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Bcl-2 and galectin-3 expression is associated with recurrence of ameloblastoma

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ABSTRACT

Background: Ameloblastoma is a benign odontogenic neoplasm with a high recurrence rate. Identifying cellular and molecular changes in this neoplasm may help predict the recurrence risk. Bcl-2 and galectin-3 are anti-apoptotic proteins associated with the prognosis of many neoplasms. However, there are a few studies focusing on the association between these two markers and recurrence of ameloblastoma. This study aimed to investigate the association of Bcl-2 plus galectin-3 expression and recurrence of ameloblastoma.

Materials and Methods: This retrospective cross-sectional study was designed on 48 paraffin-embedded blocks diagnosed as ameloblastoma from 1998 to 2019. We retrieved follow-up data from patients' records and used immunohistochemical staining for Bcl-2 and galectin-3 antibodies. Then, we analyzed their association with recurrence using Chi-square and Mann–Whitney test as well as recurrence-free survival using Kaplan–Meier curves and linear Cox regression. The level of statistical significance was $P < 0.05$.

Results: Twenty-six patients had experienced the recurrence. The mean follow-up time was 93.53 months. There was a significant association between Bcl-2 plus cytoplasmic galectin-3 staining and recurrence (both $P < 0.001$). Furthermore, in univariate analysis, high expression of Bcl-2 was associated with less recurrence-free survival (log-rank: $P = 0.020$-univariable Cox: $P = 0.033$), but in multiple Cox regression, there was no significant association ($P = 0.471$). High cytoplasmic galectin-3 expression was also associated with less recurrence-free survival (log-rank: $P = 0.007$-univariable Cox: $P = 0.015$-multiple Cox: $P = 0.044$). Furthermore, we found a correlation between Bcl-2 and cytoplasmic galectin-3 staining ($P = 0.001$).

Conclusion: It seems that Bcl-2 and cytoplasmic galectin-3 staining might predict the risk of ameloblastoma recurrence. However, only the cytoplasmic galectin-3 staining might be an independent predictor of ameloblastoma recurrence, and we recommend further studies.

Key Words: Ameloblastoma, apoptosis, Bcl-2 protein, galectin 3, recurrence

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INTRODUCTION

Ameloblastoma accounts for about 10% of odontogenic neoplasms. The annual incidence of this benign tumor is 0.5/million people.[1] Despite having a low growth potential, it has an aggressive behavior

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and a high risk of recurrence.[1,2] It is believed that the recurrence rate mostly depends on the surgical method and the complete excision of the tumor with an appropriate safe margin.[3] The recurrence rate after radical surgery (7.1%) is lower than conservative surgery (33.3%);[4] however, radical treatment can cause cosmetic and functional defects.[5] Furthermore, ameloblastoma can have late recurrence as recurrence has been reported 50 years after surgery, even after radical surgery.[6] Some researchers have shown that many radiographic and clinical factors such as cortical bone invasion, root resorption, tumor association with impacted teeth, soft tissue invasion, and pathological fracture could not be associated with the risk of recurrence.[7,8] Now, studies are mainly focusing on cellular and molecular changes of this neoplasm in order to find a risk factor predictor marker.

Bcl-2 protein is an anti-apoptotic member of Bcl-2 family, which prevents the release of cytochrome c from the mitochondria. Tumor growth is the result of a balance between proliferation and cell death.[9] Unlike Bcl-2, the expression of ki-67 (as a cell proliferation indicator[10]) is low[11] in ameloblastoma. Some researchers have reported higher Bcl-2 expression in ameloblastoma than other odontogenic tumors such as adenomatoid odontogenic tumor. They have hypothesized that Bcl-2 might be effective in tumor invasion.[12,13] Bcl-2 has been suggested as a prognostic marker in various cancers including small-cell lung cancer,[14] laryngeal squamous cell carcinoma,[15] gastric cancer,[16] as well as colon, prostate,[17] and breast cancers.[18] Limited research has been conducted on the association between Bcl-2 expression and ameloblastoma recurrence. One of these studies showed that Bcl-2 staining is higher in malignant and recurrent types of ameloblastoma.[19] Another study found that Bcl-2 expression was related to shorter recurrence-free survival of ameloblastoma.[9]

