Diagnostic Utility of Ki-67 for Histopathological Typing of Conventional Ameloblastoma, Proliferative Ameloblastoma and Ameloblastic Carcinoma – A Retrospective Study

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ABSTRACT

BACKGROUND

Ameloblastoma is a rare, benign odontogenic neoplasm which is locally aggressive and mostly present with a painless swelling. The enigma about the diagnosis of proliferative ameloblastoma and ameloblastic carcinoma is still a debate because the diagnostic criteria is not standardized or quantified which has a direct correlation on its biological behaviour and prognosis. Despite numerous studies, correlation between the histological patterns of ameloblastoma and tumour behaviour has not been consistently established. The present study was done to compare the expression levels of Ki-67 between conventional ameloblastoma, proliferative ameloblastoma and ameloblastic carcinoma and to assess the usefulness of these markers for diagnostic differentiation.

METHODS

A retrospective study of total of 18 cases of ameloblastoma were retrieved from the archives of Department of Oral and Maxillofacial Pathology, Saveetha Dental College from 2012 till 2019, which included conventional ameloblastoma, proliferative ameloblastoma and ameloblastic carcinoma. Immunohistochemical (IHC) analysis was done using the marker Ki-67 and labelling index were determined for the same.

RESULTS

The results of the current study showed that the cellular proliferative activity assessed using Ki-67 in follicular ameloblastoma was (55 %), 4 cases of plexiform ameloblastoma (22 %), 17 % of proliferative ameloblastoma and 6 % of ameloblastic carcinoma showed negative expression.

CONCLUSIONS

Immunophenotyping using the marker Ki-67 may be a useful tool for histological typing of ameloblastoma.

KEY WORDS

Ameloblastoma, Ki-67, IHC, Immunophenotyping

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BACKGROUND

Group of lesions that arise from tooth forming tissues or their remnants are the odontogenic cysts and tumours which originate from odontogenic epithelium or ectomesenchyme with various degrees of inductive tissue interactions.¹ Different odontogenic cysts and tumours have variable clinical and biological behaviors.² Ameloblastoma is a local, aggressive, benign odontogenic neoplasm that attained greater consideration in both medical and dental literature from the 19th century. According to the World Health Organization (WHO) classification, the clinical types of ameloblastoma are: conventional, unicystic, extraosseous / peripheral, and metastasizing ameloblastoma.³ Histologically, ameloblastoma is classified as follicular, plexiform, acanthomatous, granular, desmoplastic, and basal. With the radiographic features, it has been classified as unicystic or multicystic.⁴ Among all the types, about 86 % of the cases are being reported as multicystic or solid type. There are also 3 subtypes for the unicystic type which are luminal, intraluminal and mural.⁵ Ameloblastomas are the second most common odontogenic tumours following the odontome. Ameloblastoma usually presents as a painless, slow growing, non-metastatic, locally invasive swelling that histologically resembles the enamel organ of developing teeth but doesn't form dental hard tissues.

Depending on various factors like the size, histological variant, anatomical location and involvement, the treatment is decided.⁶ There are about 1 % of the cases that have been reported for malignant transformation. These malignant types of ameloblastoma may arise or may even transform from an already existing ameloblastoma. Spread from lungs, cervical lymph nodes are reported as the commonest sites of metastasis.⁷

Despite numerous studies, correlation between the histological patterns of ameloblastoma and tumour behaviour has not been consistently established. The mechanism of oncogenesis, tumour progression of ameloblastoma are still largely unknown. The enigma about the diagnosis of proliferative ameloblastoma and ameloblastic carcinoma is still a debate because the diagnostic criteria are not standardized or quantified which has a direct correlation on its biological behaviour and prognosis.⁸

Determination of epithelial proliferative activity plays a crucial role in determining the biological behaviour of the tumour, tumour classification and monitoring treatment response and relapse.⁸ Ki-67 is one of the most commonly used proliferative IHC markers to evaluate proliferative activity and biologic behaviour of most pathologic lesions. Ki-67 is a non-histone nuclear protein having correlations with cell growth. The expression of Ki-67 usually has alterations throughout the cell cycle with various expression levels in mid G1, increase in S and G2 with a peak in mitosis (M). The absence of this marker in the G0 phase helps to determine the growth fraction of a cell population. Ki-67 is detectable in the nucleus of proliferating cells. Ki-67 is one clear cut marker for proliferating cells which are mostly found during S phase.⁹ Ki-67 expression in ameloblastoma is associated with a more invasive pattern of growth with increased rate of recurrence.

