Immunohistochemical Evaluation of Bcl-2 in Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma of Salivary Glands

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Abstract

Mucoepidermoid carcinomas are the most common type of salivary gland cancer. Most start in the parotid glands. They develop less often in the submandibular glands or in minor salivary glands inside the mouth. Adenoid cystic carcinoma is usually slow growing and often appears to be low-grade when looked at under the microscope. Bcl-2 (B-cell lymphoma 2), encoded in humans by the BCL2 gene, is the founding member of the Bcl-2family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis.

Objectives: To evaluate the hisopathological immunopositivity of Bcl-2 in Mucoepidermoid carcinoma and Adenoid cystic carcinoma and compare the results with Clinicopathological parameters and histological grades by using immunohistochemistry.

Thirty cases have been studied histologically, (15) cases of Mucoepidermoid carcinoma and (15) cases of Adenoid cystic carcinomas were randomly retrieved for the period (2014 – 2018) the archives were obtained from the Specialist Surgeries Hospital, Medical city, Baghdad, Iraq Iraq. The correlations between the clinical and pathological parameters were statistically analyzed by Chi-square test the expressions of K-i67 antigen were evaluated and the level of significance was 0.05 (two-sided).

All patients were included in this study 15 (50%) men and 15 (50%) women. Men to women ratio was 1:1. The average age of patients was 52.3 years ranged (23-71 years) in males and 46.8 years ranged (35-57 years) in females. The palate was the most common site followed by the parotid gland and the other sites. For MEC 4(26.7%) cases were grade (I), 3(20%) cases were grade (II) and 8 (33.3%) cases (33.3%) were grade (III). While for ACC 6(40%) cases were grade (I), 7 (46.7%) Cases were grade (II) and 2 (13.3%) Cases were grade (III). In Two tumors cases, Positive Bcl-2 expression was observed in all studies cases with variable extent of immunostaining except 2(6.7%) cases were negative expression.

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Introduction

The growth of abnormal cells results in cancer and then develop of these cells in the process of cell division. The DNA mutations of number of cells that follow abnormal signals that produce proteins that disrupt cellular balance and lead to carcinogenesis to divide and differentiate cells continuously. that produce proteins that disrupt cellular balance. This signal autonomy then develops these cells, so that growth being uncontrolled and proliferation occurred. This proliferation will spread into cancer cells if it is left[1].

Salivary gland tumors are uncommon and comprise approximately 1% of all neoplasms in the whole body and the malignant salivary gland neoplasms account for 0.3% of human malignancies and for 3% to 6% of all head and neck cancers [2]. Salivary gland tumors are rare in children; however, the frequency of malignancy is higher in children compared to adults. About 35% of all salivary gland tumors in children are malignant and the most common malignant salivary neoplasms are mucoepidermoid carcinomas(MEC) in children and adults [3]. The classification of WHO of the head and neck

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tumors contains 24 different types of salivary carcinomas[4]. The degree of malignancy comprising high grade, intermediate grade and low grade determines more practically grouping of salivary gland tumors. Another practical grouping is based on the relative frequency of their occurrence and this distinguished mucoepidermoid carcinoma, adenoid cystic carcinoma, and acinic cell carcinoma as a group of most frequently occurring carcinomas [5].

Salivary gland neoplasms often show different growth patterns and significant morphologic variability may exist within a single tumor and between different tumors [6]. Mucoepidermoid carcinoma (MEC) consists of both epidermal and mucous cells in varying proportion within the tissue [7]. The incidence of Mucoepidermoid carcinoma (MEC) presents less than 10% of all salivary gland neoplasms [8,9]. Adenoid cystic carcinoma (ACC) is a rare slowly enlarging epithelial tumor of the salivary glands. It comprises 5% to 10% of all salivary gland tumors. It usually arises in the fifth and sixth decades of age and presents (31%) in minor salivary glands and nearly 50% of all intra oral ACC is located in the palate [10]. Apoptosis is an active distinct process of cell death that is responsible for killing of injured, altered cells in normal and in certain specific abnormal circumstances like neoplasia [11,12].

