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Epidermal Growth Factor Receptors and Ki-67 Immunohistochemical Expression in Ameloblastoma

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Research Article

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ABSTRACT

Ameloblastoma is the most frequent benign tumor of the odontogenic tumors that develops in the jaw. Nevertheless, a high rate of recurrence is reported after surgery. Epidermal growth factor receptor (EGFR) has been shown an importance in the genesis and behavior of some types of this tumor like solid multilocular ameloblastoma. Ki-67 is a molecule that can be easily detected in proliferating cells in order to gain an understanding of the rate at which the cells within a tumor are growing. The aim of this study was to examine the expression rate of EGFR and Ki-67 to clarify their role in the biological behavior of the lesions. The Oral Pathology Department of the Faculty of Dentistry in Damascus University received 25 specimens of ameloblastoma in the period October 2005 to October 2010. These specimens were studied immunohistochemically with anti EGFR and anti Ki-67 monoclonal antibodies. This study compared these with 10 normal dental buds which were taken from an aborted foetus of age 7 months in accordance with the relevant laws. Results showed that the average age of patients from which the samples were taken was 43.2 years. A tendency to affecting females was observed (72% of affected patients were females). Observed tumor types were as follows: 12 follicle type, 7 plexiform type, 6 of them were admixed. EGFR was found to be highly expressed in 19 specimens, mildly expressed in 5 specimens, and negative in only one specimen. Ki-67 expression was close to that of EGFR. There was a statistically significant relationship between the expression of the EGFR and Ki-67 monoclonal antibodies and the different patterns of the tumor islands ($p<0.01$). In conclusion, ameloblastoma is a positive EGFR tumor. There was a high Ki-67 index, which is associated with a high proliferation rate, which may indicate a high recurrence rate as proven by other studies

INTRODUCTION

Ameloblastoma is a slow growing and locally invasive odontogenic tumor with potentially destructive behavior and a high rate of recurrence [1]. There is a possibility of transformation to a malignant tumor, although they are usually noncancerous (benign). However, they grow quickly and can change and destroy bone around them. Ameloblastoma develops in the jaw, often at the site of the third molar, and rarely involves tissue from the eye sockets or sinuses [2]. The WHO classified the ameloblastoma in its 2005 tumors classification as a group of tumors; each one of tumors of this group has its own clinical behavior and radiographic appearance. Local invasion and destruction of the adjacent structures are the source of risk in ameloblastoma [3,4]. Nevertheless, a high rate of recurrence is reported after surgery. Much immunohistochemical research has been conducted in the last two decades in an attempt to elucidate the etiologic factors and treatment modalities for this tumor [5]. Epidermal growth factor (EGF), a member of the ErbB family, is a transmembrane glycoprotein that stimulates cell growth, proliferation, and differentiation by binding to its epidermal growth factor receptor (EGFR) while Ki-67 is a molecule that can be easily detected in growing cells in order to gain an understanding of the rate at which the cells within a tumor are growing [6-9].

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MATERIALS AND METHODS

This retrospective study was carried out on 25 mandibular ameloblastoma specimens received in the Oral Pathology Department of the Faculty of Dentistry in Damascus University from October 2005 to October 2010. Clinical data were retrieved from archival patient's files. Each specimen was coded and the patient's name not shown for ethical reasons. Age of the patients, sex, tumor size, site and recurrence were revised. All cases were re-examined for precise scientific diagnosis based on clinical data and other investigation in addition to histopathological reports.

This study also included 10 dental buds (with different stages of development) which were taken from one an aborted foetus of 7 months age, in accordance with relevant laws as control only.

Histological Sections

Serial sections of 4 μm thickness were taken from formalin-fixed paraffin- embedded blocks of archival ameloblastoma tissues for routine H and E, others were prepared on charged slides for immunohistochemistry The endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 15 minutes. The slides were then immuno stained. Three tumor slides from each specimen were examined with an Olympus light microscope.

