DOI: https://doi.org/10.53350/pjmhs2023171156

ORIGINAL ARTICLE

Association of Vascular Endothelial Growth Factor (VEGF) in Salivary Gland Tumors

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ABSTRACT

Aim: To identify the association of vascular endothelial growth factor (VEGF) immunoexpression in the progression of salivary gland tumor.
Study design: Comparative cross-sectional study
Place and duration of study: Department of Pathology, Peshawar Medical College.
Duration of study was six months from date of approval.
Methodology: Forty five specimen of salivary gland tumor were studied in which 30 were benign and 15 were malignant. The endpoint assessment included Hematoxylin & Eosin (H&E) and VEGF immunoexpression.
Results: The association of VEGF expression with salivary gland tumor was statistical significant (P<0.05). Maximum 18 samples (benign: 13, malignant: 5) were shown to have strong expression, 24 (benign: 16, malignant: 8) were shown moderate expression. Whereas, small and no expression was found in 2 (benign: 0, malignant: 2) and 1 (benign: 1, malignant: 0) sample, respectively.
Conclusion: Overexpression of VEGF in both malignant and benign salivary gland tumors might be associated to progress salivary gland tumors.
Keywords Benign tumor, Warthin tumor, Adenoma, Salivary gland carcinoma, Vascular endothelial growth factor

INTRODUCTION

Salivary gland tumors (SGTs) are extremely prevalent among patients.[1] Three to six percent of head and neck tumor and one percent of all body tumors are melanoma. The frequency in the development of malignant neoplasms is about 15-32% of parotid gland tumors, whereas submandibular tumors, and sublingual tumors are 41-45% and 70-90% prevalent, respectivel.[2] Forty to eighty percent, malignant salivary gland tumors affect the hard palate. The most prevalent of these are adenoid cystic carcinoma, or ACC.[3] Salviary gland tumors are a category of lesions that exhibit a wide range of pathological behaviors and complex clinical and pathological characteristics. They account for between 3 and 10% of head and neck cancers (HNN). According to WHO data, the incidence of SGT varies between 0.4 and 13.5 cases per 0.1 million individuals annually[4].

Several potential etiological factors, such as vitamin deficiency, dietary practices, radiation therapy and chemical exposures, may influence the development of SGTs[5-7]. Due to their complex and variable morphological patterns, salivary gland tumors can be challenging to diagnose by a pathologist[1]. The age, range and gender distribution of individuals with these tumors vary considerably. Since the beginning of the previous decade, numerous advancements have focused on the intracellular molecular cascade involved in the growth of these lesions. These developments have not significantly increased the proportion of chemotherapeutic procedures. It is recommended that patients with SGTs undergo surgery in order to obtain the greatest therapeutic benefit.

Vascular endothelial growth factor (cytokine) regulates angiogenesis specifically. This regulates the mitogenic response, which affects only the endothelial cells of blood vessels. The recruitment and endothelial cell cluster proliferation initiated the process of neoplastic angiogenesis[8]. Extensive research conducted that indicates an increase in VEGF levels is associated with a higher density of microvessels and a greater likelihood that patients with cancers such as lung, breast, gastric, prostate and colorectal cancers as well as a broad spectrum of other neoplasms will perish[9-12].

According to studies conducted in Pakistan, the ratio of male to female SGTs is approximately 1:1[13]. Numerous studies have been conducted on the expression of VEGF in salivary gland tumors. These previously reported studies have discovered a significant association among these factors and with clinical parameters, but their findings remain ambiguous and inconsistent.

To the best of our knowledge, none of a single study was carried out in Pakistan. This study included patients previously diagnosed with benign or malignant salivary gland tumors.

Received on 24-08-2022
Accepted on 13-12-2022

MATERIALS AND METHODS

This study was conducted at Department of Pathology, Peshawar Medical College, Peshawar, patients with salivary gland tumor were recruited and 45 formalin fixed paraffin embedded blocks of diagnosed SGT specimens. Sample size was 45, in which 30 were benign and 15 were malignant. No chemotherapy or radiation was delivered before to their procedures, regardless of whether they were curative or palliative. Separate clinical and morphological data were collected for each subject. Routinely, haematoxylin and eosin were employed to stain each of the examined slides. In each instance, immunohistochemical investigations were conducted, and VEGF expression and its association with clinicopathological variables were evaluated.

