RESEARCH ARTICLE

VEGFR-3 Expression in Oral Lichen Planus

Zahra Zolfaghari Saravi[1], Maryam Seyedmajidi[2]*, Majid Sharbatdaran[3], Ali Bijani[4], Fatemeh Mozaffari[5], Pouyan Aminishakib[6]

Abstract

Background and objective: Given the postulated the role of inflammation and possible contribution of lymphangiogenesis in oral lichen planus, this study aimed to assess any associated presence of VEGFR-3. **Material and Methods:** This cross-sectional study was performed on 52 formalin fixed and paraffin embedded blocks of oral lichen planus (pathological diagnosis based on Modified WHO criteria), comprising 25 of erosive and 27 of reticular type, along with 60 samples of normal mucosa (with minimal inflammation from clinical and histopathological aspects) obtained at crown lengthening surgery. Four micron sections were cut from paraffin blocks and stained with H and E for confirmation of diagnosis and by immunohistochemistry with primary antibodies against VEGFR-3. Negative controls were provided by omission of primary antibody and placenta was considered as a positive control. Data were analyzed by Chi-square, Mann-Whitney and Kruskal-wallis tests and $P < 0.05$ was considered statistically significant. **Findings:** VEGFR-3 expression was apparent in 61.5% of lichen planus specimens and 5% of those from normal mucosa ($p<0.001$). Also, the average number of stained vessels was significantly higher in oral lichen planus than in normal mucosa ($p<0.001$). VEGFR-3 expression in oral lichen planus ($p=0.262$) and the average number of stained vessels ($p=0.092$) demonstrated no significant difference according to the type. **Conclusion:** It appears that VEGFR-3 expression might be involved in the pathogenesis of the oral lichen planus through increasing lymphatic vessels and lymphangiogenesis.

Keywords: VEGFR-3- Immunohistochemistry- oral Lichen planus

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Introduction

Lichen planus is a chronic inflammatory and relatively common disease that affects skin and mucous membranes (Neville et al., 2009; Regezi et al., 2012; Tao et al. , 2007; Dan et al. , 2010;Seyedmajidi et al. 2011;Mardani et al. , 2012). Most researchers refer to the disease as an immune mediated disease. (Neville et al., 2009; Tao et al. , 2007; Dan et al. , 2010 ; Kimkong et al. ,2011;Seyedmajidi et al. ,2013)

Although the cause of lichen planus has remained unknown, immunological factors are generally known as its cause(seyedmajidi et al. 2010), which is formed through the accumulation of inflammatory cells such as T cells (especially CD8+) accumulate between epithelium and connective tissue (Regezi et al.,2012).

Angiogenesis and lymphangiogenesis are of particular importance in pathogenesis of chronic inflammatory diseases. Angiogenesis is formation of new vessels from existing vessels, and an indispensable component of new tissue development and tissue growth and repair. Vascularization is static in adults and angiogenesis does not occur under normal conditions. Angiogenesis plays an important role under physiological conditions such as embryonic development and wound healing and under pathological conditions such as the growth of cancers, diabetes and the development of chronic inflammatory diseases with a certain pathological origin such as rheumatoid arthritis and psoriasis (Marrelli et al. ,2011).

The vascular endothelial growth factor (VEGF) family contains large quantities of growth factors, which directly affect vascular endothelial cells and stimulate proliferation and chemotaxis of endothelial cells. VEGF was recognized as a primary molecule causing angiogenesis physiologically and pathologically (Tammela Tet al., 2005). Each type of VEGF is bonded to one type of vascular endothelial growth factor receptor (VEGFR) and has its specific effects. The VEGFR-3 is related to lymphangiogenesis and the role is taken through bonding some types of VEGF especially VEGFC and VEGFD, which increase a lymphatic vessels network. Lymphangiogenic factors such as VEGFC and VEGFA

