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Expression of Bcl-2 and clinicopathological variables in salivary glands mucoepidermoid carcinoma

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ABSTRACT

Introduction: Mucoepidermoid carcinoma (MEC) has several diagnostic, biological, and histopathological manifestations, each of which presents issues and difficulties in terms of diagnosis, grading, classification, and therapy. The goal of this study was to find out how the anti-apoptotic protein Bcl2 was expressed in salivary gland (SG) MEC and how it related to a number of clinicopathological factors.

Methods: The present study comprised 30 MECs of the SG lesions that were histopathologically diagnosed. The immunohistochemistry method was used to determine Bcl-2 expression. The spss software version 20.0 was used to find a link between Bcl-2 expression and clinical and histological features.

Results: The patients' average age was 49.93 years, and MEC of SGs was more common in females. The palate was the most commonly involved area, accounting for 13 (43.3%) of cases, followed by the parotid glands, which accounted for 8 (26.7%). High-grade tumors accounted for 14 (46.7%) of the cases, whereas mild-grade tumors accounted for 10 (33.3%) and moderate-grade tumors accounted for 6% (20.0%). Bcl-2 immunostaining was associated with gender (P = 0.047), there was also a significant (P = 0.002) difference in tumor grade and age groups. Furthermore, no significant relationships between Bcl-2 expression and the other variables were discovered.

Conclusion: Gender and age affect MEC tumor aggressiveness and grade. High-grade MEC tumors expressed Bcl-2 strongly and moderately, whereas low-grade tumors expressed it moderately. Bcl-2 may predict MEC tumor aggressiveness. MEC therapy may target Bcl-2.

Keywords: Bcl-2, grading, immunohistochemistry, malignancy, mucoepidermoid carcinoma

Introduction

Salivary gland (SG) tumors are one of the most prevalent head-and-neck neoplasms globally. Despite accounting for 0.4% of all malignancies, the tumors of SGs account for 5% of malignant tumors of head-and-neck region.[1]

The parotid gland is the most frequent location and the malignant tumors are more common in minor SGs, with the palate having the greatest proportion.[2,3]

Despite the fact that the rate of malignant transformation in SG's tumor is considerably slower, metastasis to distant organs remains a concern for clinicians. Because of these things and the fact that people do not respond well to chemotherapy and radiation, a lot of attention has recently been paid to how to treat this cancer.[3]

Mucoepidermoid carcinoma (MEC) is the most prevalent malignant SG tumor among individuals of all ages.[4]

The parotid gland is the most common location, followed by the minor SGs of the mouth and lips.[5] About 10–15% of all SG tumors and 35% of cancerous SG tumors are caused by this disease.[6]

MEC is more prevalent in female than in the male population and the incidence is highest in the 5th year of life.[7,8]

It stands for diagnostic and therapeutic, biochemical, and histopathological variability, which may complicate diagnosis, grading, and therapy.[9]

Low-grade tumors are compact, cystic masses that are well encapsulated or partially encapsulated under the microscope. Patients describe a painless growth that progressively becomes

larger. High-grade tumors, on the other hand, are solid, loosely defined masses with metastatic disease borders that are attached to nearby tissues. Patients with parotid gland tumors often complain of a painful swelling that grows quickly and gets in the way of facial nerves.[10]

The WHO divides MECs into low, moderate, and severe grades based on how many epithelial, intermediate, and epidermoid cells; they have in relation to each other.[11]

Samples may show both solid and cystic areas. Mucinous or inflammatory material is often found in cystic areas, while solid areas are usually grayish-white or brown. Under the microscope, epidermoid/squamous cells are polygonal in form, with rich eosinophilic cytoplasm and an oval, vesicular nucleus. Specific cell keratinization is rare and they construct substantial nests by intercellularly binding. Low-grade cancers have epithelial cells with pale to slightly basophilic cytoplasm and constricted nuclei. Mucin streams and a foreign-body giant-cell response may occur if mucous secretions infiltrate into neighboring tissues. Intermediate cells come in a wide range of sizes and have a small nucleus in the middle with very little cytoplasm.[12]

Low-grade tumors exhibit glandular or ductal characteristics and also a tiny number of mucous-secreting cells. Large cystic regions surrounded by predominantly columnar cells with characteristics related to general transitional and epidermoid cells characterize intermediate-grade tumors. Most of the cells in solid nests or sheets of cells that form in severe grade lesions are epidermoid cells, but there are also some cystic cells. Infiltration, apoptosis, morphological pleomorphism, and an increasing number of mitotic divisions are also seen.[13] Low-grade tumors, especially those in their early stages, have a good prognosis.[14]