The surgical procedures, whether curative or palliative, were not preceded by chemotherapy or radiation therapy. Clinical and morphological data were collected for each case. Examined were all routinely stained haematoxylin-eosin slides. To better characterize these tumors, immunohistochemistry tests were performed, and VEGF expression and its relationship to clinic pathological factors were examined in all cases.

Before using antibodies for immunohistochemical analysis, a representative paraffin block was selected in each case. For the VEGF expression immunohistochemistry analysis, we used the mouse anti-human VEGF monoclonal antibody and the EnVision Plus technology. To enhance epitope recovery from the slides, the

sections were prepared by boiling them for 20 minutes while submerged in a target retrieval solution with a pH of 9. The primary antibody was incubated for thirty minutes with the 1:25 diluted solution. DAB+ was a component of the visualization system. Hematoxylin was used as a counterstaining agent.

The immunohistochemistry VEGF expression was scored by multiplying the proportion of positively stained cells by the intensity of the staining and awarding the total score. The following formula was used to determine the proportion of positive stained cells in the microscopic field: 0 = no positive cells; 1 = less than 1%; 2 = 1–10%; 3= 11-33% and 4 = 34–66%. The intensity of the staining was graded as follows: 0 indicates no positive cells, whereas, 1, 2, and 3 were shown light, moderate, and heavy staining, respectively. The sum of the two parameters ranged from 0 to 7 for representative microscopic fields. In this study, a negative stain corresponded to a score between 0 and 1; a mild stain (+) to a score between 2 to 3; moderate stain (++) to a score between 4 to 6 and strong stain between 7 to 8, both the percentage of positive cells and the strength of the reaction product are taken into account when calculating the Allred score for the majority of carcinomas. The total of the two scores yields an eight-valued final score. Scores of 0 and 2 are deemed incorrect. Positive scores range from three to eight. The data was entered analyzed through SPSS-22. The t-test is used to compare sample mean values. If the p-value was less than or equal to 0.05, the findings were considered as statistically significant.

RESULTS

The current study includes the specimen of 30 (66.7%) males and 15 (33.3%) females. Patients diagnosed with benign tumours were 30 and malignant tumours were 15. The investigated subtypes were i) salivary duct carcinoma (SDC), ii) adenoid cystic carcinoma (ACC), iii) warthin-tumor (WT) and iv) pleomorphic adenoma (PA). The highest observed subtype was pleomorphic adenoma 26(57.8%). The adenoid cystic carcinoma was another commonly diagnosed subtype in patients 6(13.3%). Whereas, salivary duct carcinoma 3(6.7%), warthin tumor 4(9.8%) were found least.

The association of VEGF expression with type of tumor was investigated. The statistical significant association was found between these two by showing p value less than 0.05. Maximum 24 samples (benign: 16, malignant: 8) was shown to have moderate expression, 18 (benign: 13, malignant: 5) was shown to have strong expression. Whereas, no and mild expression was found in 2 (benign: 0, malignant: 2) and 1 (benign: 1, malignant: 0) sample, respectively. Table 1 is depicting the VEGF expression with type of tumors in sample of patients.

The statistical significant difference was found between these two by showing p value less than 0.05. Maximum 24 samples (PA: 13, SDC: 1, ACC: 4, WT: 3) was shown to have moderate expression. Total 18 samples were shown strong expression (PA: 12, SDC: 1, ACC: 1, WT: 1 whereas, mild expression was found in 2 samples (SDC: 1, ACC: 1) and no expression was found in 1 sample (PA: 1). Table 2 is depicting the VEGF expression with diagnosis (subtype) in sample of patients (Figs. 1-4)

Table 1: VEGF immunoexpression in benign and malignant salivary gland tumors

VEGF expression	Type of Tumor		Total	P value
	Benign	Malignant		
No expression (0-1)	1	0	1	<0.005
Mild (2-3)	0	2	2	
Moderate (4-6)	16	8	24	
Strong (7-8)	13	5	18	
Total	30	15	45	

