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Research Article

IMMUNOHISTOCHEMICAL COMPARISON OF Ki-67 EXPRESSION IN EPITHELIAL CELL PROLIFERATION AMONG VARIANTS OF AMELOBLASTOMAS

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A B S T R A C T

Introduction: Ameloblastoma has a diverse clinicopathological appearance. Several treatment modalities have been implicated based on such morphology. In this study we are evaluating cell proliferative marker Ki-67 in various histological and clinical subtypes of ameloblastomas.
Background: The primary aim of the study is to know the behaviour of various clinicopathological variants of ameloblastomas on the basis of Ki-67 expression in its epithelial cell proliferation.
Methods: Most common variants of ameloblastomas(n=20) consisting six follicular ameloblastomas(FA), six plexiform ameloblastomas(PA), two granular cell ameloblastomas(GCA) and six unicystic ameloblastomas(UA) with luminal proliferation were selected and examined morphologically and immunohistochemically for changes in proliferative activity using Ki-67 markers.
Results: The Ki-67 labelling index was significantly higher in FA,PA,UA than GCA. But ki-67marker mean labelling index difference was insignificant among FA,PA,and UA. Among various histopathological variants highest ki-67 mean labeling index was observed for unicystic ameloblastoma(46%) followed by plexiform(37.73%) and follicular ameloblastoma(37.6%) and was minimal in granular cell ameloblastoma(7.8%).
The pearson correlation (which is significant at the 0.01 level) for ki-67 expression was insignificant.(.977)among FA,PA,UA and GCA.
Conclusion: The pattern of expression of ki-67 varies among variants of ameloblastomas so it clearly indicates that it is not fruitful to compare ki-67 marker in ameloblastomas despite it is useful to assess the neoplastic behavior and recurrence of ameloblastomas. Also it can be concluded that histopathological variants have no or little role in assessing the behavior and prognosis and all ameloblastomas should be assessed clinically for treatment modalities.

INTRODUCTION

Ameloblastoma is the most frequently encountered benign epithelial odontogenic tumor(1,2) and is characterized by a locally invasive behaviour with a high risk of recurrence rate despite radical therapy.(3,4) Histopathologically, ameloblastomas show different variants including follicular, plexiform, acanthomatous, granular cell, clear cell, desmoplastic, basal cell, mucinous, hemangiomatous, papilliferous keratoameloblastoma and clinical variants like peripheral, metastasizing and unicystic ameloblastomas with variable growth pattern including luminal, intraluminal and mural proliferation (1,5).

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It has been reported that the aggressiveness of ameloblastomas may be related to the histological subtype(3), infact in a review of the literature, found for plexiform, follicular, acanthomatous and unicystic ameloblastoma recurrence rates, respectively, of 16.7%, 29.5%, 4.5%, and 13.7%.(4)Several studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors; however the detailed mechanism of oncogenesis, cytodifferentiation remain unknown(6,7,8).

Ki-67 antigen is present in all active parts of cell cycle, rises during later half of S phase and reaches a peak in G2 and M phase and rapidly degrades after mitosis with a half life of detectable antigen being an hour or less. So it correlates with other variables of cell proliferation.(9)

As we know that irreversible increased cellular proliferation is a phenomenon of tumor, so in our study we are using

proliferative marker Ki-67 to assess correlation of epithelial cell proliferative among histological variants of ameloblastomas including follicular, plexiform, granular cell ameloblastomas and unicystic ameloblatoma with luminal proliferation.

MATERIALS AND METHODS

The study material comprised of archival formalin fixed paraffin embedded specimens of patients from private oral pathology laboratory as 20 cases of ameloblastomas. The cases were selected on the basis of most commonly encountered clinicopathological variants of ameloblastomas and included 6 cases of follicular ameloblastoma, 6 cases of plexiform ameloblastoma, 2 cases of granular cell ameloblastoma and 6 cases of unicystic ameloblastoma with luminal proliferation.