Individuals with elevated and localized metastases have a worse chance of survival. Clinical phases and histopathological grades have been connected to survival and prognosis.[4] The prognostic factors linked with studied tumor have not been well studied.[5] Numerous studies have tried to realize and comprehend cancer prognostic criteria.[15] As a result, greater study of the MEC is required to develop more accurate prognostic models and better treatment methods.[16]

Bcl-2 is termed after B-cell lymphoma-2, the second member of a family of proteins discovered in follicular lymphomas on chromosomes 14 and 18. It regulates cytochrome outer membrane signal transduction and has the potential to be either pro- or anti-apoptotic. Low-grade MEC is more likely to produce Bcl-2 than moderate or high-grade MEC. It is associated with less aggressive tumors, greater improved survival, and a better prognosis, making it a good marker for detecting MEC characteristics.[17] A proto-oncogene called Bcl-2 has been found in the endoplasmic reticulum, the membrane of the nucleus, and the edge of the mitochondria.[18] It has been shown to inhibit lymphocyte and leukemic population growth in follicular B-cell lymphoma by decreasing cell function.[8]

The objective of the present research was to identify the immunohistochemical expression of the Bcl-2 marker in the various grades of MEC to estimate the severity and attitude of this cancer in our population. If we are capable of representing the severity of MEC, the attending physician may be capable to diagnose the initial cancer with high aggressiveness thus decreasing the likelihood of recurrence and distant metastasis and increasing lifespan outcomes in patients with MEC.

Materials and Methods

This study was conducted between 2014 and 2018 using (83) paraffin blocks from the Oral Diagnosis Department in the College of Dentistry/Baghdad University. Thirty cases of MEC from SGs were retrieved using a random selection technique. Those samples with defective paraffin blocks or inadequate tissue on the paraffin block slide were removed from the investigation, and the remaining samples were investigated under a light microscope (Olympus, Tokyo, Japan) following hematoxylin and eosin staining. Concurrently, two oral and maxillofacial specialists verified the diagnosis for each slide. The samples were divided into mild, intermediate, and high-grade divisions. On histopathological analysis, immunohistochemical activities against Bcl-2 (Genemed Biotechnologies, USA) were then conducted. As a substitute for the main antibody, phosphate-buffered saline was utilized as a negative control, and tonsil was utilized as a positive control.

MEC received ratings of low (0–4 points), moderate (5–6 points), and high (7–14 points) according to the grading system specified. Five histological characteristics, including the intracystic element, neurological infiltration, necrosis, proliferation, and anaplasia, have been used to determine these points. The positivity and intensity of bcl-2 were evaluated semi-quantitatively on at least 1000 cells viewed at 40 amplification and reported as the proportion of positive tumor cells compared to the total number of malignant cells in the same location. The percentage scores were then classified based on the 5% positive BCL-2 reactivity threshold. The immunoreactivity to bcl-2 was divided into four categories: [19]
0 = negative (no staining).
Score 1: + slightly positive (5-20% malignant cells).
Score 2: ++ moderately positive (20–50% of malignant cells).
Score 3: +++ highly positive (more than 50 percent of malignant cells).

SPSS version (19.0) was used to analyze the data (IBM Corp, New York, United States). The data were analyzed using descriptive statistics. Age, gender, and tumor site frequency were computed using the mean and standard deviation. The Chi-square method was used to evaluate the variables and Bcl-2 expression. A P value of 0.05 or less was considered significant.

Results

Clinicopathological findings

The present findings revealed that the patients' mean age was 49.93 years old, and the MEC of SGs occurred more often in females, with a man-to-woman ratio of 1:1.14. The palate was the most commonly affected location, accounting for 13 (43.3%) of cases, followed by the parotid glands with 8 (26.7%). According to histopathological grading, high grade accounted for 14 (46.7%) of the cases, mild grade accounted for 10 (33.3%), and moderate grade accounted for 6 (20.0%) [Table 1].

Immunostaining findings

The immunohistochemical results of cytoplasmic expression of Bcl-2 revealed 4 (13.3%) cases with negative staining in 30 samples of studied cases, 9 (30.0%) cases with score of weak and moderate staining (+1 and +2), and 8 (26.7%) cases with score of high staining (+3) [Figure 1]. The distribution of Bcl-2 expression in regarding to tumor grading was highest score in mild and sever differentiated tumor cases [Figure 2].

