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PROLIFERATIVE POTENTIALITY OF AMELOBLASTOMA BY EXPRESSION OF Ki-67

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ABSTRACT Ameloblastoma is a type of Odontogenic tumor, commonly encountered in orofacial region. It is very aggressive in nature with potential features of recurrence. Ki -67 is already a proven proliferative marker, which is used in our study to assess the proliferative potentiality of Ameloblastoma. Incisional biopsy was done from 10 cases of Ameloblastoma and 5 normal cases as control, at the department of Oral pathology and Microbiology, BIDSH, Patna. Each specimen was processed and two sections were prepared and stained with hematoxylin and eosin and Ki-67 immunohistochemical marker. Positive stained cells with with Ki-67 in ameloblastoma along with normal epithelium were counted from four randomly selected areas and were quantified using a microscope at 400x magnification. Positive correlation seen with Ki-67 positive cells and percentages, which indicates the value of one variable increases, the other also increases. The overall conclusion is that Ki-67 expression can be used as a prognostic marker in Ameloblastoma.

KEYWORDS : Ki-67 marker, Ameloblastoma, Immunohistochemistry

INTRODUCTION

Cellular proliferation is considered to be biological process of fundamental importance controlled by highly co-ordinated mechanisms. These are various regulatory networks that mediate the embryonic and normal development, where as any defect or dysregulation may result in tumour formation. [2]

Ameloblastoma is the second most common true neoplasm of enamel organ type tissue which does not undergo differentiation to the point of enamel formation.

The immunostaining with Ki-67 protein can assess the proliferative potentiality of ameloblastoma. The Ki-67 protein (also known as MKI67) is cellular marker for proliferation. It is strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0).[5,6,8] Since Ki-67 expression is detected in all proliferating cells, those molecules are often used in research studies that investigate the growth characteristics of different cells in ameloblastoma. Hence the purpose of this study is to co-relate local aggressive behaviour of ameloblastoma with Ki-67 expression and to evaluate the efficiency of this proliferative marker in detecting the chances of recurrences.[1,2]

AIMS AND OBJECTIVES

1. To examine the pattern of expression of Ki-67 in ameloblastoma.
2. To evaluate the proliferative potentiality of ameloblastoma.
3. To investigate the possible role of Ki-67 in progression and recurrence of ameloblastoma.

MATERIALS AND METHODS

Sample collection and preparation:

The samples were collected from cases reported to the Department of Oral and Maxillofacial Pathology, BIDSH Patna between 2014 and 2016 which consisted of ten patients with ameloblastoma, and five patients with normal oral mucosa as controls. Among all 15 patients, 9 were male and 6 were female. The histopathology sections were stained with Hematoxylin and Eosin (H and E) and for Ki-67 (BioGenex polymer- HRP IHC detection system).Antigen retrieval for immunohistochemistry (IHC) was done using EZ-Retriever system technique. Assessment of antigen expressing cells was performed by using light microscope (Multi viewing Microscope Model CXR5) at 40X magnifications.

Interpretation of Ki-67:

The slides for Ki-67 were observed under light microscope with a magnification of 400 x. The tissue samples were thoroughly examined and four fields randomly selected. Each field being further subdivided into nine sum-total to reduce the counting error percentage.

Evaluation of staining:

The intensity of immunohistochemical staining was graded based on subjective evaluation of color exhibited (brown color) by antigen, antibody and chromogen complex as: negative (no color). The nuclei with clear brown color of staining intensity were regarded as positive. Known positive immunostaining slides were used as positive controls.

Statistical procedures:

Data obtained was compiled on a MS Office Excel Sheet (v 2010). Data was subject to statistical analysis using Statistical package for social sciences (SPSS v 22.0, IBM).

Master Chart

Sl. No.	Age	Gender	Group	Ki-67 Positive Cells	Ki-67 Negative Cells	Percentages of Ki-67 Positive Cells
1	65 Yrs	Male	Ameloblastoma	714	1215	37.01%
2	30Yrs	Male	Ameloblastoma	373	1222	23.39%
3	45 Yrs	Male	Ameloblastoma	246	656	27.27%
4	74 Yrs	Male	Ameloblastoma	387	1568	19.80%
5	36 Yrs	Female	Ameloblastoma	219	1083	16.82%
6	32 Yrs	Male	Ameloblastoma	453	1578	22.30%
7	40 Yrs	Male	Ameloblastoma	367	1029	26.29%
8	38 Yrs	Female	Ameloblastoma	268	1210	18.13%
9	48 Yrs	Female	Ameloblastoma	359	1622	18.12%
10	24 Yrs	Female	Ameloblastoma	134	638	17.35%
11	25 Yrs	Male	Control	29	1445	2%
12	35 Yrs	Male	Control	20	665	3%
13	32 Yrs	Female	Control	323	1867	14.75%
14	42 Yrs	Male	Control	117	1218	8.76%
15	28 Yrs	Female	Control	179	1987	8%

RESULT

The expression ofKi-67protein in each group was analysed and interpreted as follows:

All samples showed positive staining with variable number of positive cells. Inter group comparison of Ki-67 positive cells, negative cells and percentages (as per lesion), there was a statistically highly significant difference found in the means of Ki-67 positive cells, and percentage of Ki-67 positive cells, between the 2 groups, but a non significant difference with Ki-67 negative cells. [Table 1]

