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Original Article

Evaluation of VEGF expression correlates with COX-2 expression in pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma

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ABSTRACT

Background: Pleomorphic adenoma (PA), mucoepidermoid carcinoma (MEC), and adenoid cystic carcinoma (AdCC) are the most common benign and malignant salivary gland tumors. Cyclooxygenase-2 (COX-2) is a key regulatory enzyme that its overexpression in various tumors is correlated with progression, metastasis, and apoptosis inhibition. Vascular endothelial growth factor (VEGF) is a potent angiogenic mediator that has an important role in neoplastic angiogenesis. The aim of this study was to immunohistochemically analyze the expression of COX-2 and VEGF and to compare the expression of benign and two malignant salivary gland tumors with varied structures.

Materials and Methods: In this cross-sectional study, 90 specimens including 30 cases of each tumor were retrieved. Immunohistochemical staining of COX-2 and VEGF was performed for all the samples. The percentage of positive tumor cells and staining intensity was evaluated by two pathologists blindly. Data were analyzed by Chi-square and Gamma test and $P < 0.05$.

Results: A statistically significant difference was noted between the expression and intensity of COX-2 and VEGF in PA, MEC, and AdCC ($P < 0.05$). A significant correlation was observed between COX-2 and VEGF expression in MEC and AdCC ($P < 0.05$). However, no significant correlation was found between the expression and intensity of COX-2 and VEGF with histologic grade and lymph node metastasis in MEC and AdCC ($P < 0.05$).

Conclusion: High expression of VEGF and COX-2 in malignant tumors compared to PA suggested the role of both markers in malignant transformation. The significant correlation of VEGF expression with COX-2 may represent the role of COX-2 in tumor angiogenesis by modulating VEGF production.

Key Words: Adenoid cystic carcinoma, cyclooxygenase-2, mucoepidermoid carcinoma, pleomorphic adenoma, vascular endothelial growth factor

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INTRODUCTION

Pleomorphic adenoma (PA) is the most common benign neoplasm with remarkable degree of morphological diversity. It usually occurs in the age range of 30–50 years. It presents with a minor preference in women.[1]

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Mucoepidermoid carcinoma (MEC) is one of the most common salivary gland malignancies, mainly affecting parotid. The tumor occurs in the second to seventh decades of life and is also the most common malignant salivary gland tumor noted in children. MEC exhibits varied clinical presentations, that is, from a slow-growing mass to a destructive rapidly growing mass. The prognosis of MEC is usually related to clinical stage and histologic grade.[1]

Adenoid cystic carcinoma (AdCC) is one of the best-recognized salivary malignancies that can occur in any salivary gland site, but approximately 40%–45% develop within the minor salivary glands. AdCC is a persistent tumor that is prone to local recurrences and eventual distant metastasis.[1]

Many immunohistochemical studies in differential diagnosis of salivary gland tumors and identifying the prognosis of malignant salivary gland tumors have been published. However, few studies have focused on the expression of cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF) and their significance. For example, CD44 expression in PA and carcinoma ex-PA and their adjacent normal salivary glands was evaluated.[2] Moreover, P63 expression was assessed in papillary cystadenoma and MEC of minor salivary glands.[3] These studies show the importance of finding immunohistochemistry markers in evaluating the prognosis of these head-and-neck tumors.

COX-2 is a key regulatory enzyme in the synthesis of prostaglandins in most tissues. The presence of COX-2 is usually associated with cellular activation including inflammation. Its overexpression has also been demonstrated in gastrointestinal tract, breast, lung, esophagus, pancreas, urinary bladder, prostate, and skin. COX-2 enzyme is not present in healthy tissues.[4] It seems that several significant processes for cancer development such as apoptosis and angiogenesis are influenced by COX-2.[5] On the other hand, the correlation of COX-2 overexpression and VEGF expression in head-and-neck cancer and oral squamous cell carcinoma has been demonstrated, but the correlation in salivary gland tumors is still elusive.[4,6]

Sakurai *et al*. showed that the expression of COX-2 in various histologic types of salivary gland adenoma and carcinoma was higher than normal salivary glands.[5]

VEGF is known as a powerful cytokine and a regulator of vasculogenesis and tumor angiogenesis in a number of malignancies. It is also related to vascular permeability and vasoactive molecule production.[7]

Lequerica-Fernández *et al*. and Fonseca *et al*. demonstrated that overexpression of VEGF in malignant salivary gland tumors might be associated with pathogenesis, progression, aggressiveness, and lymph node metastasis.[7,8]

The main aim of this study was to evaluate the combined immunohistochemical analysis of COX-2 with VEGF expression in PA, MEC, and AdCC of salivary glands.

