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RESEARCH ARTICLE

Upregulation of serum vascular endothelial growth factor and matrix metalloproteinase-3 in patients with oral squamous cell carcinoma

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Abstract Vascular endothelial growth factor (VEGF) is known as a fundamental regulator of angiogenesis that accelerates cellular proliferation, vascular permeability, and endothelial cell migration and is an inhibitor of apoptosis. Extracellular matrix degradation by matrix metalloproteinase (MMP) is necessary for endothelial cell proliferation, migration, and metastasis. Accordingly, the objective of the present study was to determine the circulating levels of VEGF and MMP3 and their relation in patients with oral squamous cell carcinoma. Using an ELISA kit, the circulating levels of VEGF and MMP-3 in the sera of 45 patients with oral squamous cell carcinoma (OSCC) and 45 healthy controls were assessed. Mean VEGF levels in the sera of patients with OSCC (122.4±36.1) were significantly higher than those in controls (65.3±23.4); however, no relation was found between VEGF levels and clinicopathologic factors. The serum MMP-3 level in OSCC patients was significantly higher (9.45±4.6 ng/ml, n=45) than that in healthy controls (5.9±3.6 ng/ml, n=45). There was no correlation in serum MMP-3 concentration with clinicopathologic features such as tumor stage, tumor size, nodal status, and histological grade. A significant relationship was found between serum levels of VEGF and MMP3. This study concludes that VEGF and MMP3 may have a potential role in the pathogenesis of OSCC but cannot be used as a tool for monitoring tumor progression. Moreover, the role of VEGF in the regulation of angiogenesis is in part due to activation of MMP-3.

Keywords MMP-3 · VEGF · Squamous cell carcinoma

Introduction

Oral malignant neoplasms are the sixth most common malignancies all over the world, and along with the malignancy of the pharynx, it accounts for the third most common malignancy in developing countries [1]. The most common type of oral malignancy is squamous cell carcinoma, which can seriously cause morbidity and mortality [2].

As the most important prognostic factor, metastasis to lymph nodes was found in more than 30 % of the patients with oral squamous cell carcinoma (OSCC) [3]. For tumor growth, invasion, and metastasis, angiogenesis and disruption of the extracellular matrix are needed [4]. Angiogenesis is essential for tumor growth, which supplies the nutritional needs of a developing tumor [5].

Vascular endothelial growth factor (VEGF) is known as a key regulator of angiogenesis that can increase vascular permeability, cellular proliferation, and endothelial cell migration and is an inhibitor of apoptosis [6]. VEGF is a heparin-binding glycoprotein, and its gene is located on chromosome 6 [7]. This protein is the most important member of the VEGF

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family and has a role in vascular permeability [8]. The inhibition of VEGF activity leads to diminished growth and decreased tumor progression which confirms its important role in the initiation of tumor angiogenesis [9].

Angiogenic and antiangiogenic factors regulate angiogenesis, which is a complex process. Matrix metalloproteinases (MMPs), members of the zinc-dependent endopeptidase group [10], can degrade matrix components and the basement membrane and have a fundamental role in this step [10].

Degradation of extracellular matrix (ECM) by the MMPs is essential for endothelial cell proliferation, migration, and metastasis [11]. Matrix metalloproteinase-3 (MMP-3, stromelysin-1) is a member of the MMP family, which is capable of degrading different substrates including collagen types II, IV, IX, X, and XI, fibronectin, gelatins, elastin, proteoglycanase, E-cadherin, and osteopontin [12, 13]. Different cells, such as macrophages, synovial cells, fibroblasts, monocytes, and chondrocytes can produce MMP-3 [14–16]. MMP-3 is produced in an inactive form and becomes active after cleavage by proteolytic enzymes like trypsin-2. The role of MMP-3 has been identified in the regulation of angiogenesis, induction of apoptosis, invasion, and metastasis in cancer [17]. In our previous study, we showed increased levels of MMP-3 in patients with OSCC [18].

