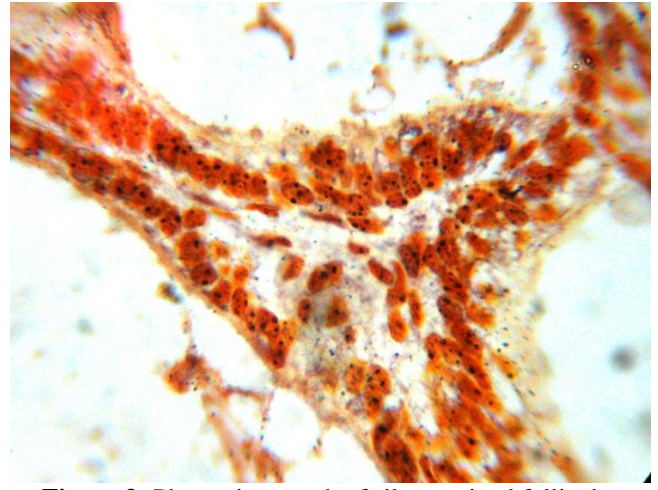


Figure 3: Photomicrograph of silver stained follicular ameloblastoma showing AgNORs (H & E 40x)

4. Results and Observations

The role of AgNOR in their cell proliferation was studied in 34 cases.

AgNOR counts

AgNORs were counted as black dots within the nuclei of the epithelial cells. A total number of 100 cells were counted in adjacent fields. The average count of AgNOR conventional ameloblastoma was $1.63 \pm .23$ while in the unicystic ameloblastoma, the value was $1.49 \pm .13$. The malignant cases showed an average count of $2.17 \pm .29$.

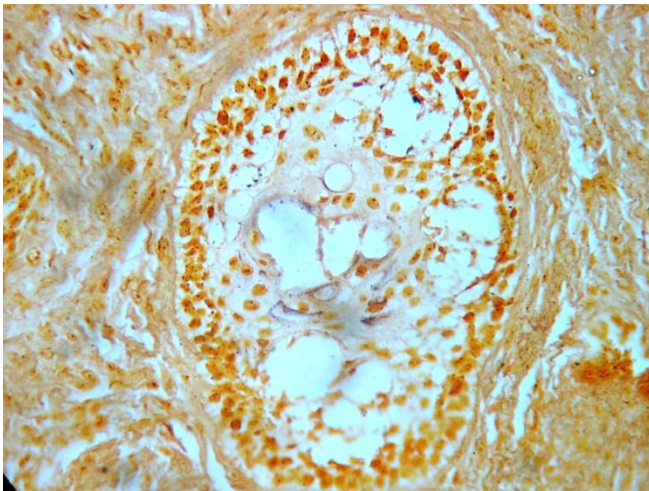


Figure 2: Photomicrograph of silver stained follicular ameloblastoma showing AgNORs (H & E 10x)

5. Discussion

The present study was aimed at studying the role of AgNOR's in cell proliferation in three broad categories of Ameloblastoma, namely, Conventional, Unicystic and Malignant types. The AgNOR technique has been useful in distinguishing benign from malignant lesions and between high and low grade tumors in diagnostic pathology⁴. It has been speculated that an increased number of AgNORs might reflect an increased transcriptional activity and might indicate increased nuclear and cellular activity⁵.

34 cases of our series were distributed into three groups based on the histological characteristics: conventional ameloblastoma, unicystic ameloblastoma and malignant type.

The study showed that there is a significant difference between conventional & malignant ameloblastomas and unicystic & malignant types and there was no significant differences between conventional and unicystic types. The findings therefore show that, AgNOR counts may be useful as a proliferative marker to differentiate between benign and malignant forms of ameloblastomas⁶.

AgNOR staining is a rapid, efficient and inexpensive procedure and provides useful information regarding cellular proliferation. The lack of a universally accepted enumeration protocol, overlapping of individual scores and intra-observer variations, however are its drawbacks.

Further, studies analyzing a combination of parameters such as AgNOR area, distribution, AgNOR volume, contour etc could be more representative of the cellular proliferation. This along with combination and comparison of AgNORs with other proliferation markers such as PCNA and Ki-67 and the use of computed image analyzers to reduce intra-observer variation might substantiate the claims of the AgNOR technique as a diagnostic marker in histopathology. Lastly, clinicopathologic analysis utilizing a larger sample size will help in better characterization of Ameloblastoma in our population.

6. Summary and Conclusion

- The AgNOR counts were not significant between conventional and unicystic types
- AgNOR counts were found to be highest in the malignant type, followed by the conventional type. Unicystic variant was seen to have the lowest observed AgNOR counts.
- AgNOR counts may be useful as a proliferative marker to differentiate between benign and malignant forms of ameloblastomas.

References

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