Galectin-3 is the only anti-apoptotic member of the galectin family[20] and the only member of this family that contains the anti-death motif of the Bcl-2 family.[21] In response to apoptotic stimulation, galectin-3 travels from the nucleus or cytosol to the mitochondria, and similar to the Bcl-2 protein, it can prevent the release of cytochrome c from the mitochondria.[22,23] Galectin-3 has been issued as a prognostic marker in various neoplasms including pituitary adenoma,[24] tongue carcinoma,[25] hepatocellular carcinoma,[26,27] cutaneous melanoma,[28] plus cystic adenoid carcinoma of the head and neck.[29] Depending on the type of tumor and pattern of expression (nucleus or cytoplasm), high expression of galectin-3 could be associated with a favorable or unfavorable prognosis. In other words, galectin-3 has dual contrasting functions, in activating or suppressing the growth mechanisms.[30-32] We found no study conducted on the association between galectin-3 expression and recurrence of ameloblastoma in English sources.

This study aims to investigate the association of Bcl-2 as well as galectin-3 expression with ameloblastoma recurrence along with the correlation between Bcl-2 and galectin-3 expression in this tumor.

MATERIALS AND METHODS

Study design

The protocol for this cross-sectional study was approved by the Tehran University of Medical Sciences Research and Ethics Committee (IR.TUMS.DENTISTRY.REC.1399.002). This study was designed on 48 paraffin-embedded blocks diagnosed as ameloblastoma from 1998 to 2019. We selected samples from the archives of the Pathology Departments of the Dental School, Imam Khomeini Hospital Cancer Institute, and Shariati Hospital of Tehran University of Medical Sciences. Inclusion criteria: (1) Samples should be diagnosed as ameloblastoma after reassessment and confirmation of the diagnosis. (2) There should be enough tumor tissue accessible. (3) The margin of the lesion should be clear. Exclusion criteria: If the lesion recurrence status was uncertain. We retrieved clinical and histological characteristics and status of recurrence (presence/absence) from the archived file of patients' records. Cases which had a diagnosis of ameloblastoma in their pathology report were included, after reviewing and confirming the diagnosis by two oral and maxillofacial pathologists.

Immunohistochemical staining: Tissue preparation

Two slides were prepared for immunohistochemical staining from each sample and stained separately. We used the Bcl-2 primary antibody (1:50, mouse monoclonal, Master Diagnóstica, Spain), the galectin-3 primary antibody (1:50, rabbit monoclonal, Master Diagnóstica, Spain), and the Master polymer plus detection system (Master Diagnóstica, Spain) as the secondary antibody. We used the normal tonsil tissue and block with papillary thyroid carcinoma diagnosis as a positive control to evaluate Bcl-2 and

galectin-3 staining, respectively. The procedure for both markers was the same as follows:

Four-micron slices were prepared from samples fixed in 10% formalin and were placed on a silicone-coated glass slide overnight. They were then deparaffinized and dehydrated. Next, they were placed in xylene three times for 8 min and then in 100% and 99% ethanol for 5 min and in 100% methanol for 2–3 min, then in methanol containing 3% hydrogen peroxide for 30 min. The samples were then placed in citrate buffer and in the microwave for 25 min. Thereafter, they were exposed to room temperature for 3–30 min, washed with running water for 10 min, and then placed in a phosphate-buffered saline (PBS) buffer for 5 min. Nonserum protein was poured onto the samples and placed in a sealed container for 30 min. The initial antibody was incubated for 1 h at room temperature with the primary antibody. It was then rinsed for 5 min and replaced in PBS. The secondary antibody was added for 30 min and again placed in PBS for 5 min. Next, 1 or 2 drops of 3,3'-diaminobenzidine were added. After 2–3 min, the samples were washed with running water. The samples were stained with hematoxylin for 5 min and washed with running water for 5 min. Finally, they were dehydrated and mounted.