Bcl-2 is an important proto-oncogene that is involved in the controlling cell death by inhibiting apoptosis in many physiologic and neoplastic conditions of cell growth [13,14]. Bcl-2 is located on the chromosome 18q21 and the B-cell lymphoma (Bcl-2) family comprises different regulators involved in apoptosis [15]. The role of Bcl-2 proto-oncogene as a new kind of oncogene is the blocking of programmed cell death (PCD)which has been seen primarily in follicular B-cell lymphomas. In these lymphomas the Bcl-2 gene is moved into juxtaposition with powerful enhancer elements in the immunoglobulin heavy chain (IgH) locus, so the result is overproduction of bcl-2 mRNAs and their encoded proteins [16]. In a number of cancers Bcl-2 expression is increased and it is thought to be involved in resistance to conventional cancer treatment [17]. Many of malignant tumors depend on the antiapoptotic activity of Bcl-2 for tumor initiation and maintenance [18]. The behavior of the tumor can be potentially detected by the antiapoptotic marker such as Bcl-2 [19]. The contribution of Bcl-2 primarily to malignant cell expansion by increasing the rate cell survival rather than to increase the rate of cellular proliferation, and accumulation of an aberrant Bcl-2 expression within the cells could be an important mark in the cause of cancer [20]. The overexpression of Bcl-2 presented in most human low-grade tumors and this antiapoptotic activity of Bcl-2 has been considered as being one of the most common steps of tumorigenesis [21].

Materials and methods

Thirty cases, histologically diagnosed as a salivary gland tumors were randomly retrieved from the archives of Specialist Surgical Hospital, Baghdad, Iraq for the period (2014 – 2018). They consisted of (15) cases of Mucoepidermoid carcinoma and (15) cases of Adenoid cystic carcinomas. The work was performed in teaching laboratories. The clinicopathological informations in regard to age, gender and lesion site were obtained from the patient files (chart). Formalin fixed paraffin embedded tissue blocks were used. In each block, one representative section was stained with hematoxylin and eosin for reassessment of histopathological diagnosis and two other sections were prepared on adhesive slides for detection of Bcl-2 by using immunohistochemistry.

Paraffin blocks were cut in 4-µm thick sections for optimum resolution with staining, and then the sections were carried on adhesive slides and left to dry upright in order to facilitate adhesion between the sections and the charged glass surface. The tissue sections were dried on the slides by heating (overnight at 65 C°) in a hot air oven to ensure that any moisture trapped under the tissue is completely eliminated by melting the paraffin and evaporation of the water droplets. A section was considered either positive or negative according to the presence or absence of brown staining in cytoplasm of tumor cells in regarding Bcl-2 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The immunostained sections were examined using light microscope to assess the prevalence of positive cases and the localization of immunostaining within the tissues. In addition, image analysis computer system was used to assess area percentage of positive cells of the immunostaining. By using the computer in Data analysis and using SPSS version 19 computer

software (Statistical Package for Social Sciences) to statistical analyses. The categorical data represent the scoring of studied parameters thus they presented as percentage and count. Frequency distributions for selected variables were done first. The relationship between categories was evaluated by Chi-square test. P value was less than 0.05 considered statistically significant.

Results

In this comparative study the range of age was divided into three groups, the first group < 40, the second group between (40−59) and the third group ≥ 60. Patients included 15 (50%) men and 15 (50%) women. Men to women ratio was 1:1. The average age of patients was 52.3 years ranged (23-71 years) in males and 46.8 years ranged (35-57 years) in females(Table 1).

	Tumor type				
	Mucoepidermoid carcinoma		Adenoid cystic carcinoma		
	N	%	N	%	P
Age in years					0.09 [NS]
<40	1	6.6	4	26.7	t-test
40-59	10	66.7	8	53.3	
60+	4	26.7	3	20	
Total	15	100	15	100	
Mean+/-SD	51.7 +/-11.8		47.4 +/-12.7		
Gender					0.01 [S]
Male	8	53.3	7	46.7	
Female	7	46.7	8	53.3	
Total	15	100	15	100	

Table 1: The difference in age and gender distribution between the MEC and ACC.

According to Site distribution in MEC and ACC, the palate was the most common site followed by the parotid gland and the other sites (Table 2).

