Dental Research Journal

Original Article

A comparative immunohistochemical study of Ki-67 and Bcl-2 expression in solid ameloblastoma and adenomatoid odontogenic tumor

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Received: May 2011
Accepted: August 2011

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ABSTRACT

Background: Solid ameloblastoma (SAB) is an invasive tumor which infiltrates adjacent normal tissues. Adenomatoid odontogenic tumor is a noninvasive tumor and never infiltrates surrounding normal tissues. The purpose of this study was to determine the biological behavior of these two epithelial odontogenic neoplasm by detecting Ki-67 and Bcl-2, which are mitotic and anti apoptotic markers respectively.

Materials and Methods: In this analytical retrospective study, 16 samples of SAB and 16 samples of adenomatoid odontogenic tumor were selected. The samples were deparafinized and antigens were retrieved. Immunohistochemistry technique was applied for evaluation of these two markers. Monoclonal antibodies MIB1 and Bcl-2 were used to detect Ki-67 and Bcl-2 protein respectively, then the labeling index (LI) was calculated for both markers according to cellular staining. Data were analyzed by "*t*" test, ($P<0.05$).

Results: The mean values of LI for Ki-67 in SAB and Adenomatiod odontogenic tumor (AOT) were 4 and 1% respectively and for Bcl-2 in SAB and AOT were 63 and 26% respectively. The indices of both markers were higher in SAB compared to AOT ($P<0.05$).

Conclusions: Higher percentage of these two markers in SAB compared to AOT confirms the aggressive behavior of SAB and the hamartomatosis behavior of AOT.

Key Words: Adenomatoid odontogenic tumor, ameloblastoma, Bcl-2 protein, immunohistochemistry, Ki-67 antigenes

INTRODUCTION

Solid ameloblastoma (SAB) is an invasive odontogenic tumor. The growth of SAB is rapid and invades adjacent normal tissues but never metastasizes.[1] Its incidence is equal in both genders. In conventional radiographs, the tumor has multilocular appearance while the smaller ones have unilocular radiolucency features.[2]

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Adenomatiod odontogenic tumor (AOT) is a noninvasive tumor. The growth is slow and is found almost always incidentally in radiographs.[3,4] This tumor is often a unilocular radiolucent defect in radiographs, behaves like a hamartoma, never invades adjacent normal tissuest, and has fibrous capsule.[4]

The best way to understand the behaviors of these tumors is to study them by means of molecular method.

Jie *et al*.[5] in their study in 2006, applied Bcl-2 marker in 75 SAB and 35 odontogenic keratocyst (OKC) cases. The labeling index (LI) of this marker in SAB was higher than OKC. Since Bcl-2 is an antiapoptotic factor, according to the molecular findings, they confirmed the fact that SAB is an aggressive tumor.

In another study by Piatelli *et al*.,[6] 22 cases of SAB, 12 cases of OKC,8 cases of dentigerous cyst and 12 cases of radicular cyst were evaluated by Immunohistochemistry (IHC) markers, Ki-67 and proliferating cell nuclear antigen (PCNA). The level of these markers was higher in SAB. Since both markers showed mitotic activity, they concluded that SAB is an invasive tumor.

In another study by Simpered[7] in 2006, 9 cases of AOT were evaluated by Ki-67 (MIB1). LI was low so the investigators concluded AOT is a noninvasive tumor and recurrence is very low.

In a survey by Moreira *et al*.,[8] in 2009, 3 samples of AOT and 13 samples of SAB were studied by methylation frequencies of cell-cycle associated genes. Methylation is an important process in cell cycle. Methylation rate of SAB was higher than that of AOT, so it indicates the higher proliferation rate in SAB.

In another study, Razavi *et al*.[9] in 2009 evaluated Bcl-2 and Ki-67 in OKC and SAB, they observed that SAB was an invasive tumor because of high expressions of Bcl-2 and Ki-67.

Ki-67 is a non-histone protein which is seen only in proliferating cells, so finding this protein reveals mitotic activity in cells.

Another prtoein which also was used in our study was Bcl-2. This protein has an antiapoptotic effect on cell proliferation, so those cells express these markers to be susceptible to behave in a tumoral manner.[10] These two molecules show the behavior of tumors through determining the proliferative and apoptotic activity in them.

We have decided to study the behavior of AOT and SAB by evaluating the Ki-67 and Bcl-2 markers.