Immunohistochemical staining: Scoring

Cytoplasmic expression of Bcl-2 and cytoplasmic and nuclear expression of galectin-3 were considered positive. We used an Olympus BX51 light microscope and selected ten hot spot fields (100X). The mean percentage of the positive cells was calculated for each slide (400X). For Bcl-2, the percentage of stained cells was scored as: 0 (<5%), 1 (5%–25%), 2 (25%–50%), and 3 (≥50%).[19] The intensity of Bcl-2 staining was also scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong).[19] For each sample, the staining percentage and staining intensity scores were multiplied to give a total score of 0–3: low and 4–9: high.

For galectin-3, the percentage of positive cells was scored as 1 (<25%), 2 (25%–75%), and 3 (>75%).[26] The intensity of cytoplasmic galectin-3 was scored as 0 (negative), 1 (weak: bright yellow), 2 (moderate: yellow-brown), and 3 (strong: Brown).[26] Scores were multiplied to give a total score total score. Total scores were divided into two groups: low (0—3) and high (4-9). Furthermore, nuclear staining of galectin-3 was scored as 0 (negative), 1 (<60%), and 2 (≥60%).[33] Then, we scored the intensity of nuclear galectin-3 staining as 0 (negative), 1 (weak to moderate), and 2 (strong).[33] The total score of 0–3 and 4–9 was considered low and high, respectively.

Statistical analysis

SPSS software version 26.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis. We used Chi-square, Mann–Whitney test, Spearman correlation test, Kaplan–Meier curves (log-rank analysis), and linear Cox regression. The level of statistical significance was $P < 0.05$.

RESULTS

Descriptive findings

The study included 48 ameloblastoma samples, of which nine were unicystic (one luminal and eight mural). Patients consisted of 20 men and 28 women. The mean age of patients was 36.34 ± 56.16 years. The mean follow-up time was 93.53 ± 54.86 months.

Association of clinical and histological characteristics with recurrence

We divided samples into two groups: Without recurrence (n = 22) and at least one recurrence (n = 26). The tumors were smaller in the group without recurrence (P = 0.037). Other clinical and histological variables were not different in the two groups [Table 1].

Association of Bcl-2 and galectin-3 staining with recurrence

Percentage, severity, and total score of Bcl-2 as well as galectin-3 staining were higher in the group with at least one recurrence. Furthermore, we considered the sum of the Bcl-2 and cytoplasmic galectin-3 total score as a variable showing a significant association with recurrence (P = 0.000002). The nuclear galectin-3 staining and the galectin-3 staining pattern did not significantly differ between the two groups [Table 2].

Correlation between Bcl-2 and galectin-3 immunohistochemical staining

The correlation between Bcl-2 and galectin-3 immunohistochemical staining is shown in Table 3.

Association of Bcl-2 and galectin-3 staining with recurrence-free survival

We assessed the association between Bcl-2 plus cytoplasmic galectin-3 total score and recurrence-free survival for 42 patients (20 patients without and 22 patients with recurrence) via the Kaplan Meyer curves (log-rank analysis). Bcl-2 and the cytoplasmic

Table 1: Association of the clinical and histological variables with ameloblastoma recurrence