	Tumor type				
	Mucoepidermoid carcinoma		Adenoid cystic carcinoma		
	N	%	N	%	P
Site					0.003[S]
Palate	6	40	6	40	
Parotid gland	3	20	4	26.7	
Lips	1	6.7	1	6.7	
Tongue	1	6.7	1	6.7	
Lymph node	1	6.7	1	6.7	
Buccal mucosa	3	20	2	13.3	
Total	15	100	15	100	

Table 2: The difference in oral sites distribution between the MEC and ACC.

In regarding to histopathological grading of Mucoepidermoid carcinoma were categorized according to growth pattern 4(26.7%) cases were grade (I), 3(20%) cases were grade (II) and 8 (33.3%) cases (33.3%) were grade (III). While for Adenoid cystic carcinoma 6(40%) cases were grade (I), 7 (46.7%) Cases were grade (II) and 2 (13.3%) Cases were grade (III). (Figure 1).

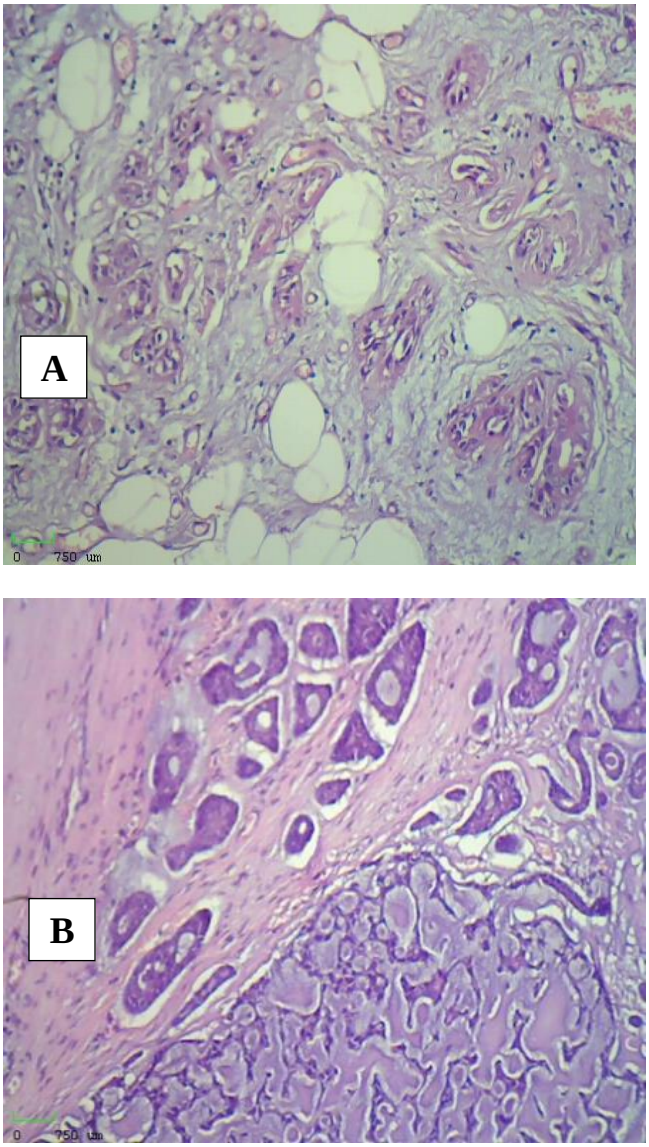
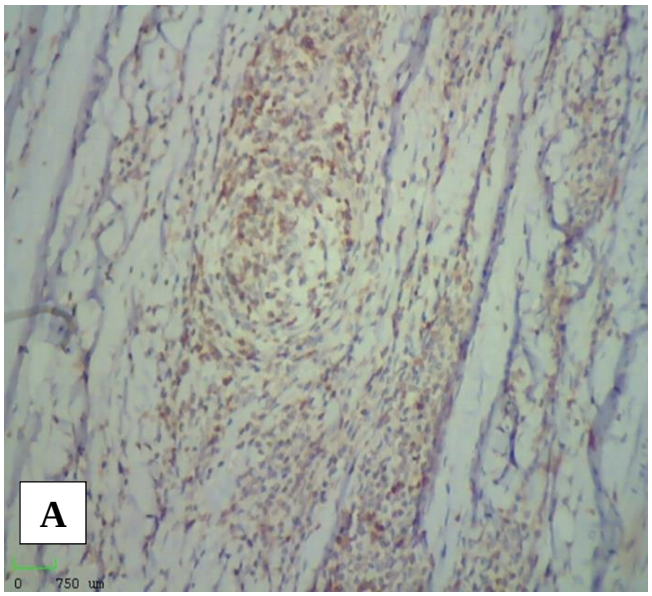
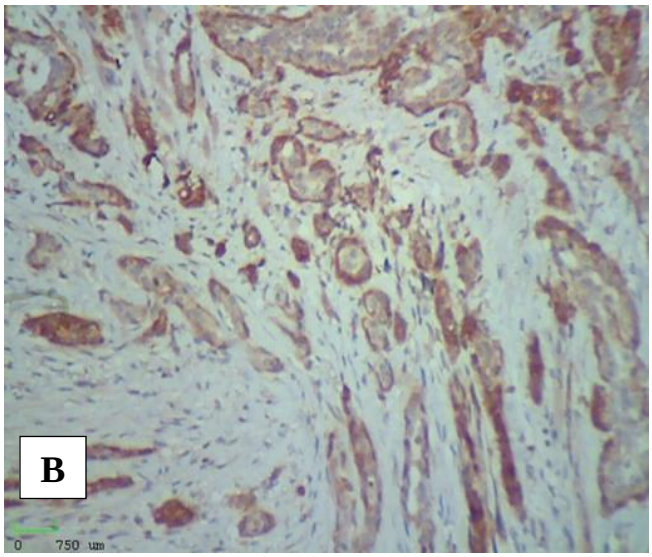