*[1]Oral and Maxillofacial Pathology Department, [2]Dental Materials Research Center, Dental Faculty, [3]pathology Department, Medical Faculty, [4]Non-Communicable pediatrics Diseases Research Center, Babol University of Medical Sciences, Babol, [5]Oral and Maxillofacial Pathology Department, Dental Faculty, Mazandaran University of Medical Sciences, Sari, [6]Oral and Maxillofacial Pathology Department, Dental Faculty, Tehran University of Medical Sciences, Tehran, Iran. *For Correspondence: ms_majidi79@yahoo.com*

are secreted in inflamed tissues by immune cells such as macrophages and resident cells such as keratinocytes and fibroblasts (Zhang et al., 2007; Otrock et al., 2007). In 2010, Huggenberger et al. studied some inflammatory skin disease in mice and realized that skin inflammation can be reduced significantly through stimulation of VEGFR-3 expression using a recombinant drug (VEGFc-156s) (Huggenberger et al., 2010). A literature review revealed that the role of lymphangiogenesis and VEGFR-3 in oral lichen planus (OLP) has not been studied yet. Therefore, this study discussed the presence and role of VEGFR-3 on OLP tissues with respect inflammatory nature of lichen planus.

Materials and Methods

After approval of study in ethics committee of Babol university of medical sciences, this cross-sectional study was done on fifty-two formalin fixed and paraffin embedded blocks of the OLP (including 25 erosive lichen planus and 27 reticular lichen planus) that were retrieved from the Archives of oral pathology department of Babol dental faculty. Diagnosis of lichen planus was based on Modified WHO criteria (Vander Meij et al., 2003). The appropriate blocks containing sufficient tissue were included in the study after preparing 4µ sections, H&E staining, and confirming diagnosis. Sixty normal tissue samples were included in the study. The tissues were obtained from a crown lengthening surgery with minimum inflammation as far as clinical and histopathological are concerned.

Primary antibody of VEGFR-3 (Mouse Monoclonal Antibody VEGFR3, product code: NCL-L-VEGFR-3, Clone: KLT, Ig, class. IgG 2b. Kappa, Nova CastraTM liquid) with 1:50 dilution was used for immunohistochemical staining. Positive control was a placental tissue and negative control was obtained by omission the primary antibody. Immunohistochemical expression of VEGFR-3 was evaluated using an optical microscope (Olympus BX41, Olympus, Tokyo, Japan) by two independent pathologists who were not aware of the clinical specifications of the samples. Using 10x magnifications, five fields with maximum hot spots were selected. Stained cells were counted using 40x magnification. VEGFR3 distribution was evaluated using semi-quantitative method at tissue level cells (Baltazar et al., 2007) which included:

Negative (0): lack of staining; slightly positive (+): staining of up to 10% of cells; moderate positive (+ +), staining in 10% to 50% of the cells; strongly positive (+ + +): staining in more than 50% of the cells.

Five areas of the histopathology slides with 100 x magnification and maximum density of vessels were selected to investigate lymphatic vessels. Density of vessels was evaluated in each area using 40 x magnifications and average of the vessels in the 5 area was registered. VEGFR-3 expression was studied among normal oral mucosa, oral lichen planus, and different types of lichen planus. The data were analyzed using SPSS 20 and Mann-Whitney test and $p < 0.05$ was considered statistically significant.

Results

Fifty-two paraffined blocks of oral lichen planus including 27 cases (51.9%) of reticular form and 25 cases (48.1%) of atrophic-erosive form, referred to 11 males (21.1%) and 41 females (78.9%) patients with the average age of 45.8±11.7 years were included Sixty samples with normal oral mucosa referred to 12 males (20%) and 48 females (80%) patients with the mean age of 34.86±10.03 years were included. Areas of biopsy of lichen planus in 6 patients (12%), 40 patients (80%), 3 patients (6%), and 1 patients (2%) were tongue, buccal mucosa, gingival and labial mucosa, respectively.

VEGFR3 expression in lichen planus samples was more than normal oral mucosa ($P<0.001$), (Figure 1). The mean score of staining in the lichen planus and normal oral mucosa were 2.5±0.6 and 1.3±0.6, respectively. The Mann-Whitney test showed that the difference was statistically significant ($P<0.001$).