The aim of this study was to examine the expression rate of Ki-67 and Bcl-2 in AOT and SAB to clarify the possible role of these factors in the different biological and clinical behaviors of the lesions and their relationship with these molecular findings. The relationship between these factors and tumoral behaviors had not yet been studied.

MATERIALS AND METHODS

In this descriptive analytical study, 16 cases of AOT and 16 cases of SAB embedded in paraffin were chosen from archives of the department of oral and maxillofacial pathology of Isfahan and Yazd dental schools Iran.

Inclusive criteria which were examined by three observers were, the existence of enough tumoral tissues stained by H and E and lack of inflammation (extremely mild inflamation less than 50 cells in a field at ×100 magnification was accepted) as inflammation destroys the architecture of tumors and exaggerates tumoral dysplasia [Figures 1 and 2].

Samples were then subject to immunohistochemical test through following steps:

1. Processing: 4 µm thick slices were prepared and adhered to microscopic slides by poly-l-lysin.
2. Deparaffinization and Rehydration: All slides were put in 60°C water bath for 45 min and deparaffinized using three step technique in Xylen and then rehydrated by five step technique in alcohol and water.
3. Antigen retrieval: All slides were immersed in citrate buffer (PH=6) and then microwaved for 10 min to fix antigens following washing with PBS (phosphate buffered saline).
4. Antigen Amplification: Slides were incubated with MIB1 (colon 124 Zymel, USA) and Bcl-2 (RES/CAT 95.96-43-1zymel, USA) for 1 h. At this time the slides were ready to be assessed by IHC technique with streptavidin biotin. Streptavidin biotin conjugates to enzymes so it makes a stable combination and reduces false positive results.

To reduce the mistakes, made by observers, three observes examined the samples with IHC staining by means of light microscope (Olympus, Tokyo, Japan). All IHC stained slides were observed in 10 HPF (high power field = ×400). To study Ki-67, brown nuclei were counted in 1000 epithelial cells of both tumors [Figures 3 and 4]. To study Bcl-2, the cells with brown cytoplasm were counted in 1000 epithelial cells of both tumors [Figures 5 and 6].

Labeling indices were calculated for both markers, regardless of staining intensity, by solving the following formula.

$$LI = \frac{\text{All positive staining cells}}{\text{1000 epitelial cells}}$$

Correlation coefficient between 3 observers was determined and the mean values of LI in Ki-67 and Bcl-2 were calculated. Data were analyzed by "*t*"-test and the results were compared.

RESULTS

Our cases in this study included 16 SAB and 16 AOT samples.

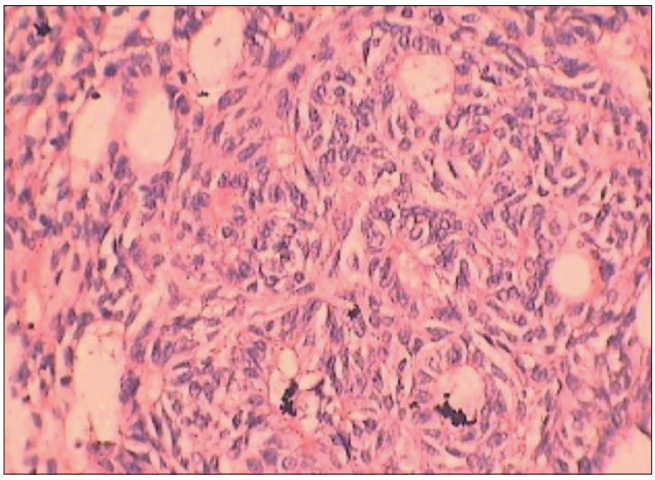

Figure 1: AOT, duct-like and rosette-like structures (H and E ×400)

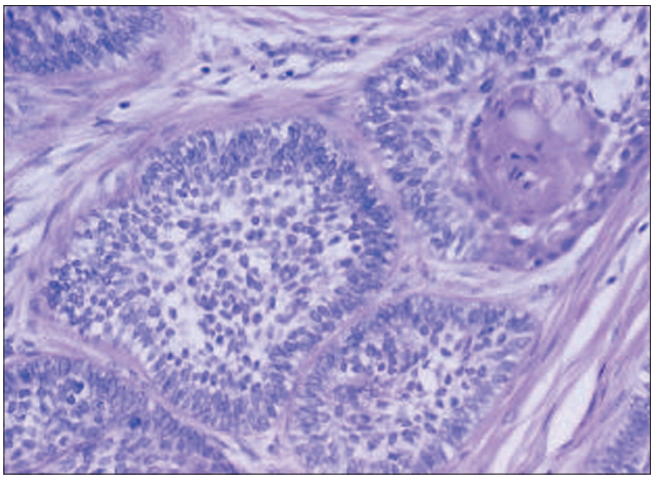

Figure 2: SAB, columnar odontogenic epithelium at the periphery of follicles and stellate reticulum in the center (H and E, ×400)