MATERIALS AND METHODS

Specimen selection

The samples of this cross-sectional study were collected from 90 formalin-fixed, paraffin-embedded tissue blocks of PA, AdCC, and MEC obtained from the archives of the Pathology Department, Amir Alam Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Thirty cases were diagnosed as PA, thirty cases as MEC, and thirty cases as AdCC. Hematoxylin and eosin-stained sections were used to confirm the diagnosis.

Clinicopathologic information on each case including age, sex, tumor location, and histologic grade was obtained from patient records and confirmed by reviewing the case slides. Cases without complete data, sufficient paraffin-embedded tumor material, appropriate fixation, incisional biopsy, and recurrent cases were excluded from the study.

Immunohistochemistry

4-μm sections were cut from all paraffin-embedded specimen blocks and mounted on silane-coated slides. The sections were deparaffinized with 100% xylene and rehydrated in graded ethanol series; they were immersed in Tris-buffered saline (TBS) of PH 6.0 and were heated in a microwave oven at 750 watts for antigen retrieval. After cooling into room temperature, the sections were incubated with primary antibodies: COX-2 (Monoclonal Mouse Anti-Human clone: SC-376861, Santa Cruz, USA) and VEGF (Polyclonal Rabbit Anti-Human clone: KLT9, Leica, USA) at 1:2000 for an hour through EnVision method. After washing in TBS, the sections were treated with a secondary antibody. DAB chromogen was applied to visualize the antibody and then counterstained with Mayer's hematoxylin. Ulcerative colitis and pyogenic granuloma were used as a positive control for COX-2 and VEGF, respectively.

Evaluation of immunohistochemistry

The COX-2 and VEGF immunoreactions in tumor cells were determined in 10 randomly selected fields by counting all positive cells (cytoplasmic staining) in each field according to the median index of positive cells obtained from 10 high-power fields and scored as follows:[9] 0 (negative), 1%–25% (score 1), 26%–50% (score 2), 51%–75% (score 3), and 76%–100% (score 4). The intensity of staining was evaluated as follows: 0 = no positive cells, + = mild, ++ = moderate, and + 3 = strong.[4,6]

Histopathologic grade of AdCC samples was classified into tubular (Grade 1), cribriform (Grade 2), and solid (Grade 3) based on the histologic type, and the grade was identified.[10] MEC was categorized into low, intermediate, and high grade according to Auclair *et al*.[11] All the slides were evaluated by two pathologists, blindly and concurrently.

Statistical analysis

Statistical analysis was performed on the tabulated data using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Gamma test were used for data analysis. The significant level of all tests was set at $P < 0.05$.

RESULTS

The general characteristics of all patients included in this study are shown in Table 1.

COX-2 was expressed in all cases of PA, MEC, and AdCC. In three groups, most cases were score 4 of expression. With respect to COX-2 intensity, 17 (56.7%) cases of PA, 27 (90%) cases of MEC, and 29 (96.7%) cases of AdCC showed strong intensity [Tables 2 and 3].

Chi-square test showed a significant difference between the intensity and expression of COX-2 in PA, MEC, and AdCC ($P < 0.001$).

Indeed, COX-2 expression showed a significant difference between MEC and AdCC ($P = 0.011$); however, there was no significant difference in COX-2 intensity between MEC and AdCC ($P = 0.612$).

VEGF expression was observed in all cases of PA, MEC, and AdCC. Twenty-three (76.7%) cases of PA, 29 (96.7%) cases of MEC, and 21 (70%) cases of AdCC exhibited score 4 of expression.

Considering VEGF intensity, 12 (40.0%) cases of PA, 27 (90%) cases of MEC, and 29 (96.7%) cases of AdCC showed strong intensity [Tables 4 and 5].

