The Differences between Benign Mixed Tumor and Papillary Cystadenoma Lymphomatosum in Proliferative, Apoptotic and Antiapoptotic Activities

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Abstract

Background: The majority of salivary glands tumors are benign mixed tumors. The present study was aimed to evaluate the differences between benign mixed salivary glands tumor and papillary cystadenoma lymphomatosum regarding the tumor cell proliferation rate, apoptotic and antiapoptotic activities. Methods: The study was performed on archived paraffin-embedded salivary glands tissue of 23 benign mixed tumor and five cystadenoma lymphomatosum. Sections were stained with hematoxylin and eosin and cases with definite diagnosis were selected for immunohistochemistry. The immunoreactivity was assessed in areas of highest positivity regarding Ki-67, P53 and bcl-2. Results: Benign mixed tumor and papillary cystadenoma lymphomatosum showed negative immune expression for Ki-67. The P53 immune staining in benign mixed tumor appeared negative while the papillary cystadenoma lymphomatosum appeared with a mild positive staining. The bcl-2 immune expression in benign mixed tumor was moderately positive while the papillary cystadenoma lymphomatosum was mildly positive. Conclusion: The benign mixed tumors showed significantly more Ki-67 and bcl-2 immune labeling indexes than papillary cystadenoma lymphomatosum, but the P53 immune expression was less (P<0.05). This indicated that the P53 expression was not correlated with the Ki-67 or bcl-2 over expression.

Keywords: Benign mixed tumors, Ki-67, P53, bcl-2, salivary gland, papillary cystadenoma lymphomatosum.

Introduction

Salivary glands are divided into major and minor salivary glands. Salivary gland neoplasms make up to 1–4% of all human tumors and each tumor shows different biological behavior [1]. According to the WHO classification, all types of salivary glands tumors are pathologically diagnosed [2,3], and the majority are benign mixed tumors [4] which have a tendency for recurrence with a malignant transformation in some cases [5]. The second common benign cystic tumor of the salivary gland is papillary cystadenoma lymphomatosum (Warthin’s tumor) [6]. Ki-67 immunoreactivity is a prognostic factor in head and neck cancer and considered as a marker for cell proliferation and a better indicator for the aggressiveness of the tumor [7].

Apoptosis is a programmed cell death and can occur physiologically or in the course of different diseases. Changes in apoptosis are mostly associated with cases of oncogenesis or pathological conditions [8]. The tumor behaviors may be affected by P53 as apoptotic marker and bcl-2 as anti-apoptosis markers. When P53 and bcl-2

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loosetheir functions, this may be considered as major cause of carcinogenesis [9]. At a cell cycle check points, the induction of apoptosis and growth arrest are controlled by P53 and cause the elimination of all damaged cells. Normally, P53 in cells is inactive and bound to a protein MDM2 which activates its degradation by acting as ubiquitin ligase. P53 activation is induced by different cancer causing agents. P53 gene-mutation decreases cells ability to repair the damaged DNA before the entry into S-phase causing a greater chance of mutational fixation into the genome and then passed onto generations of cells [10]. The bcl-2 can be expressed in various types of malignant tumors, acts as a protector to cells from apoptosis-induced DNA-damaging agents. This effect is caused by retardation of cell proliferation caused by accumulation of cells in the G0 and G1 phases of the cell cycle [11]. The differences between benign mixed salivary glands tumor and papillary cystadenoma lymphomatosum regarding the tumor cell proliferation rate, the apoptotic and antiapoptotic activities have not been studied; therefore, this study was aimed to investigste the relationship between these three factors using Ki-67, P53 and bcl-2 markers. Thus guiding dental specialists to make correct diagnosis and provide appropriate treatments.