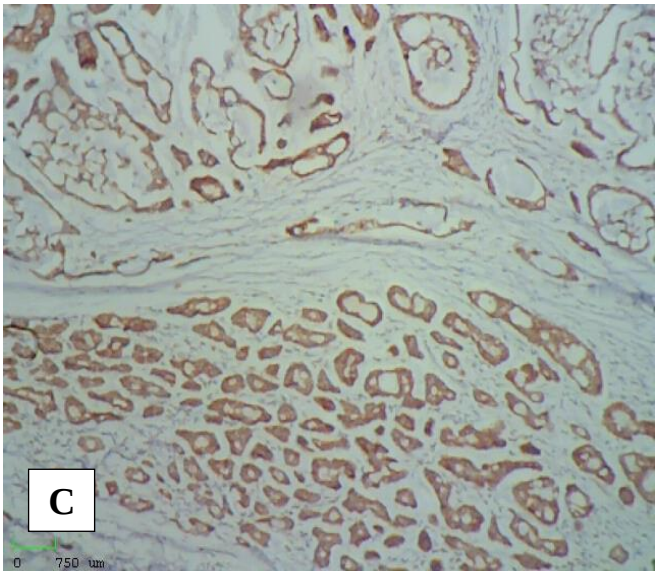
Figure 1: (A) Well-differentiated Mucoepidermoid carcinoma. H & E stain, grade (I). (X20), (B) Adenoid cystic carcinoma, tubular growth pattern. H & E stain, grade (II). (X20).

The present study revealed diffuse cytoplasmic staining for Bcl-2, immunoreactivity was found in the basal cell layer, whereas suprabasal cells showed no Bcl-2 immunoreactivity. The intensity of Bcl-2 staining was weak (1+) compared to lymphocytes within the same section in both malignant tumors. Adjacent salivary gland ducts, lymphatic tissue,

and nerves also expressed Bcl-2 in the non-neoplastic salivary gland tissue. In MEC cases, positive Bcl-2 expression was observed in all studies cases with variable extent of immunostaining except one case was negative expression. 7 (46.7%) cases with weak positive expression, 6 (40%) cases with moderate positive expression and 1 (6.7%) case with strong positive expression. In ACC, also one case (6.7%) was negative expression with Bcl-2 and 4 (26.7%) cases with weak positive expression, 6 (40%) cases with moderate positive expression and 4 (26.7%) case with strong positive expression (Figure 2).