The proliferation of cells is one necessary step that takes place in all living organisms as it has a major part in

maintaining the homeostasis of the tissues and growth of the cells.¹⁰ When it comes to neoplasms, the proliferative activity is completely altered compared to the normal process.⁸ At this time, the necessity for evaluating the cell proliferative activity through immunohistochemistry became one important technique to provide beneficial understanding upon the nature of various tumors.⁹ The incorporation of various immunologic research methods to histopathology has helped to emerge a notable increase in the microscopic diagnosis of neoplasms. Despite the fact that tissue sections stained with haematoxylin and eosin stand first when it comes to histological analysis, immunohistochemistry has become an effective tool in the armamentarium of the pathologists.¹¹ Immunohistochemistry is one technique which helps in recognizing the cellular / tissue components by antigen-antibody interactions. Immunohistochemistry mainly focuses on markers of specific cells and tumour type that guides in the diagnosis of the specific tumour.¹²

The main objective of this study was to determine the immunohistochemical expression of Ki-67 in conventional ameloblastoma, proliferative ameloblastoma and ameloblastic carcinoma, and to perform the histological typing of the ameloblastoma based on the expression of Ki-67.

METHODS

This is a retrospective study. A total of 18 cases histologically diagnosed as ameloblastoma were selected from 2012 to 2019. Based on the histopathology reports, the cases were classified into different types and selected accordingly. Different histological types of ameloblastoma included in the study were conventional follicular¹⁰, conventional plexiform⁴, proliferative ameloblastoma³ and ameloblastic carcinoma.¹ Epidemiological, clinical data and outcome were collected by retrospective review of the patient's charts from the department of oral & maxillofacial surgery of the same hospital.

Histological slides were reviewed and slides comprising the largest amount of tumour tissue and not containing any bone fragments were selected in each case and the corresponding paraffin embedded blocks were retrieved from the archives of the Department of Oral and Maxillofacial Pathology, Saveetha Dental College, Chennai.

Immunohistochemistry

3 µm sections were cut from formalin-fixed paraffin-embedded blocks mounted on gelatin-coated slides. Then sections were deparaffinized in xylene for 10 minutes & followed by dehydration in 100 % alcohol for 5 minutes and rinsed in distilled water. Following which heat mediated antigen retrieval with Tris – ethylene diamine tetra acetic acid (EDTA) buffer solution of 9.0 pH was done in a pressure cooker for 5 minutes. Depressurize the pressure cooker to 37 c under running tap water. Endogenous peroxidase was blocked for 30 minutes. Sections were incubated with primary antibody, Ki-67 (Dako, FLEX Monoclonal mouse anti-human Ki-67 antigen clone, Denmark) for 1 hour at room temperature. Sections were washed and incubated with secondary antibody (Polyexcel HRP) for 30 minutes at room temperature.

Detection was performed using polyexcel HRP/DAB detection system (Pathnsitu, conjugated by goat anti mouse/rabbit IgG, USA). The sections were then counterstained with Mayer's haematoxylin and were then dehydrated and mounted using dibutyl phthalate in xylene mountant. Negative and positive controls were used in each run.

Scoring Criteria

Positive reactivity was determined by the existence of the brown coloured reactions at the site of target antigen. Ki-67 labelling index was done by calculating the number of positive tumour cells/total number of tumour cells and expressed as a percentage. The scores are tabulated in table 1.

Statistical Analysis

All the results were tabulated and assessed for statistical analysis using Statistical Package for Social Sciences (SPSS) (IBM SPSS Statistics for Mac Version 20.0). Difference between mean values was considered statistically significant when P value was ≤ 0.05 .

RESULTS

A total of 18 histopathologically diagnosed ameloblastoma cases were included in this study. The selected cases were analysed for immunohistochemistry staining for the expression of Ki-67. Table 2 shows the patients characteristics. In the current study, the male : female ratio was 1 : 1. Age distribution ranged from 12 to 67 years, with a mean age of 34.71 years. The specimens comprised of 10 cases of follicular ameloblastoma (55 %), 4 cases of plexiform ameloblastoma (22 %), 17 % of proliferative ameloblastoma and 6 % of ameloblastic carcinoma.