All selected cases had been routinely fixed in 10% neutral formalin (24-48 h), dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Using haematoxylin and eosin-stained sections, all the slides were reviewed, the quality of the material was checked and the slides for the quantitative evaluation of the material were selected. In each case the slides selected showed the peripheral and the central zone of the tumour. Each individual tissue block was cut for one individual slides. 1 Control slide was also made for each group of cases. Standard immunohistochemistry staining protocol was performed (9) using primary antibody monoclonal mouse monoclonal mouse anti-human Ki-67 (1:100 prediluted) and super sensitive poly horse radish peroxidise (HRP) secondary antibody kit (Biogenex India private limited). Slides were checked for positive staining as crisp, brown nuclear staining for Ki-67 in respective sections. Positive controls was obtained from lymphoma for Ki-67. Negative control was included by performing duplicate assays, in which the primary antibody was replaced with phosphate buffer saline.

Evaluation of Ki-67 Staining

Representative fields were selected in each case as randomly or without bias as possible. These areas included only labelled cystic or tumor epithelial areas and excluded areas of necrosis, inflammation, and stromal cells. Representative fields were selected in each case of solid and multicystic ameloblastomas and along the cyst lining of unicystic variant. All cell counts were performed with a Olympus Ch20i microscope fitted with an eyepiece graticule, x10 oculars, a x40 objective, and a counting grid containing 400 blocks in conjunction with a hematology laboratory differential cell counter (Olympus India limited). The cases were scored by counting the positive cells per minimum of 500 tumor cells per specimen. The percentage labelling index (number of positive tumor cells/total number of tumor cells expressed as a percentage) was calculated per case. To determine intraobserver and interobserver reliability, the same examiner counted the cells in each case twice at one hour interval while a second examiner counted the cells independently in a sample of the cases using the same positions of the grid.

Statistical analysis

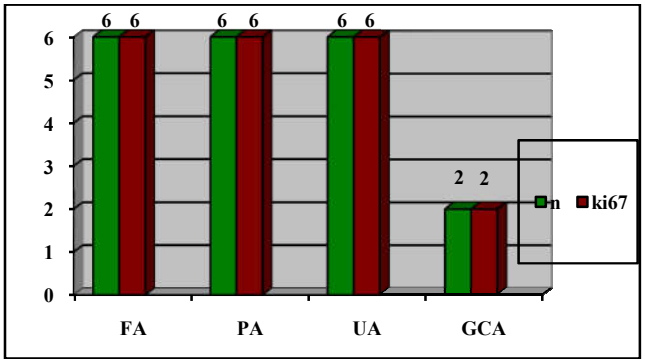
Descriptive statistical analysis was performed for each group of lesions by statistical software program SPSS (version 16.0). The mean labeling indices were compared by using an analysis of variance test (ANOVA) for Ki-67. Kruskal-Wallis Test were then performed to determine statistically significant differences in Ki-67 expression overall. Probability levels of < 0.05 were regarded as being statistically significant. To satisfy the statistical assumptions of the tests, all data were square root transformed before analysis.

RESULTS

Positive cases were selected on basis of positive staining by Ki-67 marker in same cases.(Graph 1) There was no significant difference found among follicular, plexiform and unicystic ameloblastomas for mean labelling index of Ki-67 marker but these variants shows significantly higher mean labelling index than granular cell ameloblastomas for Ki-67 marker. (p=.041)(Graph-2)

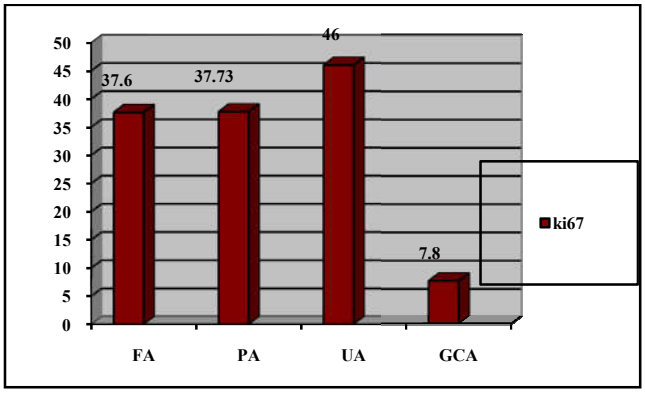
The pearson correlation (which is significant at the 0.01 level) Ki-67 expression among variants of ameloblastomas was insignificant.(.977)