Clinical or histological variable	Recurrence status						*P*
	Without recurrence			At least one recurrence			
	n	Mean rank	Sum of ranks	*n*	Mean rank	Sum of ranks	
Max size of tumor (cm)	15	13.4	202.0	7	7.2	51.0	0.037*
	Without recurrence, *n* (%)			At least one recurrence, *n* (%)			*P*
Gender							
Male	6 (30.0)			14 (70.0)			0.066
Female	16 (57.1)			12 (42.9)			
Localization (anterior/posterior)							
Anterior	1 (50.0)			1 (50.0)			0.588
Posterior	15 (57.7)			11 (42.3)			
Anterior and posterior	6 (66.7)			3 (33.3)			
Type of surgery							
Radical	7 (77.8)			2 (22.2)			0.899
Conservative	12 (80.0)			3 (20.0)			
Histopathologic type							
Multicystic	19 (48.7)			20 (51.3)			0.409
Unicystic	3 (33.3)			6 (66.7)			
Follicular							
Yes	19 (48.7)			20 (51.3)			0.567
No	3 (37.5)			5 (62.5)			
Plexiform							
Yes	5 (50.0)			5 (50.0)			0.822
No	17 (45.9)			20 (54.1)			
Basal cell							
Yes	1 (50.0)			1 (50.0)			0.927
No	21 (46.7)			24 (53.3)			
Acanthomatous							
Yes	12 (54.5)			10 (45.5)			0.324
No	10 (40.0)			15 (60.0)			
Granular cell							
Yes	3 (100.0)			0			0.059
No	19 (43.2)			25 (56.8)			
Desmoplastic							
Yes	1 (100.0)			0			0.286
No	21 (45.7)			25 (54.3)			

*Statistically significant. Incomplete medical record documentation for some patients is the reason of the missing data

galectin-3 total scores were associated with recurrence-free survival ($P = 0.020$ and $P = 0.007$) [Figure 1].

In univariate Cox regression, Bcl-2 and cytoplasmic galectin-3 total scores were associated with recurrence-free survival [Table 4]. In multiple Cox regression, the association between galectin-3 total score and recurrence-free survival remains statistically significant. On the other hand, Bcl-2 total score had no significant association with recurrence-free survival [Table 5].

DISCUSSION

Ameloblastoma is a common slow-growing odontogenic tumor with a high recurrence rate. It is believed that alteration in apoptosis mechanisms plays an important role in the development of this tumor.[11,34,35] However, there is not an accepted biomarker to predict the risk of recurrence. Bcl-2 and galectin-3 are anti-apoptotic biomarkers that are associated with the prognosis of many neoplasms.[14,15,16,18,24,28,36] The present study investigated Bcl-2 and galectin-3 expression in ameloblastoma and their association with recurrence. In our study, the percentage, severity, and total score of Bcl-2 as well as cytoplasmic galectin-3 had a significant association with the recurrence (Bcl-2: $P = 0.001$, $P = 0.015$, and $P < 0.001$, respectively. Galectin-3: $P < 0.001$, $P = 0.003$, and $P < 0.001$, respectively). Expression of these markers might be associated with a high risk of

Table 2: Association of Bcl-2 and cytoplasmic and nuclear galectin-3 staining and ameloblastoma recurrence

Immunohistochemical result	Recurrence status		*P*
	Without recurrence, *n* (%)	At least one recurrence, *n* (%)	
Bcl-2 staining			
Percentage score			
1 (<25%)	9 (90.0)	1 (10.0)	0.001*
2 (25%–75%)	8 (50.0)	8 (50.0)	
3 (>75%)	5 (22.7)	17 (77.3)	
Intensity score			
0 (negative)	1 (50.0)	1 (50.0)	0.015*
1 (weak)	8 (66.7)	4 (33.3)	
2 (moderate)	11 (52.4)	10 (47.6)	
3 (strong)	2 (15.4)	11 (84.6)	
Total score			
1 (low 0–4)	19 (73.1)	7 (26.9)	<0.001* (0.00004)
2 (high 6–9)	3 (13.6)	19 (86.4)	
Cytoplasmic galectin-3 staining			
Percentage score			
0 (<5%)	9 (100.0)	0	<0.001* (0.0000003)
1 (5%–25%)	5 (100.0)	0	
2 (25%–50%)	6 (46.2)	7 (53.8)	
3 (>50%)	2 (9.5)	19 (90.5)	
Intensity score			
0 (negative)	0	0	0.003*
1 (weak)	14 (70.0)	6 (30.0)	
2 (moderate)	7 (35.0)	13 (65.0)	
3 (strong)	1 (12.5)	7 (87.5)	
Total score			
1 (low 0–4)	20 (71.4)	8 (28.6)	<0.001* (0.00003)
2 (high 6–9)	2 (10)	18 (90)	
Sum of Bcl-2 and cytoplasmic galectin-3 total scores as one variable			
Bcl-2 total score + cytoplasmic galectin-3 total score			
2	18 (85.7)	3 (14.3)	<0.001* (0.000002)
3	3 (25)	9 (75)	
4	1 (6.7)	14 (93.3)	
Nuclear galectin-3 staining			
Percentage score			
0 (0%)	1 (100.0)	0	0.094
1 (<60%)	21 (46.7)	24 (53.3)	
2 (>60%)	0	2 (100.0)	
Intensity score			
0 (negative)	1 (100.0)	0	0.643
1 (weak to moderate)	8 (38.1)	13 (61.9)	
2 (strong)	13 (50.0)	13 (50.0)	
Total score			
1 (low 0–2)	9 (42.9)	12 (57.1)	0.718
2 (high 2–4)	13 (48.1)	14 (51.9)	