Although there is evidence of a relationship between MMPs and VEGF in tumor progression, the relation between the pretreatment serum levels of MMP3 and VEGF in OSCC and the clinicopathologic status of the patient is still unclear. Therefore, the present study aimed to determine the circulating levels of VEGF and MMP3 and also their relation in patients with oral squamous cell carcinoma.

Materials and methods

This study enrolled 45 OSCC patients (22 males and 23 females; mean age 57±16 years) and 45 healthy control subjects (22 males and 23 females; mean age 56.6±15 years). For the whole study, patients were referred from the ENT Department of the Shiraz University of Medical Sciences and had histopathological diagnoses of OSCC. Patients with other malignancies, inflammatory diseases, or infections and also the smokers were excluded from the study. Blood samples were taken from patients before they received any surgical procedures, chemotherapy protocols, or blood transfusion. Control cases were healthy nonsmoker blood donors with no evidence of systemic or inflammatory diseases or infections. The control group was matched with the study group regarding age and gender. All participants were thoroughly informed about the research and agreed to participate, then signed a written consent form. Serum samples were driven from centrifuged clotted blood at 4 °C and were stored at −80°C before analysis. VEGF and MMP-3 concentrations were measured by ELISA according to the instructions provided by the manufacturer (BMS, Bender MED Systems GmbH, Germany). An independent *t* test was conducted to compare the results of serum MMP-3 and VEGF concentrations between the control and study group. The correlation between serum MMP-3 and VEGF concentration and the correlation between the concentration of these proteins and clinicopathologic factors were calculated using Spearman's correlation test. A *P* value less than 0.05 was considered significant.

Results

Table 1 shows the clinical data of patients enrolled in this study. Mean VEGF levels in sera of patients with OSCC (122.4±36.1 pg/ml) were significantly higher than those in controls (65.3±23.4 pg/ml) ($P=0.000$), while no relation was found between VEGF levels and clinicopathologic factors ($P>0.05$).

The serum MMP-3 level in OSCC patients was significantly higher (9.45±4.6 ng/ml, $n=45$) than that of the healthy controls (5.9±3.6 ng/ml, $n=45$, $P<0.001$). The serum level of MMP-3 was higher (11.44±4.8 ng/ml) in females compared to that of males (7.35±3.6 ng/ml, $P=0.002$). Also, the serum MMP-3 level was increased with increasing age ($P=0.04$). There was no apparent correlation between serum MMP-3 concentration and the clinicopathologic features such as tumor stage, tumor size, nodal status, and histologic grade. A significant relationship was found between serum levels of VEGF and MMP3 in patients ($r=0.6$, $P<0.001$), but not in the control group ($r=0.1$, $P=0.3$) (Figs. 1 and 2).

Discussion

VEGF has been recognized as a key factor for the induction of angiogenesis and is a potent mitogen for endothelial cells [19]. In this study, the higher level of VEGF in the patient group was similar to the results of the recent studies [20–22]. Elevated VEGF level suggests its role in the pathogenesis of OSCC. According to Nayak et al. and Jaiswal et al. [21, 22], there was no association between serum level of VEGF and clinicopathologic factors in the present study, which means that VEGF level cannot be used as a tool for monitoring cancer progression.

In our study, the serum MMP-3 concentration in OSCC patients was significantly higher than that in healthy controls. Therefore, the measurement of the MMP-3 serum concentration as a marker for OSCC can be useful and may help in the diagnosis of OSCC. Linkow et al. found that the serum MMP-3 level in patients with active untreated head and neck squamous cell carcinoma (HNSCC) was lower than that in the healthy control groups and also in the treated patients [23].

Table 1 Clinicopathological features and MMP3 and VEGF levels of the patients included in this study