There was a significant correlation between Bcl-2 staining and gender variable (P = 0.047), as well as a significant difference between age groups and tumor grading (P = 0.002). Furthermore, there were no significant relationships between the Bcl-2 expression and the other variables, and there were also no significant relationships between the other variables [Table 2].

Discussion

Salivary gland tumors (SGTs) constitute 1%–4% of all body tumors.[20] Malignant SGTs account for about 5% of all malignant tumors of the head-and-neck, with MEC being the most frequent, primarily involving the oral cavity.[21,22] It is a unique epithelial neoplasm made up of epidermoid, mucous, and intermediate cells.[23]

In the present study, the average age group was 49.9 years old, and the age range was between 11 and 80 years. This result was agreed with other study findings which stated that MEC has been recognized and described in both children and adult patients.[23]

Regarding to gender, this study revealed that the women slightly more affected with MEC than men. The cause may be related to physiological processes in women body that are not found in men. This finding was concordant with several studies which showed that women predilection.[24,25] Whereas in contrast to the present study, some researchers reported that there was no sex predilection.[8,13] and other study stated that men more frequent that women.[26] Furthermore, the results revealed that there are statistically significant results between the Bcl-2 expression and gender of studied patients at $P < 0.05$%.

Table 1: Frequencies of age groups, gender, site, and grade for the patients with mucoepidermoid carcinoma of salivary glands

Clinicopathological variables	Age groups	Frequencies	(%)	*P*-value
Age groups	(11–20)	1	4.5	*P*=1.000 Non Sig.
	(21–30)	3	4.5	
	(31–40)	2	15.9	
	(41–50)	7	20.7	
	(51–60)	8	29.5	
	(61–70)	7	18.1	
	(71–80)	2	6.8	
	Mean±SD	49.93±15.503		
Gender	Male	14	46.7	*P*=0.715 Non Sig.
	Female	16	53.3	
Site		Frequency	(%)	*P*=0.088 Non Sig.
	Palate	13	43.3	
	Parotid	8	26.7	
	Buccal Mucosa	5	16.7	
	Others	4	13.3	
	Total	30	100.0	
Grade	Mild	10	33.3	*P*=0.202 Non Sig.
	Moderate	6	20.0	
	High	14	46.7	
	Total	30	100.0	

S: Sig. at $P<0.05$, NS: Non Sig. at $P>0.05$; Testing are depending on One Sample Chi-square test

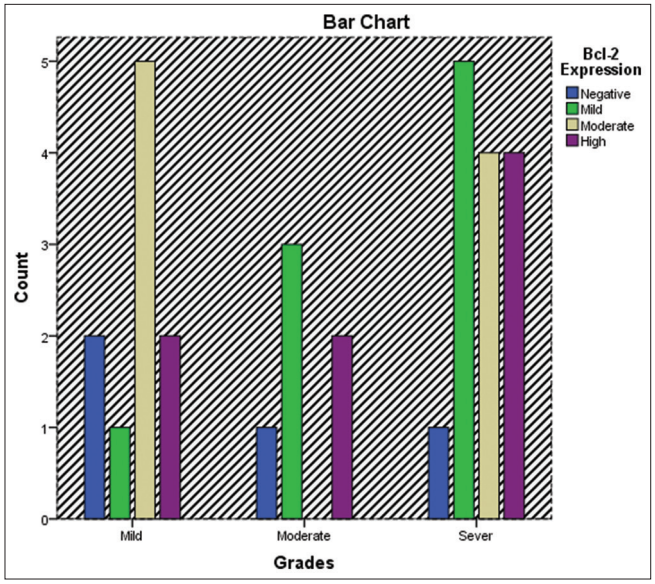

Figure 1: Bar chart presents the frequency of the Bcl-2 protein scores of different grades of studied salivary gland MEC samples