Score	Proportionality Index	Intensity of Staining
0	No labelling	Negative
1	0 to 7 % of tumour cells are labelled	Mild
2	8 to 15 % of tumour cells are labelled	Moderate
3	> 15 % of tumour cells are labelled	Severe

Table 1. Scoring Criteria for Evaluation of Expression of Ki-67

Factor	Group	Total Sample (N %)
Gender	Male	9 (50 %)
	Female	9 (50 %)
Age	< 50	14 (78 %)
	> 50	4 (22 %)
Anatomical location	Anterior	0
Site	Posterior	15 (83 %)
	Mandible	18 (100 %)
	Maxilla	0
Histopathological type	Conventional follicular	10 (55 %)
	Conventional plexiform	4 (22 %)
	Proliferative	3 (17 %)
	Ameloblastic carcinoma	1 (6 %)
Recurrence	Yes	1 (6 %)
	No	17 (94 %)

Table 2. Patient Characteristics

Histopathological Type (n=18)	Ki67 Expression	Level of Expression
Conventional follicular	Negative	NA
Conventional plexiform	Negative	NA
Proliferative	Negative	NA
Ameloblastic carcinoma	Negative	NA

Table 3. Expression of Ki - 67 According to the Histopathological Characteristics of Ameloblastoma

Tumours were predominantly located in the posterior regions of the mandible. Recurrence of the ameloblastoma occurred in 1 case out of 18 and was seen in male. The results showed that Ki-67 showed negative expression irrespective of different types of ameloblastoma.

Figure 1. Ki-67 Showing Negative Expression in a) Proliferative Ameloblastoma, b) Ameloblastic Carcinoma, c) Follicular Ameloblastoma, d) Plexiform Ameloblastoma

DISCUSSION

Immunohistochemistry is a useful diagnostic tool for both monoclonal and polyclonal antibodies to discover the definite antigen within the tissue section wherein it is widely used in the diagnosis of cancers.¹³ IHC plays a very crucial role in the diagnosis of many diseases as well. It has now become an essential part of diagnosis most widely used in medical research and clinical diagnostics.¹⁴ Ameloblastoma is a benign, local, aggressive tumour with high rate of recurrence and has a probability of malignant transformation.¹⁵

The clinical course in patients with ameloblastoma is difficult to predict and also there is no clear evidence that histologic features of ameloblastoma determine the degree of invasiveness, recurrence rate or malignant transformation. Therefore, there is a need for a predictive marker for clinical behaviour in ameloblastoma. The proliferative activity of tumour cells has been found to be an indicator of aggressiveness and aids in determining the prognosis of many tumours. The use of Ki-67 before surgical excision of the ameloblastoma may be an important step in the treatment plan to minimize the recurrence rate.

The results of the current study showed that cellular proliferative activity assessed using Ki-67 in recurrent, non-recurrent cases of ameloblastoma and ameloblastic carcinoma showed negative expression. This could be due to the small sample size of our study. The cell proliferative activity in ameloblastoma and ameloblastic carcinoma shows positive expression for Ki-67 whereas non proliferating cells that have the tendency to hark back to cell division cycle are expressed as negative for Ki-67.¹⁶

With regards to ameloblastic carcinoma, due to the rarity of this tumour, this study could include only one case. Chae et al. in his study proposed that there was no significant or clear

correlation between the proliferative activity and the clinical values.¹⁷ At the same time Carreon et al. observed a highest proliferative index in mandible with tumours measuring more than 5 cm in female patients with no recurrence.¹⁸ In this study, no significant correlations between clinical characteristics, recurrence, and protein expression were observed. Florescu et al. found a considerable variation of Ki-67 expression amidst central and peripheral cells which were considered to be zones of proliferation in an array of 14 ameloblastoma cases.¹⁹ Also in another study it was observed that few cells in the stellate reticulum like cells had positive expression for Ki-67 along with predominant peripheral cell positivity.²⁰ Wherein a study by Ahlem et al. observed that Ki-67 expression was considerably more in recurrent tumors.²¹ Another study by A. Hegab et al. also stated that there was a significant relation between the labelling index of Ki-67 and the recurrence of the disease, which was found to be higher in plexiform type followed by follicular type. He also in his study stated that there were variations in the cellular proliferating activity in different types of ameloblastoma and he also observed that statistically there was higher significance of Ki-67 in recurrent ameloblastoma than in non-recurrent ameloblastomas.²² There are also several studies which have shown that the Ki-67 labelling index is prognostically relevant.^{23,24}

CONCLUSIONS

In future, studies assessing Ki-67 expression in ameloblastoma and ameloblastic carcinoma with a longer follow-up period and with higher rates of recurrence is necessary. The current study suggests that there is no direct association between cell proliferation and clinical parameters. The significant expression of Ki-67 can be used as a prognostic marker as it is more specific for proliferating cells.

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