Table 1: Characteristics of all patients

Variables	PA	MEC	AdCC
Sex			
Male	14	16	6
Female	16	14	24
Age (mean)	38.13±16.35	40.63±20.90	43.73±15.43
Site of tumor			
Palate	18	1	2
Parotid	5	22	19
Alveolar mucosa	3	0	6
Sublingual	0	1	0
submandibular	1	1	1
Tongue	0	2	1
Flour of the mouth	0	1	0
Cheek	1	2	1
Upper lip	2	0	0
Histopathological grade			
High grade		4	
Moderate grade		7	
Low grade		19	
Solid			5
Tubular			6
Cribriform			19
Size (cm)			
Range	1-4.5	1-7.5	0.7-7.5
Mean	2.02	5.19	3.01
Lymph node metastasis		3	2

PA: Pleomorphic adenoma; MEC: Mucoepidermoid carcinoma; AdCC: Adenoid cystic carcinoma

Data analysis showed a significant difference in VEGF intensity between three groups ($P < 0.001$).

Moreover, VEGF expression in MEC and AdCC showed a significant difference ($P = 0.009$). No significant relationship was observed between COX-2 and VEGF expression and intensity with histologic grade ($P > 0.05$) and lymph node metastasis ($P > 0.05$) in MEC and AdCC.

Gamma test showed a significant correlation between VEGF and COX-2 expression in PA ($P = 0.03$). Likewise, the correlation of VEGF and COX-2 intensity was seen in both PA ($P = 0.016$) and MEC ($P = 0.001$). No significant correlation was found between VEGF and COX-2 expression and intensity in AdCC ($P > 0.05$).

Figures 1-3 demonstrate the expression of VEGF and COX-2 in PA, MEC, and AdCC, respectively.

DISCUSSION

Evaluation of COX-2 expression with invasive behavior in salivary gland malignancies has been reported. Moreover, high expression of VEGF correlated with lymph node metastasis in salivary

Table 2: Cyclooxygenase-2 expression scores in present tumors

Marker expression	COX-2 expression (%)				Total
Tumor type	Score 1 (1-25)	Score 2 (26-50)	Score 3 (51-75)	Score 4 (76-100)	
Tumor					
PA					
Count	1	2	8	19	30
Percentage	3.3	6.7	26.7	63.3	100.0
MEC					
Count	0	0	0	30	30
Percentage	0.0	0.0	0.0	100.0	100.0
AdCC					
Count	1	2	4	23	30
Percentage	3.3	6.7	13.3	76.7	100.0
Total					
Count	2	4	12	72	90
Percentage	2.2	4.4	13.3	80.0	100.0

PA: Pleomorphic adenoma; MEC: Mucoepidermoid carcinoma; AdCC: Adenoid cystic carcinoma; COX-2: Cyclooxygenase-2

Table 3: Cyclooxygenase-2 intensity in pleomorphic adenoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma

Marker intensity	COX-2 intensity			Total
Tumor type	Mild	Moderate	Strong	
Tumor				
PA				
Count	2	11	17	30
Percentage	6.7	36.7	56.7	100.0
MEC				
Count	0	3	27	30
Percentage	0.0	10.0	90.0	100.0
AdCC				
Count	0	1	29	30
Percentage	0.0	3.3	96.7	100.0
Total				
Count	2	15	73	90
Percentage	2.2	16.7	81.1	100.0

COX-2: Cyclooxygenase-2; PA: Pleomorphic adenoma; MEC: Mucoepidermoid carcinoma; AdCC: Adenoid cystic carcinoma

gland carcinomas.[7] So far, comparison of COX-2 and VEGF expression in malignant and benign salivary tumors has not been studied. In this study, we studied the comparison of expression of COX-2 and VEGF in 30 samples of PA, 30 samples of MEC, and 30 samples of AdCC and their association with histopathological grade and lymph node metastasis.