TABLE 1: INTER GROUP COMPARISON OF Ki-67 POSITIVE CELLS, NEGATIVE CELLS AND PERCENTAGES (AS PER LESION)

		N	Mean	Std. Deviation	Std. Error	p value of KW ANOVA	p value of Mann Whitney test for pairwise comparison	
Ki-67 positive cells	Ameloblastoma	10	352.00	158.707	50.187	0.009**	Amelo vs Control	0.013*
	Controls	5	133.60	124.554	55.702			
Ki-67 negative cells	Ameloblastoma	10	1182.10	350.984	110.991	0.342#	Amelo vs Control	0.254#
	Controls	5	1436.40	531.838	237.845			
Percentages of Ki-67 positive cells	Ameloblastoma	10	22.65	6.261	1.980	0.003**	Amelo vs Control	0.001**
	Controls	5	7.33	5.152	2.304			

* indicates p<0.05 i.e. significant difference
** indicates p<0.01 i.e. highly significant difference
# indicates p>0.05 i.e. non significant difference

However on pairwise comparison using Mann Whitney test, there was no statistically significant difference found between amelo vs KCOT vs Controls (p>0.05) for Ki-67 negative cells.

In groupwise comparison of Ki-67 positive cells, negative cells and percentages (as per lesion), there was a statistically highly significant difference found in the means of Ki-67 positive cells, Ki-67 negative cells and percentage of Ki + cells in the group ameloblastoma, and control group. [Table:2]

On pairwise comparison using Mann Whitney test, there was statistically highly significant difference found for all the individual pairs of Ki+ vs. Ki- vs. Percentage of Ki+ cells in both ameloblastoma, and control group. [Table: 2]

TABLE 2: GROUPWISE COMPARISON OF KI-67 POSITIVE CELLS, NEGATIVE CELLS AND PERCENTAGES (AS PER LESION)

		N	Mean	Std. Deviation	Std. Error	p value of KW ANOVA	p value of Mann Whitney test for pairwise comparison	
Amelo	Ki + cells	10	352.0000	158.70658	50.18743	0.000**	Ki+ vs Ki -	0.000**
	Ki – cells	10	1182.1000	350.98384	110.99084		Ki+ vs Perc +	0.000**
	% of Positive cells	10	22.6473	6.26057	1.97976		Ki – vs Perc +	0.000**
	Total	50	332.8462	475.62424	67.26343			
Controls	Ki + cells	5	133.600	124.5544	55.7024	0.000**	Ki+ vs Ki -	0.008**
	Ki – cells	5	1436.400	531.8381	237.8452		Ki+ vs Perc +	0.008**
	% of Positive cells	5	7.331	5.1516	2.3039		Ki – vs % of +	0.008**
	Total	25	335.635	606.4431	121.2886			

DISCUSSION

In our study, ameloblastoma showed Ki-67 positivity. It indicates that ameloblastoma has more proliferative activity. Our findings were in accordance to A. R. Gadbail et al., who found the Ki-67 was expressed in 100% cases of ameloblastoma.[1]

In the present study, Ki-67 proliferation index of the follicular ameloblastoma was higher than that of the plexiform ameloblastoma. The results were comparable with Sandra et al. and Li et al. The values did not show any significant difference in proliferation activity, suggesting that the follicular type and the plexiform type have little difference in proliferating activity. Proliferation is a key feature of the progression of tumor; Li et al compared radiographic border and cell proliferation in 97 cases of ameloblastoma and found an interesting correlation. Tumors with well-defined edges with sclerosis showed the lower index of Ki-67 whereas the index was higher in tumors with ill-defined borders. The review of literature suggests that different histological patterns do not correlate with different clinical behavior, especially in term of local recurrence and at present the major prognostic factor appears to be the surgical treatment that strongly affects the recurrence rate.

In the study of Nafarzadeh Shima et al, the expression of PCNA and Ki-67 as markers of cell proliferation in 15 paraffin embedded samples of each dental follicle, dentigerous cyst, unicystic ameloblastoma and ameloblastoma belonging to a total of 30 male and 30 female paients using immunohistochemistry method and found a significant differences in the expression of Ki-67 and PCNA in these four lesion.[9]

Nafarzadeh Shima et al in their study, compared the proliferating index of dental follicle, dentigerous cyst,unicystic ameloblastoma and ameloblastoma by using Ki-67 and PNCA and revealed that unicystic ameloblastoma has greater Ki-67 and PNCA when compared with SMA. Hence we can infer that the high expression of Ki-67 in cases of ameloblastoma goes hand in hand with our result.[9]

CONCLUSION

The Ki-67 protein expression has a tendency to be expressed in an increasing quantitative and qualitative manner, as the biologic behavior of odontogenic cyst or tumors becomes more aggressive. Ki-67 over expression may promote cell proliferation in odontogenic lesions. Thus, it can be stipulated that Ki-67 protein expression can be used as a prognostic marker in ameloblastoma. It may be a useful adjunct to evaluate the aggressiveness and also to determine the prognosis as well as the recurrence of ameloblastoma.

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