Table 2: VEGF expression in subtype of salivary gland tumor

Diagnosis	VEGF Expression				Total	P value
	No n (%)	Mild n (%)	Moderate n (%)	Strong n (%)		
Pleomorphic adenoma (benign)	1	0	13	3	16	<0.05
Salivary Duct carcinoma (malignant)	0	1	2	2	4	
Adenoid Cystic carcinoma (malignant)	0	1	4	1	5	
Warthin tumor (benign)	0	0	13	1	14	
Polymorphous low grade adenocarcinoma (malignant)	0	0	4	2	6	
Total	1	2	36	9	45	

Fig. 1: Lymphoid Tissue showing Positive-Control (VEGF). (A) 10X, (B) 40X

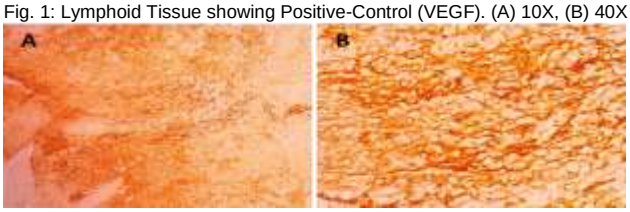


Fig. 2: Pleomorphic Adenoma. (A) H&E , (B) IHC

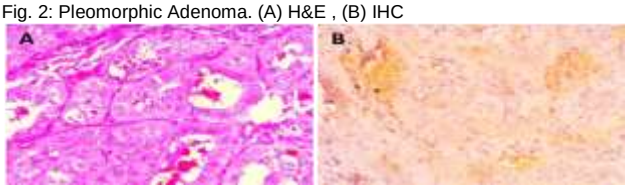


Fig. 3: Adenoid Cystic Carcinoma – (A) H&E , (B) IHC

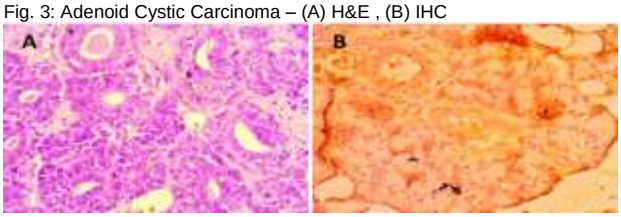


Fig. 4: Warthin Tumor – (A) H&E , (B) IHC

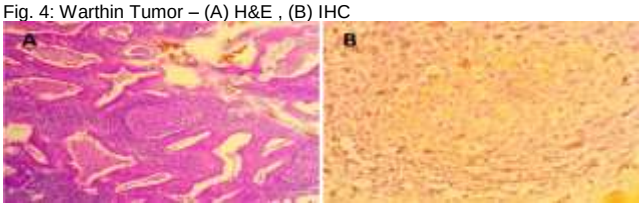
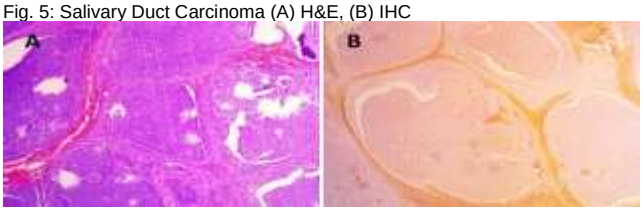


Fig. 5: Salivary Duct Carcinoma (A) H&E, (B) IHC



DISCUSSION

Extensive research has been conducted on the prognostic significance of VEGF expression in gastrointestinal tumors, lung, breast, prostate and oral squamous cell carcinomas[14-17]. On the role of VEGF expression in salivary gland tumor, only a few studies have been conducted, and their results are controversial[18-21]. Moreover, an association of Allred scoring with VEGF immunoexpression was an out-of-focus area in the treatment of

disease. The findings of this study, we believe, add to our understanding of the VEGF and Allred score in terms of salivary tumors, specifically salivary gland tumor. When initiating the treatment, both VEGF expression and Allred scoring must be considered. Particularly in Pakistan, this is the first study that not only identifies the role of VEGF expression in salivary gland adenoma but also addresses its association with Allred scoring for the treatment of neoplasm.