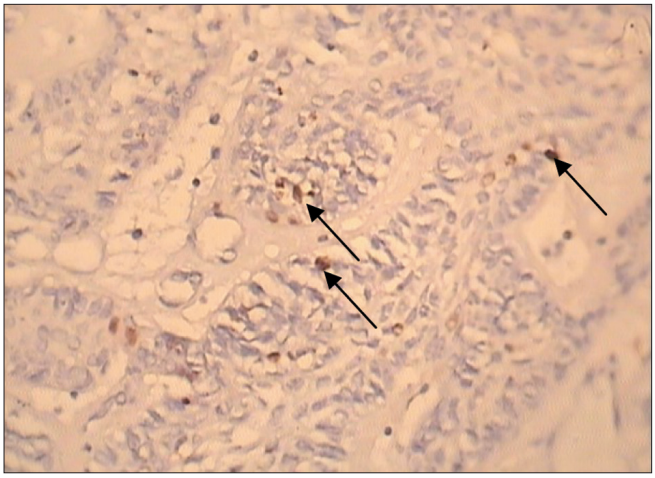

Figure 3: Arrows showing the positive nuclei in SAB (Ki-67 ×400)

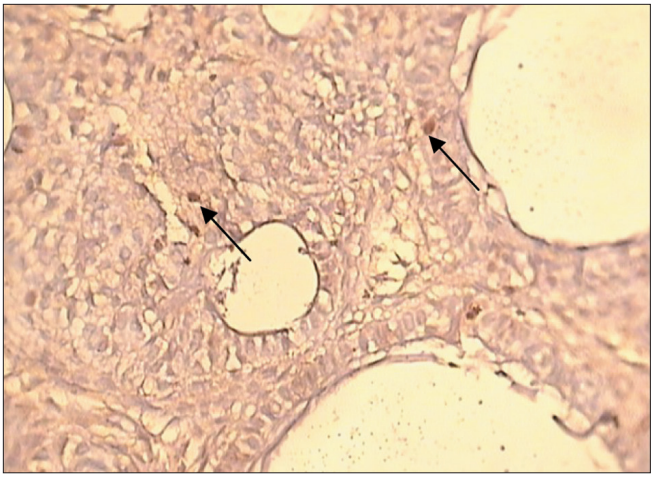

Figure 4: Arrows showing the positive nucleus in AOT (Ki-67 ×400)

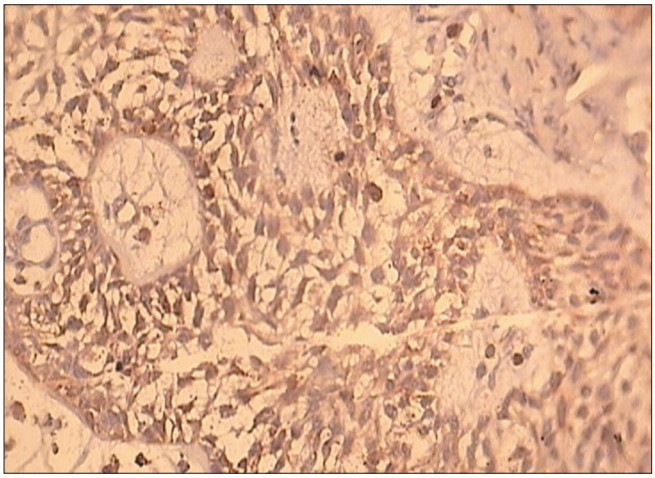

Figure 5: Positivity of epithelial odontogenic cells in SAB (Bcl-2 ×400)