*Statistically significant

recurrence by preventing apoptosis and prolonged cell survival. In addition to its anti-apoptotic functions, galectin-3 is effective in cell growth, cell adhesion to the extracellular matrix, immune system suppression, and progression of angiogenesis in cancers.[37] We hypothesize that the association between cytoplasmic galectin-3 staining and the recurrence may be due to similar functions in ameloblastoma.

In univariate Cox analysis, high expression of Bcl-2 was associated with less recurrence-free

Table 3: Correlation between Bcl-2 and galectin-3 immunohistochemical staining

Correlation between	Spearman's rank correlation coefficient	*P*
Bcl-2 percentage score and cytoplasmic galectin-3 percentage score	0.449	0.001*
Bcl-2 intensity score and cytoplasmic galectin-3 intensity score	0.323	0.025*
Bcl-2 total score and cytoplasmic galectin-3 total score	0.495	<0.001* (0.0003)
Bcl-2 percentage score and nuclear galectin-3 percentage score	−0.038	0.800
Bcl-2 intensity score and nuclear galectin-3 intensity score	0.155	0.292
Bcl-2 total score and nuclear galectin-3 total score	−0.116	0.433
Cytoplasmic galectin-3 percentage score and nuclear galectin-3 percentage score	0.149	0.312
Cytoplasmic galectin-3 intensity score and nuclear galectin-3 intensity score	0.264	0.69
Cytoplasmic galectin-3 total score and nuclear galectin-3 total score	0.210	0.152

*Statistically significant

Table 4: Association of Bcl-2 and galectin-3 staining with ameloblastoma recurrence-free survival (univariable Cox regression)

Variable	Hazard ratio	95% CI		*P*
		Lower	Upper	
Bcl-2 percentage score	1.885	0.935	3.800	0.077
Bcl-2 intensity score	1.516	0.820	2.803	0.184
Bcl-2 total score	2.952	1.089	8.005	0.033*
Cytoplasmic galectin-3 percentage score	2.846	1.345	6.025	0.006*
Cytoplasmic galectin-3 intensity score	2.324	1.106	4.883	0.026*
Cytoplasmic galectin-3 total score	3.390	1.270	9.052	0.015*
Bcl-2 total score + cytoplasmic galectin-3 total score (as one variable)	2.110	1.119	3.711	0.010*
Nuclear galectin-3 percentage score	2.042	0.425	9.803	0.372
Nuclear galectin-3 intensity score	0.778	0.356	1.702	0.530
Nuclear galectin-3 total score	0.673	0.271	1.675	0.395

*Statistically significant (*n*=42). CI: Confidence interval

Table 5: Association of the age, sex, lesion location, type of surgery, histological type, histological pattern, and Bcl-2 total score with the recurrence-free survival of ameloblastoma (multiple cox regression)