The average age of patients with PA, MEC, and AdCC was 38.13, 40.63, and 43.7 years, respectively, which was similar to the studies of Fonseca *et al*.,[8] Aoki *et al*.,[12] Cho *et al*.,[13] and Merza[14] and inconsistent with Zyada *et al*.[15] and Stárek *et al*.[16]

In our study, the most common site of PA was palate which was consistent with the studies of Aoki *et al*.[12] and in contrast with the studies of Cho *et al*.,[13] Merza,[14] and Faur *et al*.[17] The most common location of MEC and AdCC was parotid which was consistent with the studies of Lequerica-Fernández *et al*.,[7] Fonseca *et al*.,[8] Cho *et al*.,[13] Zyada *et al*.,[15] Stárek *et al*.,[16] and Faur *et al*.[17] and was inconsistent with the study of Lim *et al*.[9]

In this study, immunohistochemical expression of COX-2 was seen in all samples of PA, MEC, and AdCC. Score 4 of COX-2 expression was 100% in MEC, 76.7% in AdCC, and 63.3% in PA. These results were consistent with the studies of Sakurai *et al*.,[5] Zyada *et al*.,[15] and Yi *et al*.[18] 29 (96.7%) samples of AdCC, 27 (90%) samples of MEC, and 17 (56.7%) samples of PA exhibited strong COX-2 intensity.

In the present study, the expression of VEGF was observed in all samples of PA, MEC, and AdCC, which was in score 4 in most samples. In 27 (90%) of MEC, 29 (96.7%) of AdCC, and 12 (40%) of PA samples, VEGF intensity was strong. These results were consistent with the studies of Fonseca *et al*.,[8] Faur *et al*.,[17] Lim *et al*.,[9] Ou Yang *et al*.,[19] and Gupta *et al*.[20] Our results about COX-2 and VEGF expression were in contrast to Cho *et al*.,[13] Merza,[14] Rocha Tenorio *et al*.,[21] Lequerica-Fernández *et al*.,[7] and Li *et al*.[22] studies. This discrepancy may be attributed to different antibody manufactures and incubation time of primary antibody.

In our study, COX-2 expression in MEC and AdCC was higher than PA ($P < 0.001$). This result was consistent with the studies by Sakurai *et al*.,[5] Cho *et al*.,[13] Merza,[14] and Yi *et al*.[18] In this study,

Table 4: Vascular endothelial growth factor expression in pleomorphic adenoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma

Marker expression / Tumor type	VEGF expression				Total
	1-25	26-50	51-75	76-100	
Tumor					
PA					
Count	3	1	3	23	30
Percentage	10.0	3.3	10.0	76.7	100.0
MEC					
Count	1	0	0	29	30
Percentage	3.3	0.0	0.0	96.7	100.0
AdCC					
Count	2	1	6	21	30
Percentage	6.7	3.3	20.0	70.0	100.0
Total					
Count	6	2	9	73	90
Percentage	6.7	2.2	10.0	81.1	100.0

VEGF: Vascular endothelial growth factor; PA: Pleomorphic adenoma; MEC: Mucoepidermoid carcinoma; AdCC: Adenoid cystic carcinoma

Table 5: Vascular endothelial growth factor intensity in pleomorphic adenoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma

Marker intensity / Tumor type	VEGF intensity			Total
	Mild	Moderate	Strong	
Tumor				
PA				
Count	2	16	12	30
Percentage	6.7	53.3	40.0	100.0
MEC				
Count	0	3	27	30
Percentage	0.0	10.0	90.0	100.0
AdCC				
Count	0	1	29	30
Percentage	0.0	3.3	96.7	100.0
Total				
Count	2	20	68	90
Percentage	2.2	22.2	75.6	100.0

VEGF: Vascular endothelial growth factor; PA: Pleomorphic adenoma; MEC: Mucoepidermoid carcinoma; AdCC: Adenoid cystic carcinoma

the intensity of VEGF and COX-2 in malignant tumors was higher than the benign tumor which was consistent with Sakurai *et al*.'s[5] study.