Nuclear Ki-67 reactivity was exhibited by ameloblastomas, mostly in basal and parabasal odontogenic epithelial cells in many areas of different variants of ameloblastomas. Among various histopathological variants highest Ki-67 mean labelling index was observed for unicystic ameloblastoma followed by plexiform and follicular ameloblastoma and was minimal in granular cell ameloblastoma. (Figure1-4)



Graph 1 Immunopositive Samples of Ameloblastomas

(FA-Follicular Ameloblastoma,PA-Plexiform Ameloblastoma, UA- Unicystic Ameloblastoma, GCA- Granular Cell Ameloblastoma, n=Number Of Cases)



Graph 2 Mean Labeling Index For Ki-67 In Ameloblastomas

(FA-Follicular Ameloblastoma, PA-Plexiform Ameloblastoma, UA- Unicystic Ameloblastoma, GCA- Granular Cell Ameloblastoma)

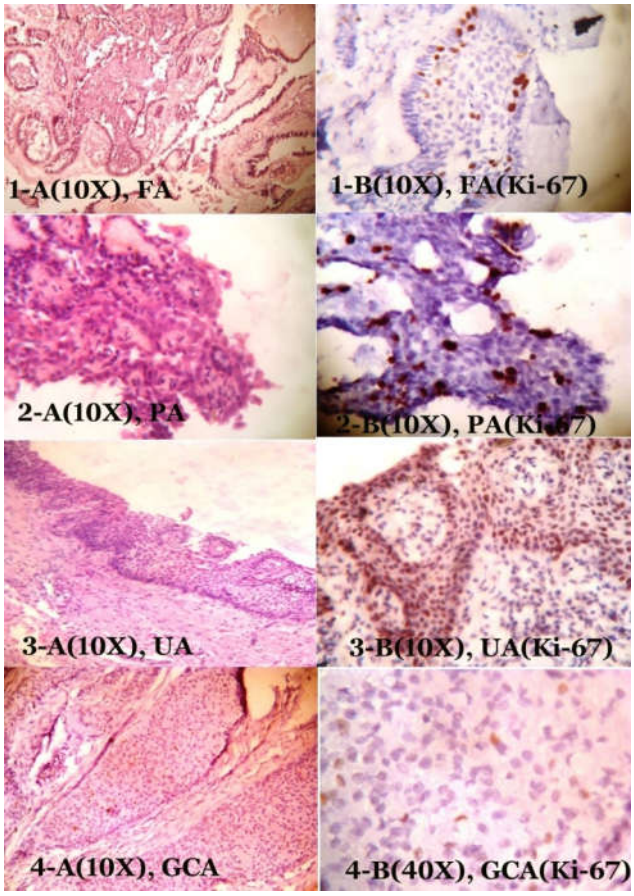


Figure Legends-1(A-B) Follicular ameloblastoma(FA) ,1A-H&E section of follicular ameloblastoma (10x),1B Positive Ki-67 expression in peripheral & central cells of follicular ameloblastoma(10x), 2 (A-B) Plexiform ameloblastoma (PA), 2A-H&E section of plexiform ameloblastoma (10x),2B-Positive Ki-67 expression in peripheral & central cells of plexiform ameloblastoma (10x), 3 (A-B) Unicystic Ameloblastoma,3A-H&E section of unicystic ameloblastoma (10x),3B- Positive Ki-67 expression in basal & suprabasal cells of unicystic ameloblastoma(10x),4 (A-D) Granular cell ameloblastoma(GCA),4A-H&E section of granular cell ameloblastoma (10x), 4D-Sparse positive Ki-67 expression in basal & central cells of granular cell ameloblastoma(40x)

DISCUSSION

Many investigation of tumor cell proliferative activity in tumorigenesis have been evaluated using Ki-67 in oral and other systemic tumors.(10-13)

In this study, assessment of cellular proliferation markers was shown to be accurate, reliable and reproducible and produced excellent comparable results. The use of labelling indices has, however, proven to be extremely tedious for routine application in the assessment of cellular proliferation.