Variable	Hazard ratio	95% CI		*P*	Variable	Hazard ratio	95% CI		*P*
		Lower	Upper				Lower	Upper	
Age (year)	1.060	0.945	1.188	0.320	Age (year)	1.089	0.950	1.249	0.219
Gender	0.020	0.000	3.861	0.145	Gender	0.004	0.000	1.785	0.076
Localization					Localization				
Anterior	57.959	0.187	17941.537	0.165	Anterior	63.617	0.145	27921.108	0.181
Posterior	132.148	0.000	5.568E+248	0.987	Posterior	1059.895	0.000	5.159E+176	0.973
Type of surgery	29.226	0.612	1396.406	0.087	Type of surgery	54.856	0.725	4148.406	0.070
Histopathologic type	36.084	2.645	492.244	0.007*	Histopathologic type	37.576	2.141	659.522	0.013*
Histologic pattern					Histologic pattern				
Follicular	0.004	0.000	7.568E+207	0.982	Follicular	0.011	0.000	2.946E+151	0.980
Plexiform	0.043	0.000	8.328E+208	0.990	Plexiform	0.906	0.008	2.450E+153	1.000
Basal cell	0.021	0.000	7.123E+146	0.982	Basal cell	0.247	0.000	3.116E+103	0.991
Acanthomatous	0.507	0.014	18.080	0.709	Acanthomatous	0.338	0.000	14.852	0.574
Granular cell	0.001	0.000	1.420E+207	0.977	Granular cell	0.012	0.000	3.140E+151	0.980
Desmoplastic	0.150	0.000	5.919E+245	0.995	Desmoplastic	0.010	0.000	4.446E+171	0.982
Bcl-2 total score	1.933	0.322	11.603	0.471	Cytoplasmic galectin-3 total score	11.717	1.063	129.128	0.044*

*Statistically significant (*n*=30). CI: Confidence interval

survival (log-rank: $P = 0.020$-univariable Cox: $P = 0.033$). However, in multiple analyses, we found no significant association ($P = 0.471$). High cytoplasmic galectin-3 expression was also associated with recurrence-free survival (log-rank: $P = 0.007$-univariable Cox: $P = 0.015$-multiple Cox: $P = 0.044$). In the present study, Bcl-2 and galectin-3 cytoplasmic staining were the predictors

