The differences between benign mixed tumor and papillary cystadenoma lymphomatous in proliferative, apoptotic, and antiapoptotic activity

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ABSTRACT
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 lymphomatous regarding the tumor cell proliferation rate, apoptotic and antiapoptotic activity. The study was performed on archived paraffin-embedded salivary glands tissue of 23 benign mixed tumors and five cystadenoma lymphomatosis. Sections were stained with hematoxylin and eosin and cases with the definite diagnosis were selected for immunohistochemistry. The immunoreactivity was assessed in areas of highest positivity regarding Ki-67, P53, and bcl-2. Our results refer that benign mixed tumor and papillary cystadenoma lymphomatous showed negative immune expression for Ki-67. The P53 immune staining in benign mixed tumor appeared negative, while the papillary cystadenoma lymphomatous appeared with mild positive staining. Bcl-2 immune expression in the benign mixed tumor was moderate positive while the papillary cystadenoma lymphomatous was mild positive. We conclude that the benign mixed tumors showed significantly more Ki-67 and bcl-2 immune labeling indexes than papillary cystadenoma lymphomatous, but the P53 immune expression was less (p<0.05). This indicates that the P53 expression was not observed to be correlated with the Ki-67 or bcl-2 overexpression.

Key words: Benign mixed tumors, Ki-67, P53, bcl-2.

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Introduction
The salivary glands are divided into major and minor salivary glands. Salivary gland neoplasms make up 1–4% of all human tumors and each tumor shows different biological behavior. According to the WHO classification, all types of salivary glands tumors are pathologically diagnosed, and the majority is benign mixed tumors which has a tendency for recurrence with a malignant transformation in some cases. The second common benign cystic tumor of the salivary gland is papillary cystadenoma lymphomatous (Warthin’s tumor). 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 tumor. Apoptosis is 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. The tumor behaviors may be affected by P53 as an apoptotic marker and bcl-2 as anti-apoptosis markers. When P53 and bcl-2 lose their functions, this may be considered as the major cause of carcinogenesis.

At a cell cycle checkpoints, 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 an ubiquitin ligase. P53 activation is induced by different cancer-causing agents. P53 gene mutation decreases the cell's 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. The bcl-2 can be expressed in various types of malignant tumors, act as a protector to the cells from the apoptosis-induced DNA-
damaging agents. This effect is caused by the retardation of cell proliferation caused by a cell’s accumulation 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 activity has not been studied; therefore this study 
was based on investigating the relationship between these three factors using Ki-67, p53, and bcl-2 markers, thus 
guiding dental specialists to make correct diagnosis and provide the 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 lymphomatosum). They were 
retrieved from the archives of Pathology Laboratory of Al-Kafel in Karbala/ Iraq in 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 Ministry of Health in Karbala/ Iraq. 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 
the 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, DakoEnVision™, 
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. Biopsies cases of oral squamous cell carcinoma, breast ductal carcinoma, and tonsils were immunostained for Ki 67, P53 and bcl-2 respectively and considered as a positive 
control slides.

With a light microscope, all slides were examined and then photographed with a digital camera at 100 and 400 
magnifications. The Ki-67, P53 and bcl-2 immunoreactivity was quantitatively evaluated / 1000 cells examined at 
x400 magnification and recorded as the percentage of positive tumor cells. The levels of Ki-67, P53 and bcl-2 positive expression were evaluated: The percentage of positively stained cells was calculated as: Absent: < 1%, 
Mild: 1 - 10%, Moderate: 10 - 50%, and Strong: > 50% 12. Data were presented as mean ± standard deviation. To 
compare Ki-67, P53, and bcl-2 immune reactivity between them, a t-test was used. The result of P ≤ 0.05 was 
considered significant. SPSS (Statistical Package for Social Science) software was used to analyze the data.

Results
Hematoxylin and eosin results are seen in Figure-1. The benign mixed tumors and papillary cystadenoma 
lymphomatosum showed negative immune expression for Ki-67 (0.74 ± 0.08 % and 0.16±0.13%) respectively as 
seen in Figure-2. Statistical analysis showed significant differences present between them (P=0.0003). The p53 
immunostaining in benign mixed tumors appeared as negative (0.14±0.04). While the papillary cystadenoma 
lymphomatosum appeared as a mild positive (2.26±1.22) and the positivity was mostly seen associated with 
epithelial cells as seen in Figure -3. Statistical analysis showed significant differences present between them 
(P=0.008).