Table 1 shows that the mean of the lymphatic vessels

Table 1. Lymphatic Vessels Count in Oral Lichen Planus and Normal Oral Mucosa

Group	N	Mean±SD	Pvalue
Oral lichen planus	52	23.7±7.8	0.000
Normal oral mucosa	60	10.5±4.1	

*Mann-whitney test

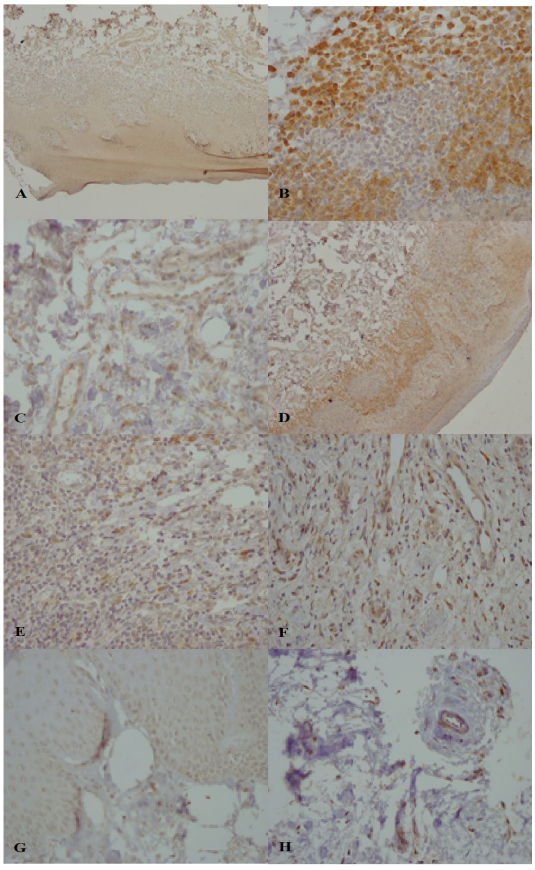

Figure 1. Immunohistochemical Staining of VEGFR-3 in Atrophic-Erosive Oral Lichen Planus in Epithelium, Band-Like Infiltration and Vessels (A,B,C) , Reticular Oral Lichen Planus Epithelium, Band-Like Infiltration and Vessels (D,E,F) and Normal Oral Mucosa in Epithelium and Laminaproperia(G,H)(40X Magnification)

Table 2. Comparison of Stained Cells and Lymphatic Vessels in Different Types of Lichen Planus for VEGFR-3

Variable	Group	N	Mean±SD	Pvalue
Stained cells(score)	Reticular	27	2.6±0.6	0.262
	Atrophic-erosive	25	2.4±0.8	
Lymphatic vessels(count)	Reticular	27	21.7±6.7	0.092
	Atrophic-erosive	25	25.2±7.7	

*Mann-whitney test

in lichen planus was significantly higher than normal oral mucosa (P<0.001). Table 2 shows no significant difference between the VEGFR3 expression and lymphatic vessels count in different types of lichen planus.

Discussion

This study aimed the evaluation of presence and role of VEGFR-3 in oral lichen planus in compare to normal oral mucosa. The results showed that none of the Lichen Planus was negative in terms of presence of this marker and mean of staining score in Lichen Planus group was significantly higher than normal samples. The results also showed that the average number of stained vessels in the OLP was significantly more than normal mucosa. The study of Metwaly et al. showed that VEGF expression in epithelial cells of oral lichen planus was more than the control group. It was concluded that VEGF plays an important role in the pathogenesis of OLP through activating angiogenesis (Metwaly et al., 2014). Serumal level of VEGF in the patients with OLP was more than the ones of control group according to study of Mardani et al., (2012) which confirms the role of VEGF in OLP. Rhodus et al., (2005) showed that a series of angiogenesis triggered cytokines including IL8, IL6, IL1, TNF-α rises inherently in OLP patients. These factors may raise VEGF level and lead to increased VEGF serum levels. The findings have been observed in some other diseases of the immune system. For instance, in the study of Young et al. , plasma levels of VEGF and FLT1 (VEGFR-1) increased considerably in the patients with psoriasis as compared with the control group and mean of plasma levels of KDR (VEGFR-2) was higher in the patients with psoriasis as compared with the control group (Young et al.,2004). The study of Lee et al., (2008) showed that serum level of FLT4 (VEGFR3) in the patients with psoriasis was higher the normal control group. They suggested that VEGFA and FLT4 are related in the pathogenesis of psoriasis indirectly and VEGFA may lead to production of inflammatory cells indirectly, which may lead to production of VEGFC and VEGFD from inflammatory cells that bond to FLT4 (VEGFR-3).