	Number (%)	Mean MMP3 level±SD	*P* value	Mean VEGF level±SD	*P* value
Sex					
Male	22 (48)	7.3±3.6	0.002	124.2±22.3	0.05
Female	23 (52)	11.4±4.8		122.3±20.1	
Tumor size					
T1	6 (13.4)	10.5±6.8		110.9±57.1	
T2	26 (57.7)	9.2±4.1	0.8	128.4±27.8	0.6
T3	6 (13.4)	6.7±2.3		100.8±29.1	
T4	7 (15.5)	12.1±5.9		128.5±49.6	
Lymph node involvement					
N0	17 (37.8)	9.6±4.1		131.2±35.2	
N1	8 (17.7)	7.7±5.6	0.8	109.6±37.2	0.3
N2	5 (11.2)	13.8±5.5		125.9±46.5	
N3	15 (33.3)	8.7±4.3		116.7±34.9	
Grade					
G1	24 (53.3)	9.3±4.5		126.2±27.7	
G2	15 (33.3)	9.3±5.7	0.4	112.9±45.6	0.05
G3	6 (13.4)	10.1±3.1		135.7±42.7	
Stage					
I	2 (4.5)	16.5±2.6		173.1±3.3	
II	12 (26.7)	9.6±3.4	0.8	135.9±27.4	0.6
III	8 (17.7)	7.4±5.4		104.2±41.4	
IV	23 (51.1)	9.4±4.6		117.3±34.4	

They applied the LabMAP technique and evaluated HNSCC from different anatomic locations, while we used the ELISA kit and evaluated oral SCC, and these may justify the difference between the results obtained from these two studies. No correlation was found between serum MMP-3 concentration and clinicopathologic features of the tumor, such as tumor size, tumor stage, nodal status, and histopathological grade. Several previous studies have found correlations between MMP-3 expression and clinicopathologic features of OSCC [24, 25]. In the mentioned studies, researchers employed immunohistochemistry (IHC) in order to evaluate MMP-3 tissue expression; however, we utilized an ELISA kit to

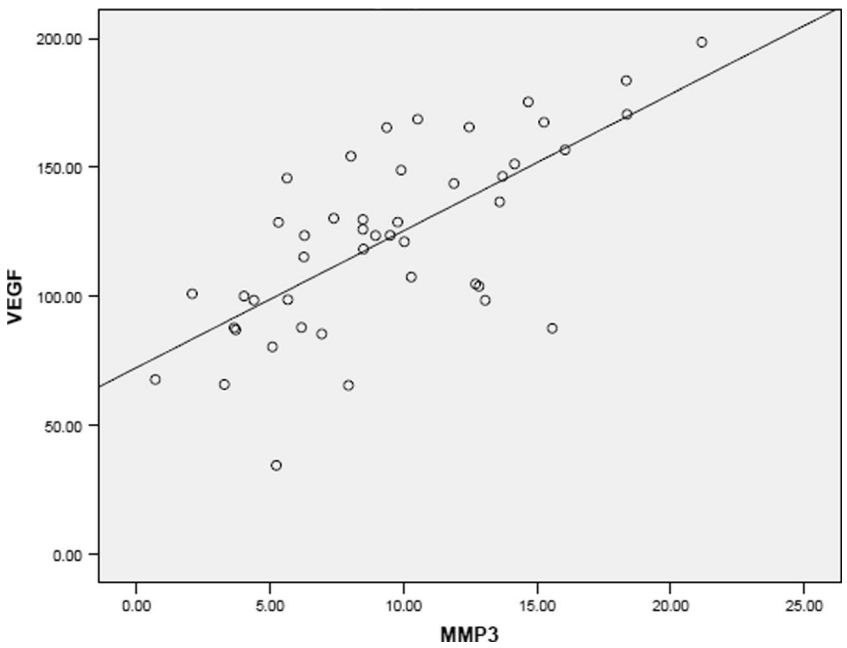

Fig. 1 Correlation between serum VEGF (pg/ml) and MMP-3 (ng/ml) levels in patients with OSCC (r=0.69, P<0.001, n=45)