Immunohistochemistry

Immunohistochemical analysis for EGFR and Ki-67 with a labelled streptavidin-biotin-peroxidase complex technique was performed on tumor sections. The antibodies used were monoclonal antibodies against EGFR. (DAKO, DakoCytomation) and Ki-67 (clone MIB-1, N1633, Dako Corporation). EGFR immunostaining required antigen retrieval with 0.2% trypsin, Ki-67 immunostaining required pretreatment with 1 mM EDTA (at pH 8.0) for 20 minutes in a microwave oven. Proper positive and negative controls were performed. Buds which were taken from the aborted 7 month foetus (taking in consideration approved ethical standards) and were used as a positive control for EGFR, tonsils for Ki-67(as recommended by the manufacturer data sheet). As a negative control, sections were stained without the addition of a primary antibody.

Immunohistochemical Analysis

Two pathologists conducted the immunohistochemistry assessment. Slides were viewed in X40 magnification. Ten cellular areas were selected and evaluated at X400 magnification.

Assessment of Egfr

When the IHC ended, the target antigen reacted with the added antibody to give brown color in the section - meaning positive reactivity compared with the control.

All sections were evaluated (according to the manufacturer data sheet) for the EGFR. Staining intensity was evaluated as +++; ++ and + for positive immunohistochemical staining and – for negative [10-13].

Assessment of Ki-67

Ki-67 labelling of nuclei in relation to expressed staining intensity was evaluated as +++; ++ and + for positive immunohistochemical staining and – for negative [14].

Statistical Analysis

All clinical and immunohistochemical parameters were evaluated by statistical analysis, using (SPSS) program version 17. Proportion was compared using Chi-square test. A probability value (p-value) of less than 0.05 was considered statistically significant.

RESULTS

This retrospective study included 25 samples. The patients from whom these samples taken were aged between 21 and 80 years old with an average age of 43.2 years. The proportion of patients below 50 years of age was 72%, while the number of cases after the age of 50 years was 28%. The arithmetic mean of the age of the patients was 43.2 years. Further information about the age distribution of patients is shown in **Table 1**. Male patients accounted 7 (28%) and females were 18 (72%).

This study found that the most prominent histological type was the follicular type, which was present in 12 cases. The plexiform was present in 7 cases and both these types with cystic islands were present in the remaining 6 cases **(Figure 1 and Table 2)**.

Immunohistochemical Findings

EGFR was highly expressed in 8 cases and moderately appeared in 11 cases. From these 19 cases, 12 were follicular type **(Figures 2 and 3 and Table 3)**.

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Ki-67 was highly expressed in 7 cases, moderately in 13 cases, faint in 4 cases and didn't appear in only one sample **(Figure 4 and Table 4)**. All cases with high positive expression were of the follicular pathological type. Study of the relationship between the age of the patient and Ki-67 showed no relationship; the chi-square Pearson correlation showed no significant relation (P>0.05).

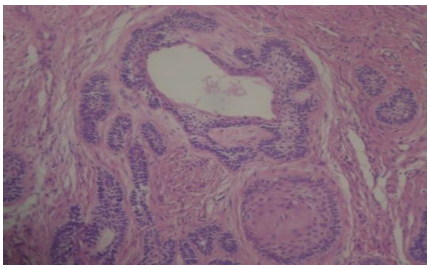

Figure 1. Follicular type of ameloblastoma x100.

Figure 2. EGFR immunostaining membranous in basal and stellate reticulum like cells x100.

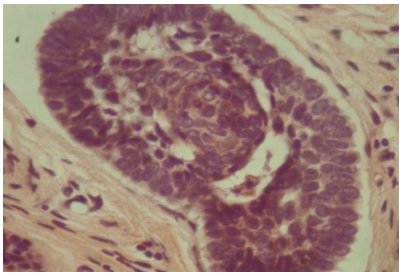

Figure 3. EGFR immunostaining cytoplasmic expression in basal and stellate reticulum like cells x100.