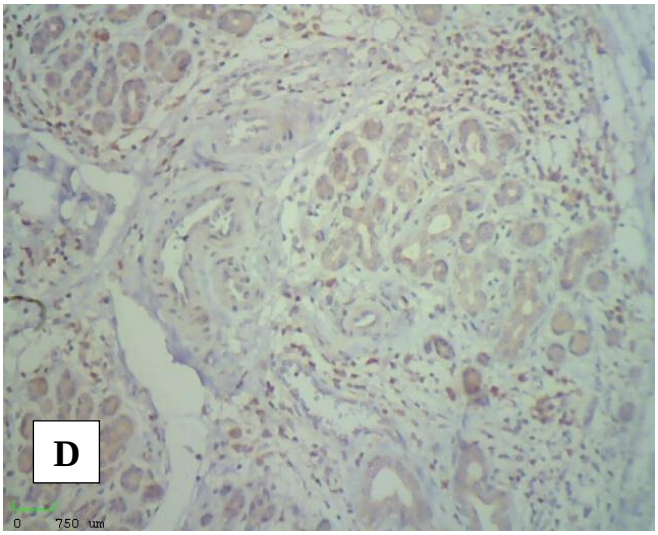
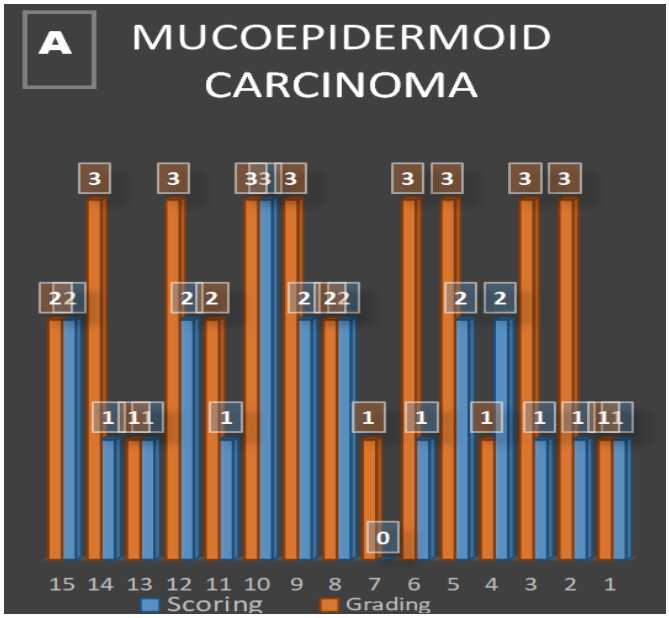


Figure 2. (A) Moderate positive Bcl-2 immunohistochemical expression in Mucoepidermoid carcinoma, high grade (X20), (B) High positive Bcl-2 immunohistochemical expression in Mucoepidermoid carcinoma, high grade (X20), (C) High positive Bcl-2 immunohistochemical expression in adenoid cystic carcinoma, moderate grade (tubular pattern) (X20), (D) Moderate positive Bcl-2 immunohistochemical expression in adenoid cystic carcinoma, low grade (Cribriform pattern) (X20).

The interpretation of the results by correlating and cross tabulating with the clinical and histopathological categories. For MEC the highest expression of Bcl-2 staining levels on the high grade (poorly differentiated MEC) were 8 (53.3%) cases and the lowest expression on the

moderately grade (moderately differentiated MEC) were 3 (20%), while for ACC the highest expression of Bcl-2 staining levels on the moderately differentiated (tubular pattern) were 7 (46.7%) cases and the lowest expression on the poorly differentiated (solid pattern) were 2 (13.3%) cases. The correlation between the expression Bcl-2 staining and the tumor grade level for MEC and ACC was non-significant (P= 0.79) and (P=0.95) respectively (Figure 3).