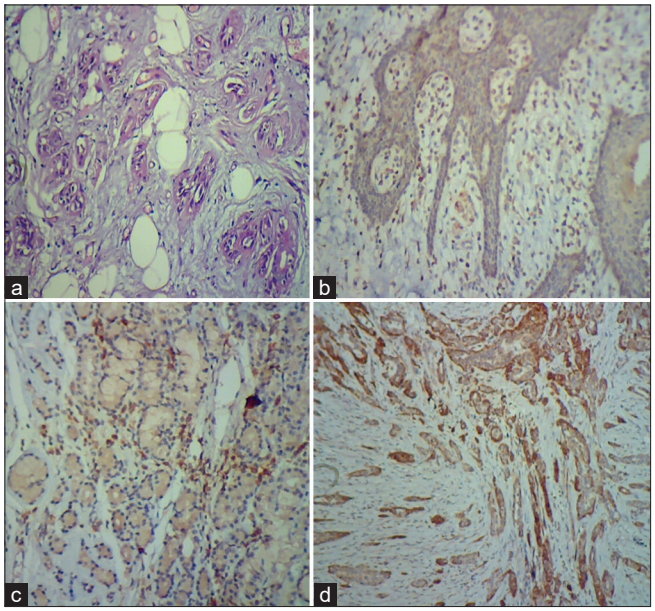

Figure 2: (a) Photomicrograph of poorly differentiated MEC of salivary glands (H&E stain), (×20), (b) weak positive Bcl-2 expression in well-differentiated MEC (×20), (c) moderate positive BCl-2 expression in poorly differentiated MEC (×20), and (d) strong positive Bcl-2 expression in poorly differentiated MEC (×20)

The findings of this research recognized that the minor salivary glands of the palate were the most frequent location then followed by the parotid gland and finally followed by buccal mucosa. This is indicated in different studies conducted on MEC,[27,28] while other researches stated that the parotid gland was the most often affected gland, followed by minor salivary glands.[25,26]

The Bcl-2 proto-oncogene provides a favorable protective effect on B cells, encouraging their oncogenic progression.

Table 2: The relationships of the Bcl-2 expression with the clinicopathological characteristics of the studied patients with mucoepidermoid carcinoma of salivary glands

Variables	Age	Gender	Site	Grade
Bcl-2 expression				
Pearson Correlation	0.031	0.365*	0.062	0.045
Sig. (2-tailed)	0.869	0.047	0.744	0.813
n	30	30	30	30
Age				
Pearson correlation	-	-0.052	0.055	0.540**
Sig. (2-tailed)	-	0.783	0.771	0.002
n	-	30	30	30
Gender				
Pearson Correlation	-	-	0.000	0.000
Sig. (2-tailed)	-	-	1.000	1.000
n	-	-	30	30
Site				
Pearson Correlation	-	-	-	0.319
Sig. (2-tailed)	-	-	-	0.086
n	-	-	-	30

*Correlation is significant at the 0.01 level, **Correlation is significant at the 0.05 level (2-tailed)

The Bcl-2 study found the assumption that inappropriate programmed cell death leads to cancer, indicating a remarkable improvement in our present knowledge of carcinogenesis. Clinical trials of experimental medicines targeting Bcl-2 family members have raised confidence that a special generation of chemotherapeutic medications is in the future.[29]

According to tumor grading, the results of the study showed that most of the MEC samples were in high grade (46.7), while the lowest cases were in intermediate grade (20%). This result was agreed with other study conducted with MEC.[28] Whereas several studies were disagreed with current study which stated that most of MEC found in our population are of low grade.[26,30] The study reported that there is a significant correlation between the tumor grading and the age groups at $P < 0.05\%$.

In this study, positive immunoexpression of Bcl-2 protein was 86.7%, while negative immunoexpression was 13.3% because of differences in tissue sections. The greatest levels of strong and moderate positive Bcl-2 expression were found in high grades, whereas the highest levels of intermediate positive expression were found in low grades. However, this discovery contrasted with the previous studies that found positive expression of Bcl-2 in 60% of MEC patients, as well as low-grade MEC having greater expression of Bcl-2 than intermediate and high-grade MEC. This difference may be due to a discrepancy in research conditions.[30,31]

According the current research, the diagnostic behavior of a MEC is mostly determined by its tumor grade, which may range from a low-grade malignancy to an extremely aggressive tumor with a high death rate. Studies show that the patient's

prognosis and treatment depend on the histological grade of a MEC.[17,23]

The findings of this research suggest that Bcl-2 expression be used in the standards used to grade MEC. Bcl-2, in combination with other diagnostic and clinicopathological biomarkers, may assist in evaluating tumor activity and outcome. This may aid treating oncologists in formulating a treatment strategy based on the severity of the tumor, thereby reducing relapse and enhancing the rate of survival.

Conclusions

MEC behavior may be altered by gender, and the grades and aggressiveness of MEC tumors can be identified by predicting the patient's age. The highest levels of strong and moderate positive Bcl-2 expression were detected in MEC tumors with high grades, whereas the highest levels of intermediate positive expression were observed in tumors with low grades. As a result, the presence of Bcl-2 in MEC lesions may aid in predicting the behavior of these tumors in terms of their aggressiveness. In the future, targeted therapy against BCL-2 could be used to help MEC patients get better care.

Authors' Declaration Statements

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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