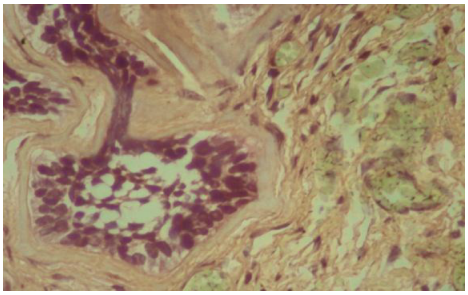

Figure 4. Nuclear Ki-67 immunostaining x100.

Table 1. Distribution of cases according to age group.

Age group	21-30	31-40	41-50	51-60	61-70	71-80
No. of cases	9	4	5	2	3	2
%	36	16	20	8	12	8

Table 2. Distribution of cases to the histological types.

	Follicular	plexiform	Follicular with cystic egeneration	Plexiform with cystic degeneration
No. of cases	12	7	1	5
percentage	48%	28%	4%	20%

Table 3. The immunhistochemical expression of EGFR.

EGFR				
Intensity	+++	++	+	-
No. of cases	8	11	5	1
Percentage	32%	44%	20%	4%

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Table 4. The immunhistochemical expression of Ki-67.

Ki-67				
Intensity	+++	++	+	-
No. of cases	7	13	4	1
Percentage	28%	52%	16%	4%

DISCUSSION

The EGFR gene mutation can lead to uncontrolled cell division and the development of cancer [15-17]. EGFR up regulation appeared to be selectively expressed in a number of tumors as lung cancer [18-20].

This study showed the impact of human growth factor receptor detector. There was a clear impact of the EGFR between peripheral and central cells in neoplastic epithelial islands in each specimen. Proliferation activity is expected to increase as the size of the tumor islands increases. Thus it was recorded that 8 samples (32%) showed strong EGFR expression while 11 samples (44%) were moderate, and 5 samples (20%) were weak. Only one sample was negative impact to the EGFR. Some studies showed that both membranous and cytoplasmic parts of tumor cells were stained immunohistochemicaly with EGFR while others showed only membranous staining [6,7].

According to these studies, it must be expected that ameloblastoma invades adjacent tissues so it has a tendency to recur. The treatment of these tumors is suggested to be radical resection of the involved bone.

EGFR was highly expressed in 12 follicular cases of the histological types of the tumors (48%) and there was a significant relationship ($P<0.05$) between follicular type and EGFR. Other histological types showed less expression (rate $P>0.05$). This indicates that the traditional type of the neoplastic islands representative central satellite like cells of the follicular type associated with EGFR. Other studies suggested that some biological factors in these cells influence the proliferation of the tumor. We can indicate the biological behavior of the tumors and their response to treatment and prognosis by measuring the cell division index based on their histological classification [5].

In relation to Ki-67, this study like others showed high expression rates which may be explained by a high recurrence rate of the tumor and may also indicate its prognosis. Ki-67 is a proliferation marker specific to mitoses and is never found in the non-proliferative phase of the cell cycle. Ki-67 was highly expressed in the follicular type about (60%) and significantly associated with it like EGFR. Less appeared in other types and this may be due to the small sample size in this study [21-23].

In this study there is an inverse correlation between the age of the patient and the expression of EGFR. The younger patients showed the most positive immunohistochemical expression; the tumor on more youthful patients is more active in its biological behavior and that is why it is highly recurrent if not actively treated with complete resection. While there is no significant relationship between age and Ki-67, this could be explained by the active proliferating rate of tumor cells in spite of different age groups [14,24-29].

CONCLUSION

Evaluation of Ki-67 status together with conventional histological evaluation can help in providing more information about the biologic behavior of the tumor, while EGFR could be a target of an expanding class of anticancer therapies. Since ameloblastomas are EGFR-positive tumors, anti-EGFR agents could be considered to reduce the size of large tumors and to treat unresectable tumors that are in close proximity to vital structures. Identification of proliferating activities in tumors may be useful to predict their biological behavior. Ki-67 protein is a nuclear non-histone protein which is required for maintaining the cell cycle. Ki-67 is expressed by proliferating cells in all phases of the active cell cycle (G1, S, G2 and M phase) but is absent in resting (G0) cells that's why Ki-67 has been used to determine the proliferation rate of ameloblastomas.

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