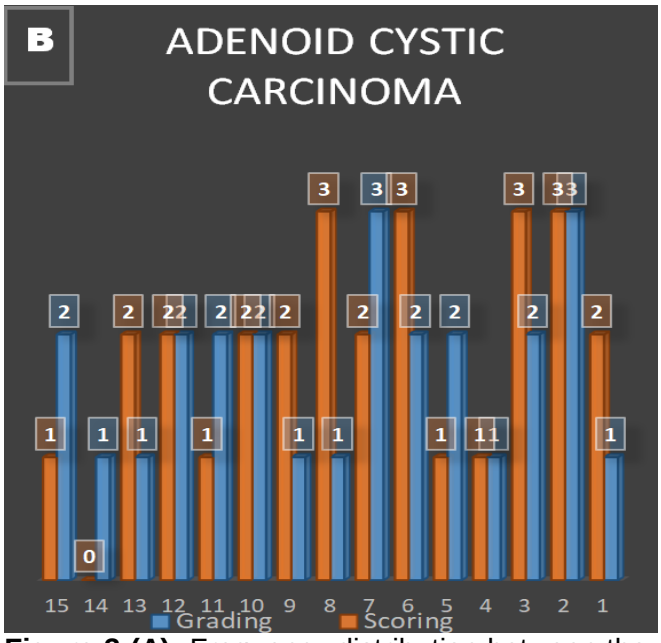


Figure 3 (A): Frequency distribution between the grading of MEC and scoring of Bcl-2 expression **(B):** Frequency distribution between the grading of ACC and scoring of Bcl-2 expression.

Discussion

In present study, men to women ratio was 1:1, while in most other studies the ratio was 1:1.3 which showed women predominance for ACC and other salivary gland tumors[22]. However, there are studies reporting men predominance in ACC[23], and in others reporting an equal gender distribution[24,25]. The mean age of patients was 48.6 years but in other studies was 46 years and 53% of them were in 4th and 5th decades. This finding of mean age correlated with other previous studies[26,27]. The origin of MEC of salivary gland from pluripotent reserve cells of excretory ducts which differentiates into columnar, mucous and squamous cells[28].

The gross appearance of Low-grade MEC are small and partially encapsulated tumor tissues and microscopically characterized by the presence of more mucous producing cells[29]. MEC is characterized by prominent cystic structures lined by mature mucous, intermediate, or epidermoid cells in addition to solid areas are not evident and prominent fibrous stroma often is present. ACC is a rare malignant tumor accounting for 2% to 4% of all head and neck malignancies [27]. It most commonly occurs in minor salivary glands, and palate is the most common site (>= 50% of cases), while in our study the ratio was 40%, so this finding is nearly accordance with other findings [23,30]. Microscopically, the tumor cells of ACC are two types, first cells that lined the duct and second the myoepithelial cells [10].

The characteristic feature of ACC is the perineural or perivascular spread without stromal reaction. In many studies, the most common pattern of ACC was the cribriform pattern with incidence of (50%) of cases, but in current study was tubular pattern (46.7 %) of cases[27,31]. The family of Bcl-2 gene has an important role in the regulation the death and survival cycle of cells without affecting cell proliferation. Overexpression of Bcl-2 prevents the death of epithelial cells, but it is cannot immortalize cells nor cause tumorigenesis in immortalized cells [32]. However, the early event in carcinogenesis may appear to be overexpression of the antiapoptotic Bcl-2 and subsequent cooperation with cellular and viral oncogenes [33].The immunohistochemical expression of Bcl-2 has been evaluated in some salivary gland tumors such as pleomorphic adenoma, monomorphic adenoma, Warthin's

tumor, basal cell adenoma, mucoepidermoid carcinoma, adenocarcinoma(NOS), acinic cell carcinoma, adenoid cystic carcinoma, anaplastic carcinoma, squamous cell carcinoma, and basal cell adenocarcinoma in addition to its use as an immunohistochemical marker in the differentiation between two malignant tumors of salivary glands is not been reported. Bcl-2 reactivity in ACC was found to be the strongest when compared to other malignant salivary gland tumors such as acinic cell carcinoma, mucoepidermoid carcinoma, and undifferentiated carcinoma and this was attributed to the fact that other tumor genes that influence apoptosis may be present in salivary gland tumors but bypass the Bcl-2 associated apoptotic pathway; such genes include p53, Rb tumor suppressor genes, and c-myc proto-oncogene and this is accordance with current study [34].