Gilbert et al[22] investigated the expression of VEGF in salivary gland tumor. This factor was found to be more abundant in cancerous cells than in healthy cells. It was discovered that high-grade cancers had it more frequently than low-grade cancers. VEGF does not appear to be a diagnostic biomarker for SGTs, according to their study.

According to another study, patients of SGT with high VEGF level have increased susceptibility of death. They identified the frequency of VEGF expression in patients with SGCs and its correlation with other diagnostic markers (such as p53, Ki67).[19]

Patients with adenoid cystic carcinoma (i.e. ACC) of the salivary glands have high levels of inducible nitric oxide synthases (iNOS), nuclear factor kappa b (NFkB), and vascular endothelial growth factor (VEGF) in their salivary glands. Immunohistochemical staining assays were used to determine the levels of NFkB, iNOS, p65, and VEGF protein expression. After conducting an analysis, they discovered that patients with salivary gland tumors that expressed VEGF had the highest risk of death.[23] The study of Lequerica-Fernandez et al[24] looked at the same expression of VEGF and found similar outcomes in salivary gland cancers.

However, many studies have failed to find a link between VEGF expression and an increased risk of death in patients with salivary gland tumors. Caveolin-1 expression in salivary gland mucoepidermoid carcinoma (MEC), as well as the number of small blood vessels and biochemical results had a strong correlation. Caveolin-1 and VEGF expression, as well as intra tumoral micro vessel density (MVD) (labelled with CD34) in 75 patients with MEC, were studied using immunohistochemistry (IHC), and statistical correlations with clinical and pathological parameters were investigated. Although many research were not found with statistical significant outcome, but found significant effect of VEGF with high expression on the death ratio of diagnosed patients. This shows an inferior survival for diagnosed patients. The author conclude that the insignificant analysis was may be due to the relatively small sample size of malignant neoplasm in the study.

Moreover, many previous studies found increased expression of VEGF and concluded it as local metastasis and distant metastases; whereas, the study of deFaria et al[21] not significantly found the biological potential of VEGF expression.

As per the outcomes found in the present study and the studies of by deFaria et al[21] and Doi et al[25] in which the expression of VEGF is statistically significant in high grade malignancies and this predicting poor diagnosis, the present study hypothesized that VEGF expression produces vital part in the development of SGTs pathogenesis, however more literature is required to identify some other variables and biological inter and intra cell receptors (such as semaphorins and neuropilins). These receptors are well known to interact with VEGF via interacting negatively or positively and thus enhanced angiogenic potential in the development of neoplasms. Moreover, previously reported findings shown an enhanced expression of VEGF in patient diagnosed with malignancies as compared with patients diagnosed with benign tumors and a significant effect in the specific survival rate of diagnosed carcinoma patients showing high protein level.

Moreover, the study of Błochowiak et al[26] identify the expression of VEGF, EGF, and HGF in the tissues sample of enrolled population effected with and without tumor. The results show insignificant differences and no significant correlations with stages of tumor. The level of VEGF in saliva was statistically significant and increased in diagnosed patients of pleomorphic-adenoma (PA) and Warthin-tumor (WT). Whereas, insignificant correlation between expression of VEGF165b and VEGFR2 was found in tumors and non-tumor surgical margins.

Similarly, the study of Faur et al[27] interested to found the difference in morphology and evolution of salivary gland in terms of neo-angiogenesis, VEGF protein expression and the diagnostic value of the outcome. They collected 45 surgical specimens (carcinoma ex pleomorphic adenoma 6, acinic cell carcinomas: 5, adenoid cystic carcinomas: 4 mucoepidermoid carcinomas: 6, basal cell adenomas 5, pleomorphic adenomas: 8, Warthin tumors 7, and adeno carcinomas: 4). All samples were passed through immunostaining. They found that VEGF protein expression is significantly more than malignant salivary gland tumors than benign ones. The VEGF protein expression and the micro vascularization in SGTs are the one of the vital factors that needs to be measured during diagnosis of disease and investigating case evolutions in enrolled patients of such tumors. However, in current study, the association of Allred Score with stained cells was investigated. The statistical significant association was found between these two by showing p value less than 0.05.

CONCLUSION

Over-expression of VEGF in both malignant and benign salivary gland tumors might be associated in the pathogenesis and aggressiveness of SGTs.

Conflict of interest: Nil

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