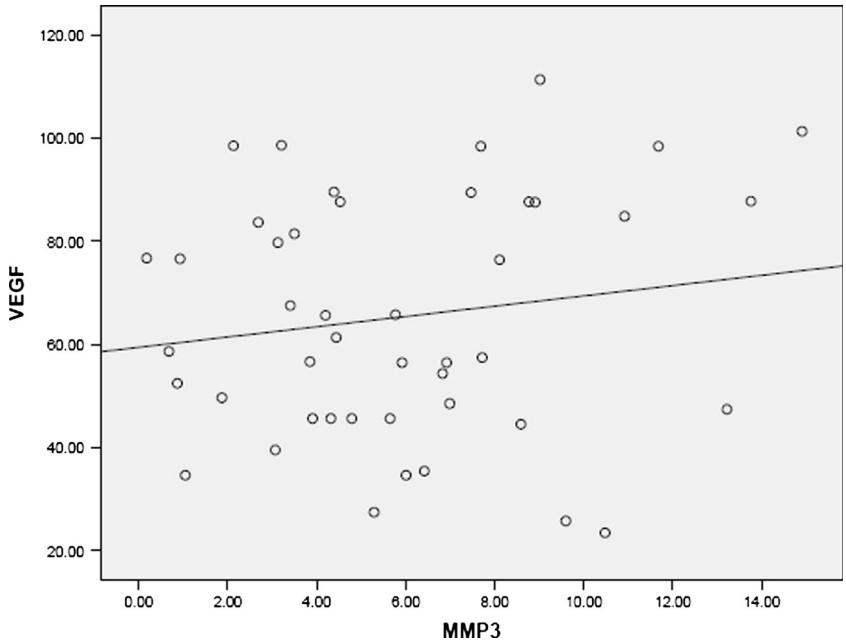

Fig. 2 Correlation between serum VEGF (pg/ml) and MMP-3 (ng/ml) levels in the control group (r=0.1, P=0.3, n=45)

evaluate the serum MMP-3 level in the subjects; therefore, these different results may probably be due to different methods used to evaluate the MMP-3 expression. It is possible that all tissue MMP-3 is not released into the serum; therefore, the serum level of MMP-3 cannot show the tissue level of MMP-3. This indicates that the evaluation of both serum and tissue MMP-3 will simultaneously be useful. Moreover, MMP-3 releases inactively, and ELISA can also assess the inactive form of MMP-3, hence applying other methods to assess the active form of MMP-3 is useful to evaluate the correlation between the serum MMP-3 level and clinicopathological features of OSCC patients.

In our study, MMP3 and VEGF levels correlated with each other, which may indicate that these two factors have a functional interaction with each other. This result was similar to the result of Liu et al. which found a correlation between MMP9 and VEGF levels in OSCC [4]. Among different factors affecting tumor metastasis, changes of angiogenesis and factors related to angiogenesis such as MMPs are vital in proliferation, migration, and metastasis of tumor cells.

In other tumor models, a functional interaction between VEGF and MMPs has been found. Huang et al. found a fundamental role of macrophage-derived MMP9 in angiogenesis and growth of ovarian carcinoma using in vivo models [26]. Recent reports suggest that VEGF lead to the upregulation of MMP2 in human melanoma cells [27]. Belotti et al. showed that activated MMP9 is correlated with high VEGF bioavailability in cultured ovarian carcinoma [28]. Tas et al. found that a significant relationship exists between serum levels of VEGF and MMP3 in melanoma [10].

The expression of VEGF is widely seen in the tumor parenchyma, stroma, and endothelial cells. VEGF has an important role in tumor angiogenesis, proliferation, growth, and metastasis in an autocrine and paracrine manner [29]. However, its direct role in angiogenesis remains unclear. The literature reveals that in a concentration-dependent manner, VEGF promotes endothelial cell proliferation. Therefore, VEGF stimulates angiogenesis through direct interaction with endothelial cells. In addition to its degradation function, MMPs play a role in tumor-induced angiogenesis [10].

VEGF may enhance MMP expression in endothelial cells. The obtained result reveals that VEGF is involved in the degradation of ECM which leads to endothelial cell proliferation, migration, and metastasis [10].

One of the limitations of our study was that we do not have the data of the patient's survival, so for further studies, we recommended to investigate the relation of serum levels of these markers with the long-term survival of the patients.

Conclusion

The results obtained from this study suggest that VEGF and MMP3 may have a potential role in the pathogenesis of OSCC; however, they cannot be used as tools for monitoring tumor progression, and the role of VEGF in the regulation of angiogenesis is in part due to the activation of MMP-3.

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Conflicts of interest None

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