In non-neoplastic tissue of salivary glands, the adjacent salivary gland ducts, lymphatic tissue, and nerves are expressed Bcl-2 as in cases of this study, so Bcl-2 positivity was observed in ductal but not in acinar cells, while Other studies have also reported Bcl-2 expression in basal cells of striated and excretory ducts, indicating that these cells are reserve cells that acinar, myoepithelial, and most luminal cells are negative for Bcl-2 [35]. The staining intensity of solid pattern of ACC was significantly lower than cribriform and tubular subtypes according to Freier et al study, an unexpected finding since solid ACC is clinically more aggressive and may inherit a higher number of cytogenetic aberrations [36]. A higher expression of Bcl-2 was noted in the solid and cribriform types of ACC suggesting that Bcl-2 expression might be associated with myoepithelial cells as these types have more myoepithelial than ductal cells [37]. Furthermore, in the regulation of apoptosis there was a significant inverse correlation between Bcl-2 reactivity and the apoptotic index that found to be supporting the suppressing mechanism of Bcl-2[35]. Soini et al. compared Bcl-2 expression in pleomorphic adenomas and in malignant salivary gland tumors comprising of mainly ACC and MEC, found 100% positivity in pleomorphic adenoma and 64% positivity in malignant tumors, in contrast, present study showed a higher expression of Bcl-2 in ACC (26.7%) and lesser expression in MEC (6.7%).

Thus the cell survival rate is generally higher in ACC than MEC. This finding is in accordance with Garlifante et al study and others [35,38]. The degree of malignancy of the most malignant tumors is related to the activity of carcinoma cells [39]. The increased expression of Bcl-2 in epidermoid cells of MEC can be correlated with increased survival rate of these cells and tumors with predominance of these cells. A recent report of Bcl-2 expression in MEC by Yin et al that concluded that Bcl-2 is one of the potentially useful markers for survival in patients with MEC in minor salivary glands [40]. The selective survival advantage of the B cells that promotes their neoplastic expansion is provided by Bcl-2 proto-oncogene. The concept that defective programmed cell death contributes to malignancy was established by studies of Bcl-2, representing a major step forward in current understanding of tumorigenesis. In recent years there are increased hopes of administration of new experimental therapies targeting Bcl-2 family proteins as an anticancer drug [41].

The immunohistochemical expression of Bcl-2 is highly variable in epithelial malignancies. The cell type and degree of differentiation may effect on the Bcl-2 expression in addition to other factors such as viral infection may also contribute to high levels of Bcl-2 in epithelial malignancies [32] . Elevated Bcl-2 expression was related to the possible protection against apoptosis, so some studies stated that 90% (18 out of 20) of cases showed positive expression and both layers of the neoplastic epithelium (basal and luminal cells) showed positive immunoreactivity, with cytoplasmic localization, surrounded by a negatively stained stroma and this agreed with this study [42]. The lack of Bcl-2 expression in these cells may be linked to the degree of differentiation. Terminal differentiated cells like the mucous cells, normal acinar cells, salivary gland duct cells also do not express Bcl-2, but basal cells of the normal oral epithelium express Bcl-2 [43,44].

Genetically, there are certain domains of homology between family members termed Bcl-2 homology domains that are critical for various aspects of their activities, including the induction or suppression of cell death. However, the mechanism of the apoptosis of cells that is regulated by proteins of Bcl-2 gene family is still unclear. Collected evidence has also showed that these proteins hetero-dimerize and homodimerize with each other and that the relative proportions of these dimers appear to

determine whether a cell becomes apoptotic or not, Thus, the evaluation of several proteins in the Bcl-2 family might be more important than changes within single protein level for the understanding of the role that played by the Bcl-2 family in the regulation of apoptosis [45,46].

Conclusions

In conclusion, the palate was the most common site followed by the parotid gland and the other sites in MEC and ACC. The immunohistochemical expression of Bcl-2 could be used as an effective biomarker to predict aggressive biologic behavior of MEC and ACC. The evaluation of several proteins in the Bcl-2 family that play an important role in the regulation of apoptosis. The correlation between the expression Bcl-2 staining and the tumor grade level for MEC and ACC was non-significant. However, further investigations with larger samples are needed to reach further results.

Declaration of Interest

The authors declare that there is no conflict of interests.

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