The results of animal studies are also consistent with this study, as Baldwin et al. stated that the increase of VEGFC through lymphangiogenesis and lymph flow creation are effective in improving cutaneous chronic inflammation. They report that mouse VEGF-D fails to bind mouse VEGFR-2 but binds and cross-links VEGFR-3 as demonstrated by biosensor analysis with immobilized receptor domains and bioassays of VEGFR-2 and VEGFR-3 cross-linking and concluded that the anti-inflammatory effect of VEGFC in chronic cutaneous inflammation is through lymphangiogenesis stimulation not through angiogenesis (Baldwin et al., 2001).

The study of Pytowski et al. on mice specified that VEGFR-3 blocking prevents completely and specifically from VEGFC-caused lymphangiogenesis in normal tissues and tumors in mature mice. However, they stated that VEGFR-3 blocking has no effect on hemangiogenesis or function and survival of lymphatic vessels (Pytowski et al., 2005).

Some similar clinical studies are consistent with the results of present study. Some studies proved that anti-angiogenic treatments - through specific inhibitors of VEGF may reduce severity of some diseases such as arthritis and cancer and postpone progression of chronic inflammatory diseases chronic in some inflammatory and autoimmune diseases (Ferrara et al.,2005; Takahashi et al., 2005). Kataru et al., (2009) proved that inhibition of VEGF - C/D by Soluble VEGFR-3 reduces considerably lymphatic flow in coetaneous bacterial inflammation. Huggenberger et al., (2010) stated that angiogenesis inhibition through inhibiting VEGFR-2 to improve chronic inflammation of the skin, and in contrast to that, specific reduction of lymphangiogenesis by inhibiting of VEGFR-3 improve edema caused by disrupting lymphatic drainage. VEGFC release, which is a lymphangiogenic factor, inhibits chronic coetaneous inflammation, epidermal hyperplasia, abnormal differentiation of cells, and CD8+ T cells. In addition, intradermal injection of recombinant VEGF-c-156s, which only activates VEGFR-3, significantly reduces coetaneous inflammation.

The results of present study showed no significant difference between staining score in different types of lichen planus for VEGFR-3. As far as number of the lymphatic vessels was concerned, no significant difference was seen among different types of lichen planus.

The study of Tao et al., (2007) showed that angiogenesis and VEGF expression are strongly associated with different clinical forms of OLP lesions at tissue level, as VEGF expression and MVD (microvessel density) are higher in reticular form than erosive -atrophic type of lichen planus. Of course, this study examined angiogenesis and it aimed at discussing lymphangiogenesis. In this regard, the two studies are different. It seems that angiogenesis and lymphangiogenesis are not consistent. Thus, according to inflammation process in lichen planus and higher expression of VEGFR3 in oral lichen planus in compared to normal oral mucosa, it seems that we can reduced inflammation process and severity of lichen planus with increasing lymphangiogenesis from VEGFR3 pathway by recombinant VEGFC-1565.

The results showed that the percentage of stained cells and the lymphatic vessels for VEGFR-3 in oral lichen planus was more than normal oral mucosa and it seems that VEGFR-3 involves in pathogenesis of oral lichen planus and it takes this role by increasing lymphatic vessels and lymphangiogenesis. However, VEGFR-3 expression did not have a significant difference among different types of lichen planus disease.

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