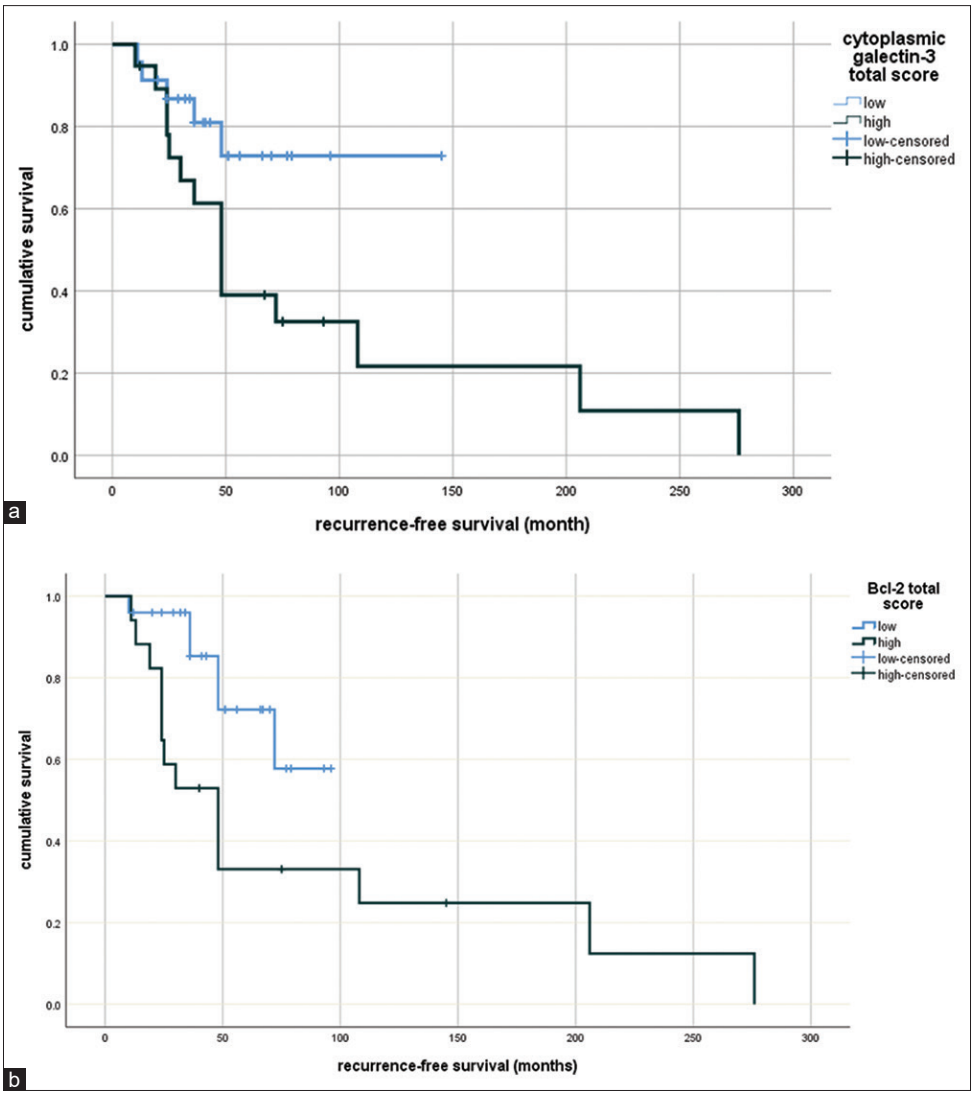

Figure 1: (a) Recurrence-free survival curves according to Bcl-2 total score in ameloblastoma (n = 42). (b) Recurrence-free survival curves according to cytoplasmic galectin-3 total score in ameloblastoma (n = 42).

of ameloblastoma recurrence, but only galectin-3 cytoplasmic staining was the independent predictor of recurrence in ameloblastoma. Wang *et al.* reported higher Bcl-2 staining in recurrent and malignant ameloblastoma than in primary cases (both $P < 0.01$). Other studies demonstrated higher Bcl-2 expression in malignant ameloblastoma than in recurrent ameloblastoma ($P < 0.01$).[19] In Luo *et al.*'s study, there was no significant difference regarding Bcl-2 staining between primary and recurrent ameloblastoma.[38] Their different results from our findings might be due to the difference in methods used: They only assessed the percentage of Bcl-2 staining, and the percentage scoring system was different. Kim *et al.*[9] showed an association between high Bcl-2 staining and decreased recurrence-free survival (log-rank: $P = 0.007$-multiple Cox: $P = 0.018$). Their results are consistent with the present study where Bcl-2 staining was associated with reduced recurrence-free survival (log-rank: $P = 0.020$). We did not find any studies about the association between galectin-3 expression and ameloblastoma recurrence in English sources; however, some studies showed the association between galectin-3 expression and the prognosis of other neoplasms.

In the present study, age, gender, type of ameloblastoma (multicystic/unicystic), type of surgery, and the follicular or plexiform pattern had no significant association with recurrence ($P = 0.822$, $P = 0.567$, $P = 0.264$, $P = 0.899$, $P = 0.066$, and $P = 0.260$, respectively). In Reichart *et al.*'s study, the recurrence rate in follicular ameloblastoma (29.5%) was higher than in plexiform (16.7%) ($P < 0.1$).