The bcl-2 immune expression in the benign mixed tumor was moderate positive with a mean 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 cystadenomalymphomatosum was mildly positive (7.8 ±0.75), and the positive cells were 
seen mainly in the epithelial cells. Immunohistochemical expression of p53 and bcl-2 in papillary
cystadenomalyphomatosum may indicate the growth of the proliferating epithelial cells. Statistical analysis showed significant differences present between them (P=0.00001).

**Figure-1:** Benign mixed tumor showing the epithelial and mesenchymal components (H&Ex400). Papillary cystadenoma lymphomatous shows dense lymphoid stroma with the surrounding of epithelial cells (B, H&Ex400).

**Figure-2:** Negative Ki-67 immune expression in benign mixed tumor (A), and papillary cystadenoma lymphomatous (B). The positive cells are mostly associated with the epithelial cells, arrows (Immunohistochemistry X400).

**Figure-3:** Negative P53 immune expression in benign mixed tumor (A) and positive P53 immune expression in papillary cystadenoma lymphomatous (B), the positive cells are mostly associated with the epithelial cells (Immunohistochemistry X400).
Discussion

Ki-67, a cell proliferation marker, is considered as a prognostic factor in salivary gland tumors (13). In the present study, the Ki-67 showed a negative immune expression in benign mixed tumor (0.74 ± 0.08 %). Mythily and Saranya (14) found that the Ki-67 labeling index in the benign mixed tumor was 1% which is nearly similar to that of Kazanceva et al. result (15). But Kaza et al. (7) found a mild positive immune expression of Ki-67 in the benign mixed tumor which was 1.6%, while Kitayama et al. (16) study found that the labeling index of the 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%). Kaza et al. (7) also found a negative expression. These results disagree with that of Sangeetha et al. study (17), they found that the papillary cystadenoma lymphomatosum showed mild positive immune expression with a labeling index of 3% which was also correlated with Kazanceva et al study (15, 37).
Apoptosis occurs physiologically and associated with many diseases. Changes in apoptosis are always associated with oncogenesis (18). The overexpression of P53 was an early event in the malignant transformation (19, 20). Active P53 may be induced by ultraviolet radiation and different DNA damaging drugs (21). The p53 gene mutation results in a conformation change in the protein, which becomes stabilized, thus allowing for immunohistochemical detection (22, 36). In the present study, the p53 immune staining in benign mixed tumor and papillary cystadenoma lymphomatous 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 (23, 24). On the other hand, our results are in contrast with another study that revealed positivity in the benign mixed tumor (25). Tarakji et al. (26) results showed nuclear P53 were expressed strongly in 20.7% of the pleomorphic benign mixed tumors. Kitayama et al. (16) found that in benign mixed tumor the P53 LI was 1.5%. The positivity of the P53 in the nuclei of tumor cells of epithelial and myoepithelial components could be caused by the accumulated mutations which may lead to the formation of carcinoma ex-PA, especially when it stays for a long period of time without any treatment. Kuzenko et al. (27) study found that the immunohistochemical staining of Warthin’s tumor was positive for P53. Alnour (28) found that all slides showed total negativity for P53 and neither the pleomorphic adenoma nor Warthin’s tumor had any degree of positivity for P53 in this study.

In the present study, the bcl-2 immune staining in benign mixed tumor and papillary cystadenoma lymphomatous were 34±8.18 and 7.8±0.75 respectively. Yanez et al. (29) study found that bcl-2 has an important role in the development of a benign mixed tumor and all the cases examined were positive for it and expressed especially in the tubuloductal, solid and trabecular areas. Atarbashi et al. (30) found that (71%) of the benign mixed tumor was positive for bcl-2, and localization of bcl-2 was in ductal and myoepithelial cells. But Aoki et al. (31) found that the localization of bcl-2 was in the neoplastic myxomatous stromal cells and plasmacytoid cells. Regarding the papillary cystadenoma lymphomatous, the bcl-2 was previously detected in 55.6% of cases, while the normal parotid gland tissues were found negative for Bcl-2 32. Abd-Elhamid and Elshafei (33) found that 90% of papillary cystadenoma lymphomatous showed positive expression of bcl-2. They suggested a protective role of tumor cells from apoptosis to maintain the survival of cells, but not increase their malignant potentiality. A study by Soini et al. (34) found that bcl-2 expression was observed in all benign mixed tumors and papillary cystadenoma lymphomatous in which most cases showed strong expression. Pammber et al. (35) found that bcl-2 expression in benign mixed tumors was mainly in basal cells of tubule ductal 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 lymphomatous showed negative immune expression for Ki-67. The p53 immune staining in benign mixed tumor appeared negative, while the papillary cystadenoma lymphomatous appeared as mild positive. While the bcl-2 immune expression in the benign mixed tumor was moderate positive while the papillary cystadenoma lymphomatous was mild positive.

References

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