Materials and Methods
The study was performed on archived paraffin-embedded salivary glands tissue samples derived from 28 patients with benign salivary gland tumors (23 benign mixed tumor and five cystadenoma lymphomatous). They were retrieved from the archives of Pathology Laboratory of Al-Kafeel Hospital in Karbala, Iraq during the period between October /2016 and December /2018. All tumors examined were primary. Exclusion criteria included the presence of previous salivary gland surgery and other salivary gland pathologies. Tumor diagnosis was performed independently by two pathologists. Sample collection was authorized by Health Directorate in Karbala, Iraq. Also, at Al- Hussain University College, the research project was approved under protocol by the Research Ethics Committee. Using microtome, sections were made and then stained with hematoxylin and eosin and reviewed. Then cases with definite diagnosis were selected for IHC. The immunoreactivity, regardless of its intensity, was assessed in areas of highest positivity. Immunostaining for Ki-67 was performed using monoclonal Mouse Anti-Human Ki-67 Antigen, Code No. M 7240 staining system, Clone MIB-1 and N-series primary antibody, Dako EnVision™, EnVision™ double staining with LASAB™ 2 systems. Immunostaining for P53 and bcl-2 were done using monoclonal Mouse anti-human P53 (clone D07, IgG-ready to use) and monoclonal anti-human bcl-2 (clone 100/D5, IgG-ready to use). Positivity of the tumor cells for Ki-67 and P53 were demonstrated by a brown precipitate in the nucleus, while Bcl-2 was identified by brown cytoplasmic staining. With each batch of stain, positive and negative control tissue specimens were run. Biopsy cases of oral squamous cell carcinoma, breast ductal carcinoma and tonsils were immune-stained for Ki 67, P53 and bcl-2, respectively and considered as positive control slides. With a light microscope, all slides were examined and then photographed with digital camera at 100 and 400 magnifications. The Ki-67, P53 and bcl-2 immunoreactivity was quantitatively evaluated/ 1000 cells examined at 400x magnification and recorded as the percentage of positive tumor cells. The levels of Ki-67, P53 and bcl-2 positive expression were evaluated. Moreover, the percentage of positively-stained cells was calculated as: Absent, Mild, Moderate and Strong when it is <1, 1-10, 50% or >50%, respectively [12]. Data were presented as mean±standard deviation. To compare Ki-67, P53, and bcl-2 immune reactivity between them, t-test was used. Results with P ≤0.05 were considered significant. SPSS (Statistical Package for Social Science) software was used to analyze the data.

Results
Hematoxylin and eosin results were presented in Figure (1). The benign mixed tumors and papillary cystadenoma lymphomatous showed negative immune expression for Ki-67 (0.74±0.08% and 0.16±0.13%), respectively (Figure 2). Statistical analysis showed significant differences between them (P= 0.0003). The p53 immune staining in benign mixed tumor appeared as negative (0.14±0.04), while the papillary cystadenoma lymphomatous appeared as mild positive (2.26±1.22) and the positivity was mostly seen associated with epithelial cells (Figure 3).
Statistical analysis showed significant differences between them ($P = 0.008$). The bcl-2 immune expression in benign mixed tumor was moderately positive with a mean±SD immune labeling index of (34±8.18) and the localization of bcl-2 immune-positive cell was seen in ductal, myoepithelial cells, neoplastic myxomatous stromal cells and plasmacytoid cells (Figure 4). One case showed strong positive bcl-2 immune expression. It may represent an early stage of formation of carcinoma in pleomorphic adenoma. The bcl-2 immune expression in papillary cystadenoma lymphomatosum was mild positive (7.8±0.75) and the positive cells seen were mainly epithelial cells. Immunohistochemical expression of p53 and bcl-2 in papillary cystadenoma lymphomatosum might indicate the growth of the proliferating epithelial cells. Statistical analysis showed significant differences between them ($P = 0.00001$).

![Figure 1](image1.jpg)

**Figure 1:** Benign mixed tumor showing the epithelial and mesenchymal components (A; H&Ex400). Papillary cystadenoma lymphomatosum showed dense lymphoid stroma with surrounding of epithelial cells (B; H&Ex400).

![Figure 2](image2.jpg)

**Figure 2:** Negative Ki-67 immune expression in benign mixed tumor (A) and papillary cystadenoma lymphomatosum (B). The positive cells are mostly associated with epithelial cells, arrows (Immunohistochemistry 400X).
Figure 3: Negative P53 immune expression in benign mixed tumor (A) and positive P53 immune expression in papillary cystadenoma lymphomatosum (B). The positive cells were mostly associated with the epithelial cells (Immunohistochemistry 400 X).