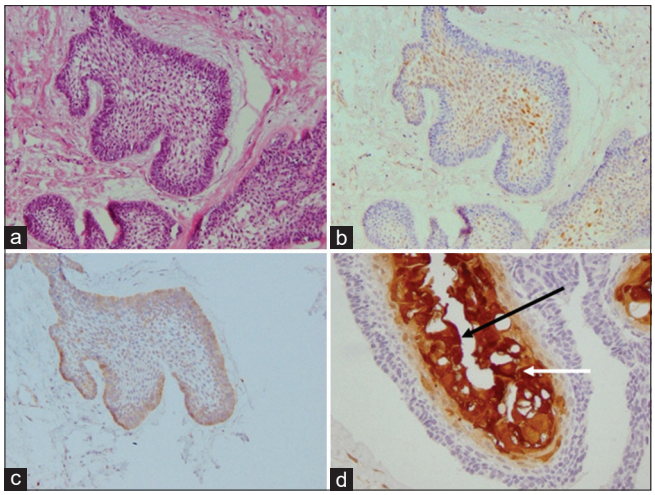

Figure 2: (a) Photomicrograph of H and E-stained section of ameloblastoma tumor (×100). (b) Moderate to strong staining of stellate-like cells with the galectin-3 marker (×100). (c) Moderate to strong staining of ameloblast-like cells and weak-to-moderate staining of stellate-like cells with Bcl-2 marker (×100). (d) Severe staining of stellate reticulum cells with the galectin-3 marker in ameloblastoma islands with squamous metaplasia (white arrow) demonstrating microcytic change (black arrow) (×400).

Furthermore, they found a higher percentage of recurrences in multicystic ameloblastoma (22.7%) than in unicystic ameloblastoma (13.7%) ($P < 0.1$).[39] Hertog *et al*. found no significant association between histological type (follicular, plexiform, and mixed) and ameloblastoma recurrence ($P = 0.213$).[40] Milman *et al*. found no significant association between age, gender, location (maxilla/mandible), as well as pathologic pattern and recurrence ($P = 0.28$, $P = 0.97$, $P = 0.26$, and $P = 0.48$, respectively) which was in the line with our results ($P = 0.002$).[41] Furthermore, another study found no association between age, gender, or tumor location and recurrence-free survival ($P > 0.05$).[7]

We found limited studies in English about galectin-3 expression in ameloblastoma.[42,43] In Pereira-Prado *et al*.'s study, metaplastic and cystic areas showed positive galectin-3 staining[43] which was similar to our findings [Figure 2].

Galectin-3 is the only member of the galectin family that contains the anti-death motif of the Bcl-2 family.[21] In addition to structural similarities,[44] cytoplasmic galectin-3 has functional similarities to the Bcl-2 protein and can prevent the release of cytochrome c from mitochondria.[22,23] Unlike the anti-apoptotic function of cytoplasmic galectin-3, nuclear galectin-3 could play a pro-apoptotic role in some cancer cells.[30,45] van den Brûle *et al*. found positive nuclear and cytoplasmic galectin-3 staining in the normal prostate-epithelium, but only positive cytoplasmic staining in cancer cells.[32] In the present study, Bcl-2 and cytoplasmic galectin-3 staining had a significant correlation (percentage, intensity, and total score correlation of the two markers: $P = 0.001$, $P = 0.025$, and $P = 0.001$, respectively), but Bcl-2 did not correlate with galectin-3 nuclear staining (correlation of percentage, intensity, and total score of two markers: $P = 0.800$, $P = 0.292$, and $P = 0.739$, respectively). There was no significant correlation between nuclear and cytoplasmic staining of galectin-3 (correlation of percentage, intensity, and total score of the two markers, respectively: $P = 0.889$, $P = 0.690$, and $P = 0.281$). A few studies[46-48] have investigated the correlation between galectin-3 and Bcl-2 expression. It has demonstrated a correlation between galectin-3 staining and Bcl-2 intensity score ($P = 0.010$), while positive nuclear galectin-3 staining was not correlated with Bcl-2 intensity score ($P = 0.058$).[47] These findings are in line with our results.

The limitation of the present study was that clinical variables had been incompletely recorded in patients' records. Hence, 15 samples were censored in multiple Cox analyses. Possibly due to small sample sizes (30), confidence intervals were high in multiple Cox analyses [Table 5]. We recommend longitudinal studies with larger sample sizes as well as recording all the clinical, radiographic, and histological variables.

CONCLUSION

It seems that high expression of Bcl-2 and cytoplasmic galectin-3 has an association with higher recurrence and lower recurrence-free survival in ameloblastoma. If further studies confirm these findings, both these markers might be the predictor of ameloblastoma recurrence, while only cytoplasmic galectin-3 expression can serve as an independent predictor.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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