Keeping the concept of tumor in mind the most common property is excessive proliferation of tumor cell, we carried out this study with selection of Ki-67 epithelial proliferative marker to assess the clinicopathological behaviour of various histopathological variants of ameloblastomas. Variable expression of Ki-67 noted among histopathological variants of ameloblastomas.

Unicystic ameloblastoma showed statistically significantly higher Ki-67 labelling indices than solid variants. It was similar to results of Meer *et al* (9) and this variation can be attributed to difference in methodology, especially the counting protocol, used. Unfortunately it is very difficult to compare these parameters because published reports frequently fail to provide sufficient detail or explanation. Another possible reason might be the difference in the morphology of the tumors, with the solid lesions providing large follicles or plexiform sheets for analysis, whereas only a thin lining is available in the unicystic cases. This may have resulted in the inclusion of greater numbers of basal and parabasal cells in the unicystic group, thus resulting in higher mean labeling indices. This is unlikely as cells in the entire thickness of the epithelial linings of the unicystic lesions were included in the count (9). Inclusion of granular cell variant has resulted in decreased overall expression of Ki-67 marker. Granular cell variant minimal cellular proliferation can also be explained on the basis of loss or inactivation of signaling pathways related to cell proliferation and differentiation in the granular cells, suggesting that these cells are functionally inactive and their synthesis and secretion activities have become irregular (17).

Ki-67 proliferating marker was observed predominantly in peripheral cells of tumor islands and cystic lining. This could explain the locally infiltrating growth of ameloblastomas and its aggressive behaviour. Difference in morphology of ameloblastomas facilitates more difficulty in surgical accessibility of conventional ameloblastomas than unicystic ameloblastomas. So it can explain recurrence and worse prognosis in conventional ameloblastomas than unicystic ameloblastomas. (9)

The result of Ki-67 expression for follicular, plexiform and unicystic variants were significantly higher than granular cell ameloblastoma. However comparision among different variants of ameloblastomas for ki-67 markers was insignificant. (18)

CONCLUSION

So overall result clearly indicates the pattern of expression of ki-67 varies among variants of ameloblastomas so it is not fruitful to compare Ki-67 marker in ameloblastomas variants however it is useful to assess the neoplastic behaviour of ameloblastomas.

Ki-67 on the other hand could provide useful prognostic markers for proliferative activity and good prognostic indicators for recurrence of ameloblastomas. Our study clearly indicated that except granular cell ameloblastom, other variants of ameloblastomas have high proliferative potential. Granular cell ameloblastoma shows minimal proliferation and can be explained on molecular and pathogenetic basis as initially we use term granular cell changes in ameloblastoma suggesting that these changes are initiating in central stellate reticulumn like cells of either foliicular or plexiform ameloblastoma and in course of time granular cell changes involves even peripheral ameloblastoid cells and presents as extensive granular cell changes in whole tumor mass and at this time term granular cell ameloblastoma should be applied.(14-16,19) So granular cell ameloblastom can be considered as mature tumor showing minimal proliferation based on our results as well as previous molecular studies. So apart from proliferative potential, it is the ease with which a

tumor can be removed will decide the tumor behaviour. So all ameloblastomas should be assessed clinically for treatment modalities.

However further study with large sample size of different clinicopathological variants and other relevant tumor marker analysis will be required to confirm these findings and to make a definite cause and effect relationship.

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