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Original Article

Serum Level of Vascular Endothelial Growth Factor in Patients with Different Clinical SubtypeS of Oral Lichen Planus

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Abstract

Background: Oral lichen planus is a chronic inflammatory disease with a poorly understood etiology. The role of angiogenesis in the development of different chronic inflammatory diseases is of great concern. Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis. We aimed to evaluate the serum level of VEGF in patients with oral lichen planus compared with normal individuals and consider its clinical significance.

Methods: In this case-control study, 36 serum samples from patients diagnosed with oral lichen planus admitted to the Oral Medicine Department of the School of Dentistry at Shiraz University of Medical Sciences (14 men, 22 women, mean [±SD] age: 38.8 [±6.07] years) and 23 serum samples from healthy individuals (9 men, 14 women, mean [±SD] age: 38.7 [±4.9] years) were collected. VEGF concentration was measured using the ELISA method. The Mann-Whitney test was used for statistical analysis.

Results: The serum VEGF level was significantly higher in patients with oral lichen planus compared with the healthy controls (112.97 [±63.2] vs. 66.21 [±56.2] ngr/ml, $P<0.001$). A similar difference was also observed between the two types of oral lichen planus, being more pronounced in the erosive form ($P<0.001$).

Conclusion: Serum VEGF can be used as a useful and suitable marker to scrutinize the disease activity.

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Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease affecting 1-2% of general adult population.[1] Andreasen's classical classification was modified by other authors who sub classified OLP to reticular, atrophic, and erosive forms.[2,3] In the erosive form the surface epithelium desquamates and the areas of ulceration and erosion remain.[2]

The etiology of OLP is still not well understood. One theory is that in OLP, autocytotoxic CD^{8+} T-cells initiate the programmed cell death of oral epithelial cells.[4] Other etiologies include stress, several drugs, genetic background, infectious agents, certain dental materials, or an association with autoimmune disorders.[5] The role of angiogenesis in the development of chronic inflammatory diseases is of considerable concern. Angiogenesis is the development of new blood vessels from existing ones and is an imperious feature in the new tissue formation, and healing of the tissues. The adult vasculature is mostly quiescent, and angiogenesis does not

happen under normal conditions. Therefore, this process has a role in physiological conditions such as embryonic development and wound healing, and in pathological conditions such as growth of cancer, and the development of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis.[6]

Vascular endothelial growth factor (VEGF) is a key regulator of vasculogenesis and angiogenesis.[7] Different cells such as endothelial cells, macrophages, fibroblasts, and smooth muscle cells produce VEGF.[8] It is a chimerical glycoprotein with a molecular weight of 34-45 KDa, consisting of two subunits.[9] Different physiological and pathological conditions accompanied by hypoperfusion and/or hypoxia can cause upregulation of VEGF.[10]

Elevated levels of VEGF have been reported in the serum of patients with rheumatoid arthritis, polymyositis/dermatomyositis, and active systemic lupus erythematosus.[11] Scardina and colleagues reported that 64.2% of OLP samples show VEGF expression and they found that a considerable neoangiogenesis occuring in OLP.[12]

Tao and co-workers assessed the microvessel density and expression of VEGF in patients with OLP and found that angiogenesis and VEGF expression were closely correlated to the different clinical forms of OLP lesions.[1]

However, there is no data on the correlation between serum VEGF levels and different clinical forms of OLP. Therefore, we aimed to evaluate the serum VEGF level in patients with OLP and to investigate its clinical significance.

Materials and Methods

In this case-control study, 36 serum samples from patients diagnosed with OLP (14 men, 22 women, mean [±SD] age: 38.8 [±6.07] years) and 23 serum samples from healthy individuals (9 men, 14 women, mean [±SD] age: 38.7 [±4.9] years) were collected.

The patients were admitted to the Oral Medicine Department at the School of Dentistry, Shiraz University of Medical Sciences and were diagnosed with OLP both clinically and histopathologically. The Ethics Committee of Shiraz University of Medical Sciences approved the study. Written informed consent was obtained from all the participants.

The controls were healthy blood donors, who were matched for age and gender. The types of OLP were subclassified into two clinical forms; reticular (n=22) and erosive/atrophic lesions (n=14).

Exclusion criteria for both groups were the presence of any systemic disease, existence of periodontal disease, use of corticosteroid or non-steroid anti-inflammatory medications at least 3 months prior to the study, or a history of malignancy of any type.

Serum samples were drawn from clotted blood following centrifugation at 4°C and stored at -80°C until analysis. VEGF concentrations were measured by Sandwich enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (BMS Bender Med System GmbH, Germany) (8) as follows:

1 Coating microtiter plate wells with 100 µl of the appropriate coating antibody, at a concentration between 1-10 µg/ml in coating buffer and then cover the plate and incubate overnight at 4°C.
2 Add 150 µl of blocking solution to each well and incubate for 60 minutes at 37°C.
3 Add 100 µl of suitably diluted samples to the relevant wells and incubate for 90 minutes at 37°C or overnight at 4°C.
4 Add 100 µl of biotin-conjugated detection antibody (appropriately diluted in wash buffer) to each well and incubate them for 1 hour at 37°C.
5 Add 100 µl of enzyme-conjugated streptavidin (appropriately diluted in wash buffer) to each well and incubate them for 60 minutes at 37°C.
6 Add 100 µl of the appropriate substrate solution to each well and incubate at room temperature for 30 minutes, or until desired color change is attained.
7 Read absorbance values immediately at the appropriate wavelength.