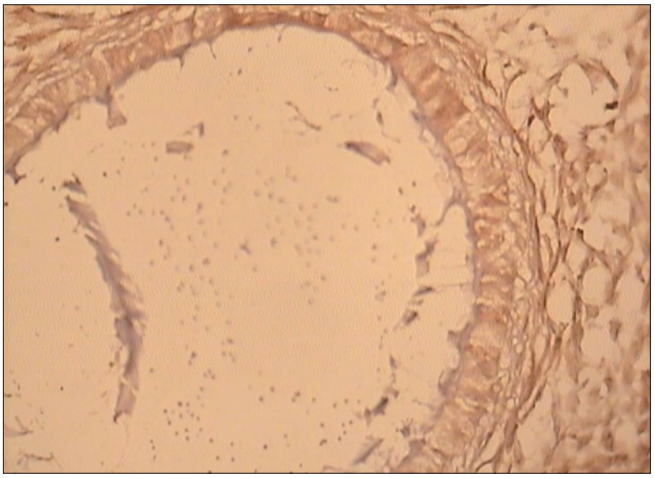

Figure 6: Positivity of epithelial odontogenic cells in AOT (Bcl-2 ×400)

Mean values of Bcl-2 expression (LI) in SAB was 62.93±13.46, and in AOT was 25.93±9.78 [Table 1].

Mean values of Ki-67 expression (LI) in SAB was 4.31±6.27, and in AOT was 0.91±1.32 [Table 1].

Table 1: Bcl-2 and Ki-67 expression in AOT and SAB

Maximum	Minimum	95% Confidence interval for mean		Std. error	Std. deviation	Mean	*N*	Marker	Samples
		Upper bound	Lower bound						
21.00	0.00	7.6585	0.9665	1.56984	6.27937	4.3125	16	SAB	Ki-67
4.00	0.00	1.6228	0.2147	0.33031	1.32122	0.9188	16	AOT	
21.00	0.00	4.3408	0.8904	0.84588	4.78500	2.6156	32	Total	
90.00	40.00	70.1116	55.7634	3.36584	13.46338	62.9375	16	SAB	Bcl-2
43.00	12.00	31.1493	20.7257	2.44518	9.78072	25.9375	16	AOT	
90.00	12.00	52.3962	36.4788	3.90227	22.07456	44.4375	32	Total	

SAB: Solid ameloblastoma, AOT: Adenomatiod odontogenic tumor

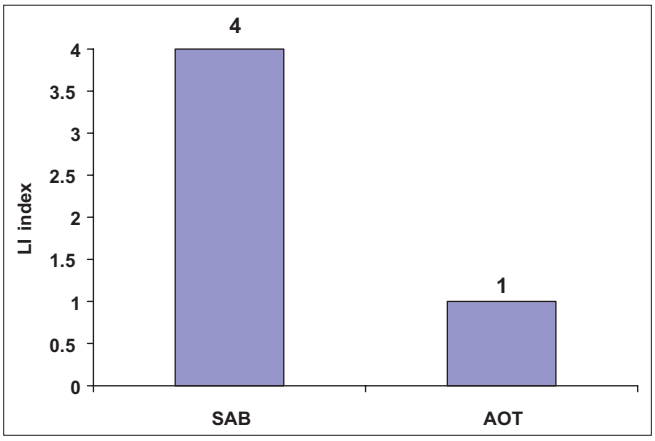

Figure 7: Comparison of AOT and SAB in Ki-67 (*P*=0.043)

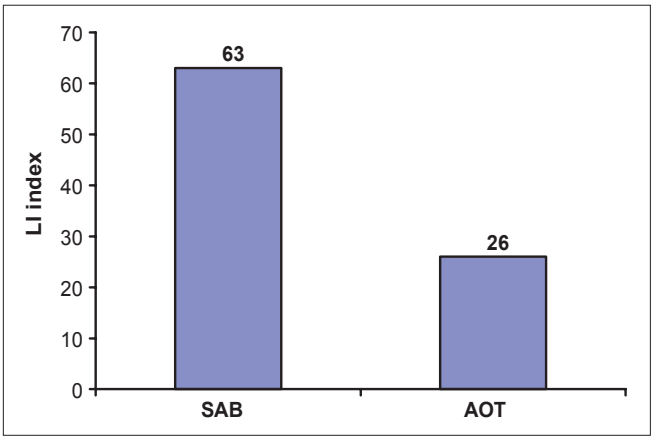

Figure 8: Comparison of AOT and SAB in Bcl-2 (*P*<0.001)

The findings were analyzed using "*t*"-test. The Bcl-2 LI of SAB was higher than that of AOT (*P*<0.001). The Ki-67 LI of SAB was higher than that of AOT (*P*=0.043) [Figures 7 and 8].