In this study, from 30 samples of MEC, 19, 7, and 4 cases were low, intermediate, and high grade, respectively. The expression of COX-2 was score 4 in all samples and for VEGF expression 29 samples were score 4. There was no significant relationship between histopathological grade of MEC and expression of VEGF and COX-2. This finding was consistent with the studies of Cho *et al*.[13], Zyada *et al*.[15] and Li *et al*.[22] and contrary to the study of

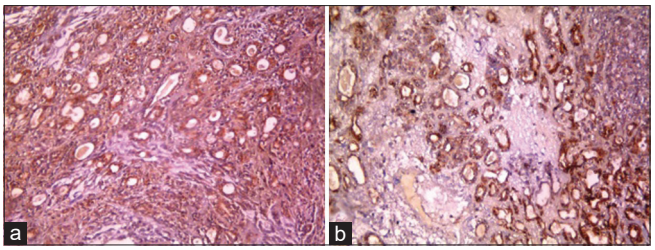

Figure 1: Strong cytoplasmic expression of vascular endothelial growth factor (a) and cyclooxygenase-2 (b) in pleomorphic adenoma, ×200.

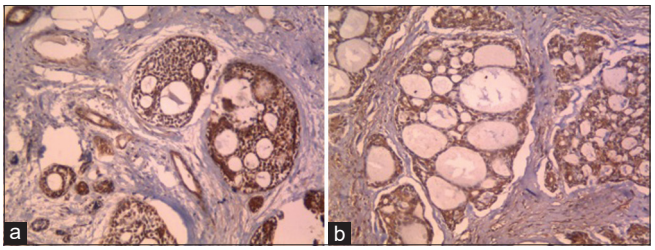

Figure 2: Strong cytoplasmic expression of vascular endothelial growth factor (a) and cyclooxygenase-2 (b) in adenoid cystic carcinoma, ×200.

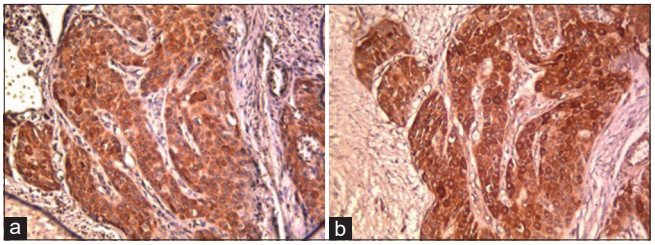

Figure 3: Strong cytoplasmic expression of vascular endothelial growth factor (a) and cyclooxygenase-2 (b) in mucoepidermoid carcinoma, ×200.

Lim *et al*.[9] The difference may be due to different scoring methods.

In the present study, from 19 samples of AdCC with cribriform pattern, 13 samples were score 4 for COX-2 and VEGF expression. All samples of AdCC with tubular pattern and 4 samples of AdCC with solid pattern were score 4 for COX-2 expression, and 5 samples of AdCC with tubular pattern and 3 samples of AdCC with solid pattern were score 4 for VEGF expression. There was no significant relationship between the expression of COX-2 and VEGF with histologic grade in AdCC. This result was consistent with Lim *et al*.'s[9] study and in contrary to Li *et al*.[22] study.

In our study, three samples of MEC showed lymph node metastasis and all of them were score 3 for COX-2 and VEGF expression. Chi-square test

showed no statistically significant difference between the expression of both markers and lymph node metastasis in MEC ($P > 0.05$). This finding was consistent with the study of Ou Yang *et al*.[19] and in contrary to the studies of Lequerica-Fernández *et al*.[7] and Zyada *et al*.[15] Furthermore, only two samples of AdCC showed lymph node metastasis and both of them were score 4 for VEGF expression, and, in one sample, the expression of COX-2 was score 4. With Chi-square test, no significant difference was observed. This finding was in contrary to the studies of Lequerica-Fernández *et al*.[7] and Li *et al*.[22] As the number of samples with metastasis in our study was few, it seems that there could not reach a proper conclusion.

In this study, the coexpression of VEGF and COX-2 in salivary gland tumors was examined, in which there was a significant difference between the expression and intensity of both markers in PA and intensity of them in MEC, but no significant correlation was observed in AdCC. According to this study and Baghban *et al*.'s study,[4] COX-2 has probably a synergistic effect with VEGF in angiogenesis and progression of malignancies.

CONCLUSION

The high expression of VEGF and COX-2 in malignant tumors comparing PA suggests the role of both markers in malignant transformation. The significant correlation of VEGF expression with COX-2 may represent the role of COX-2 in tumor angiogenesis by modulating VEGF production.

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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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