Statistical analysis was performed using Mann-Whitney test. $P<0.05$ was considered as statistically significant. To determine under curve area and suggestion for diagnostic test, receiver operating characteristics curve (ROC) was used.

Results

22 (61.1%) women and 14 (38.9%) men were diagnosed as having OLP in our study. The mean [±SD] serum VEGF level was higher in patients with OLP compared with the healthy controls (112.97 [±63.2] vs. 66.21 [±56.2] ngr/ml, $P<0.001$).

A cut-off point of 71 ngr/ml was found to differentiate the patients with OLP from the controls (sensitivity: 77.8%, specificity: 82.6%, C index: 0.791, ROC analysis).

The mean (±SD) serum VEGF level was significantly higher in patients with erosive OLP compared with the patients who had the reticular form (178 [±51.62] vs. 71.59 [±20.19] ngr/ml, $P<0.001$).

However, we found no statistically significant difference in serum VEGF levels between the men and women ($P=0.885$). Moreover, there was no significant correlation between serum VEGF levels and the patients' age.

Discussion

Recently, many studies have focused on the role of angiogenesis and microvascular endothelial injury in the pathogenesis of different diseases.[13] Furthermore, angiogenesis is correlated with disease activity of some chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and osteoarthritis.[14-16]

VEGF is an important key regulator in the process of new vessel formation.[10] We found that the serum VEGF level was significantly higher in patients with OLP. VEGF expression may be induced by numerous inflammatory mediators including IL6, IL1, and IL8 and regulated by the oxygen concentration of the tissue, with hypoxia stimulating its expression.[17]

As an autoimmune disease with an inflammatory origin and chronic progression, Ding and colleagues found that oral mucosa in patients with OLP is under a hypoxic condition; under which increased angiogenesis and VEGF levels can be expected.[18]

Another study showed that a series of pro-angiogenic cytokines, including tumor necrosis factor-α (TNF-α), IL-1, IL-6, and IL-8 substantially increased in the tissue of lesions and different oral fluids in patients with OLP.[19] These factors can upregulate the expression of VEGF and lead to increased serum VEGF levels.

The significantly increased serum VEGF level in our patients, may point out that angiogenesis in OLP is a systemically driven process. Moreover, because of its high sensitivity (77.8%) and specificity (82.6%), measuring serum VEGF levels can be used as a diagnostic tool.

VEGF is expressed in OLP tissues within keratinocytes, fibroblasts, inflammatory cells and endothelial cells;[1] thus, the spill over of VEGF from different cells can be the source of elevated serum VEGF concentration.

In our study, the serum VEGF level was altered in different clinical subtypes of OLP with the erosive/atrophic form showing a particularly increased level. This finding was consistent with another study showing that angiogenesis and VEGF expression were strongly correlated to different clinical form of OLP lesions.[1]

The serum VEGF level is associated with disease activity in a large number of autoimmune diseases including rheumatoid arthritis, psoriasis and osteoarthritis.[20] Increased level of VEGF in the atrophic erosive group implies that angiogenesis can be an implicit indicator of disease activity of OLP.

Previous immunohistochemical studies have revealed that angiogenetic phenomenon is present in the malignant transformation of many precancerous lesions of the oral epithelium, including OLP.[21] Thus, greater tendency for malignant transformation reported in erosive OLP lesions,[21] may be related to the increased angiogenesis.[21]

Some studies have demonstrated a reduction in serum VEGF concentrations after therapeutic intervention.[22] Considering the expression of the inducing factors of angiogenesis, new therapeutic modalities based on the use of anti-angiogenic medicine should be considered. These medications are already used in other pathologies with chronic inflammatory pathogenesis and are yielding good results.[12]

Many studies have shown that anti-angiogenic treatment, can decrease disease severity and delay the progression of chronic inflammation in some autoimmune and inflammatory diseases through the specific inhibition of VEGF.[23,24]

Considering the poor response to routine immunosuppressive or anti-inflammatory treatment in some patients with OLP, and regarding the role of angiogenesis and VEGF expression in OLP, angiogenesis can be used as an efficient target for therapeutic strategies.[20]

One of the limitations of our study was that we did not have the post treatment serum samples of the patients and therefore, we could not compare VEGF levels before and after treatment.

Conclusion

The analysis of serum VEGF may serve as a useful and convenient marker to scrutinize the activity of OLP. However, further studies are recommended to measure serum VEGF level after therapeutic intervention using anti-angiogenic strategies to evaluate the role of VEGF in the treatment of OLP.

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Conflict of interest: None declared

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