DISCUSSION

In a study, Barboza *et al*.,[3] in 2005 applied PCNA and P53 markers in 16 cases of SAB and 8 cases of AOT. PCNA is a marker for mitosis activity and is somewhat similar to Ki-67. P53 is another marker that indicates proliferative and anti apoptotic activities. Both markers were higher in SAB compared to AOT. So the investigators concluded that SAB is more aggressive than AOT.

In this study, levels of Bcl-2 and Ki-67 were evaluated in SAB and AOT. Bcl-2 is an antiapoptotic marker, it is more specific than P53 in determining the apoptosis.[11]

P53 is a tumor suppressor protein; mutation of this protein causes increased tumoral activities.[1]

Ki-67 is a proliferation marker specific to mitoses and is never found in nonproliferative phase of cell cycle. This protein is somewhat similar to PCNA but the former is more specific.[11] Similarly, Barboza *et al*. showed a higher tumoral activity in SAB compared to AOT.

In another study in 2005, Medeiros *et al*.,[12] applied two markers, P53 and PCNA, in 8 cases of AOT, IHC markers were very low so they concluded that AOT is a hamartoma rather than a tumor.

In a survey by Leon *et al*. in 2005, Ki-67 IHC marker was evaluated in 39 cases of AOT. Expression of this marker was very low in AOT (LI<1%) so the investigators concluded that this tumor growth is very slow and it cannot invade adjacent tissues.[4]

According to these studies, AOT had hamartomatosis behavior and it does not have tendency to recur so the treatment for AOT is just an enucleation.[7]

In another survey by Apple *et al*.,[13] 24 cases of SAB were evaluated for P53 gene via IHC method. Both markers were high so the authors concluded that SAB is invasive and tends to recur.

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

Some surgeons advocate that the margin of resection should be at least 1 to 1.5 cm far from the radiographic limits of the tumor, although, recurrence rates of up to 15% is evident.[14]

Nowadays IHC markers have been used widely, especially in tumor progression tests. One of the oldest markers is P53. Naturally, P53 is a tumor suppressor gene for abnormal mitotic activity.[15]

PCNA and Ki-67 are proteins with expression in mitotic cells. They are widely used in new studies to determine tumoral activity, especially Ki-67.[16,17]

Ki-67 is a diagnostic factor for many of cancers, for example, non Hodgkin lymphoma, cancers of prostate and salivary tumors.[18]

Bcl-2 is an anti apoptotic protein, it is now one of the most useful markers to determine the aggressiveness of many tumors' behavior such as follicular B cell lymphomas, cancers of prostate skin, cancer like melanoma and SCC.[19]

In addition to cellular pleomorphism and atypism which are important factors to determine tumoral behavior; mitotic activity is another important factor to indicate tumoral behavior and grading as well.

In fact the mitotic and apoptotic index in contrast to cellular pleomorphism is a quantitative index and will be used in tumor grading with higher validity.[20]

It is realized that higher expression rate of Bcl-2 means more stable cell cycle and resistance to death and subsequently more aggressive clinical behavior of SAB, therefore, the radical surgery is needed.

In contrast to SAB, AOT showed less Bcl-2 expression, so the stability of cell cycle is not as high as SAB; therefore, we may expect less aggressive behavior of AOT and the treatment of choice is conservative surgery.

It is also found that the Ki-67 expression, and subsequently, proliferative activity being higher in SAB compared to AOT, which result in more aggressive clinical behavior of SAB.[2-10]

CONCLUSIONS

Although the LI of Ki-67 was very low in SAB but it was higher than that of AOT, these findings confirmed the aggressive behavior of SAB and also the hamartomatosis behavior of AOT. And similar to previous studies, we also suggest further studies about this subject.

ACKNOWLEDGMENTS

This study was supported in part by Vice Chancellery for research of Isfahan University of Medical Sciences (grant number: 389221). The authors would like to thank F. Mahmoodi for her kind help in the laboratory works.

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How to cite this article: Razavi SM, Tabatabaie SH, Hoseini AT, Hoseini ET, Khabazian A. A comparative immunohistochemical study of Ki-67 and Bcl-2 expression in solid ameloblastoma and adenomatoid odontogenic tumor. Dent Res J 2012;9:192-7.

Source of Support: This report is based on a thesis which was submitted to the School of Dentistry, Isfahan University of Medical Sciences, in partial fulfillment of the requirements for the M.Sc degree in Pathology (#389221). This study was financially supported and approved by Isfahan University of Medical Sciences, Isfahan, Iran.,
Conflict of Interest: None declared.