Figure 4: Moderate (A1 and A2) and strong (A3 and A4) immune expression of bcl-2 in benign mixed tumor. Mild positive (B1 and B2) bcl-2 immune expression in papillary cystadenoma lymphomatosum (A1, A3 and B1), immunohistochemistry 100 X, A2, A4 and B2, immunohistochemistry 400X).
Discussion

Ki-67, a cell proliferation marker, is considered a prognostic factor in salivary gland tumors. In the present study, Ki-67 showed negative immune expression in benign mixed tumor (0.74±0.08%). On the other hand, found that Ki-67 labeling index in benign mixed tumor was 1% which is nearly similar to that of. In addition, found a mild positive immune expression of Ki-67 in benign mixed tumor which was 1.6% while found that the labeling index of benign mixed tumor was 2.8%. The mean labeling index in papillary cystadenoma lymphomatosum regarding the Ki-67 immune expression was (0.16±0.13%). Also, found a negative expression. These results disagreed with those of who found that papillary cystadenoma lymphomatosum showed mild positive immune expression with a labeling index of 3% which also correlated with. Apoptosis occurs physiologically and is associated with many diseases. Changes of apoptosis are always associated with oncogenesis. The over expression of P53 was an early event in the malignant transformation. Active P53 may be induced by ultra violet radiation and different DNA damaging drugs. The P53 gene mutation results in a conformational change in the protein, which becomes stabilized, thus allowing for immunohistochemical detection. In present study, the P53 immune stainings in benign mixed tumor and papillary cystadenoma lymphomatosum were 0.14±0.04 and 2.26±1.22, respectively. Some studies found that the benign mixed tumor showed negative P53 expression in the tumor cells. On the other hand, our results were in contrast with other studies that revealed positivity in benign mixed tumor. Moreover, results showed nuclear P53 was strongly expressed in 20.7% of pleomorphic benign mixed tumor. Also, found that in benign mixed tumors, P53 LI was 1.5%. The positivity of P53 in the nuclei of tumor cells of epithelial and myoepithelial components could be caused by the accumulated mutations which may lead to formation of carcinoma ex-PA, especially when it stays for a long period of time without any treatment. Furthermore, found that the immunohistochemical staining of Warthin’s tumor was positive for P53. However, found that all slides showed a total negativity for P53 and neither the pleomorphic adenoma nor Warthin’s tumor had any degree of positivity for P53 in that study. In present study, the bcl-2 immune staining in benign mixed tumor and papillary cystadenoma lymphomatosum were 34±8.18 and 7.8±0.75, respectively. In addition, study found that bcl-2 has an important role in the development of benign mixed tumor and all cases examined were positive for it and expressed especially in the tubuloductal, solid and trabecular areas. Also, found that (71%) of benign mixed tumors were positive for bcl-2 and localization of bcl-2 was in ductal and myoepithelial cells. Nonetheless, found that the localization of bcl-2 was in the neoplastic myxomatous stromal cells and plasmacytoid cells. Regarding papillary cystadenoma lymphomatosum, the bcl-2 was previously detected in 55.6% of cases while normal parotid gland tissues were found negative for bcl-2. Furthermore, found that 90% of papillary cystadenoma lymphomatosum showed positive expression of bcl-2. They suggested a protective role of tumor cells from apoptosis to maintain survival of cells, but not increase their malignant potentiality. A study by found that bcl-2 expression was observed in all benign mixed tumors and papillary cystadenoma lymphomatosums in which most cases showed strong expression. However, found that bcl-2 expression in benign mixed tumors was mainly in basal cells of tubuloductal structures. All the differences can be attributed to the racial and environmental factors or due to the different methods used for laboratory detection and calculation of positive cells.

Conclusion

Benign mixed tumors and papillary cystadenoma lymphomatosum showed negative immune expression for Ki-67. The P53 immune staining in benign mixed tumor appeared negative while the papillary cystadenoma lymphomatosum appeared mild positive. Also, bcl-2 immune expression in benign mixed tumor was moderate positive while the papillary cystadenoma lymphomatosum was mild positive.
Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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