The Assessment of Angiogenesis by Microvessel Density in Patients With Atrophic/Erosive or Reticular Oral Lichen Planus

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Abstract- The role of angiogenesis in the development of chronic inflammatory diseases, including oral lichen planus (OLP), is of great concern. The most representative method for the assessment of angiogenesis is the semi-quantification of microvessel density (MVD) using specific markers such as CD105. We aimed to assess the MVD in patients with OLP and find its clinical significance to differentiate the atrophic/erosive forms from reticular ones. This cross-sectional study was conducted on 82 patients with clinically and histopathologically proven cases of OLP, including reticular (n=52) or atrophic/erosive (n=30) lesions. The control group comprised 82 age- and sex-matched subjects without any oral disease. To assess the MVD using CD105, tissue blocks were sliced, and the immunoexpression of CD105 was measured by the standard immunohistochemical staining procedure. The mean value of MVD in OLP patients was significantly higher than that in the controls (14.61±12.48 *vs*. 8.67±1.76, *P*<0.0001). Furthermore, there was a significant difference in the mean MVD value between reticular and atrophic/erosive lesions (8.19±7.13 *vs*. 25.73±12.06, *P*=0.001). However, no significant difference was observed between the reticular lesions and normal tissues (*P*=0.58). An increased level of CD105 in OLP patients can improve our knowledge about the causes and mechanisms of the disease. The CD105-MVD assessment might be a useful method for semiquantitative measurement of angiogenesis in OLP patients as well as differentiating its clinical forms; therefore, it can open new vistas for formulating strategies based on antiangiogenic treatments for the management of OLP and other precancerous lesions.

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Introduction

Oral lichen planus (OLP) is a chronic disease of the stratified squamous epithelium with inflammatory pathogenesis. There are commonly encountered in clinical dental practice and primarily found on the buccal mucosa and the tongue (1-3). The clinical history of OLP is characterized by a subepithelial lymphocytic infiltration with periods of relapses and remissions that directly influences the patient's quality of life; however, there is no effective way to predict or prevent such event. OLP is estimated to affect 0.5% to 2.0% of the general adult population, and the middle-aged or elderly females are generally more susceptible than males (2,4). There are no widely accepted diagnostic criteria for OLP; however, these lesions can manifest in six clinical subtypes, including reticular, popular, plaque-like, atrophic, erosive, and bullous forms. Atrophic and erosive forms are the main ulcerative subtypes with a higher risk of malignant transformations; however, the reticular form being the most common form of OLP (2,5,6).

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The etiopathogenesis of OLP has yet been poorly understood, but several mechanisms, including the T cell-mediated immunity and inflammatory pathways have been proposed for this complex disease (7-9). Factors such as stress, genetic background, drugs, infectious agents, certain dental materials, and autoimmune disorders are thought to play a role in the pathogenesis of OLP (10,11). The angiogenetic phenomenon, a fundamental mechanism of the pathogenesis of chronic inflammatory processes, has also been suggested to contribute to the development and progression of OLP. Furthermore, angiogenesis is considered a vital phenomenon of malignant processes and has a substantial role in the aggressive behavior of lesions (11-13). It primarily depends on a delicate balance between the stimulators and inhibitors; therefore, an angiogenic phenotype can be triggered by any physiological or pathological change in this balance. It seems that prolonged inflammation can stimulate aberrant angiogenesis and neovascularization can facilitate the inflammation (14-16).

The microvessel density (MVD) is a widely accepted index for quantitative analysis of angiogenesis through the immunohistochemistry (IHC) assay (16,17). A variety of molecules, including vascular endothelial growth factor (VEGF), von Willebrand factor, factor VIII, CD31, CD34, and Endoglin (CD105) have been proposed for MVD assessment in human tissues (18-20); however, CD105 is considered as a more specific and sensitive angiogenesis marker than other pan-endothelial markers (21,22). CD105 is a transmembrane glycoprotein of the vascular endothelium and involved in blood vessel development. It is one of the transforming growth factor β (TGF-β) co-receptors. CD105 is predominantly expressed in the angiogenic endothelium during tumor angiogenesis and inflammation; therefore, its over-expression is usually considered as a powerful marker of newly-formed vessels (23-25). The role of angiogenesis in OLP has not been fully elucidated; therefore, the current study aimed to investigate the expression of CD105-MVD in patients with OLP lesions and compare the results with that observed in healthy ones.

Materials and Methods

Ethics

The protocol of this study was approved by the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Approval ID: IR.SUMS.REC.1393.S7105). All participants signed the written informed consent according to the declaration of Helsinki and its later amendment.

Study population

A total of 82 adult patients with a confirmed diagnosis of OLP, including those with reticular (n=52) or atrophic/erosive (n=30) lesions, were enrolled in this cross-sectional study. They were referred to the Pathology Department of Dentistry School, Shiraz University of Medical Science, Shiraz, Iran. All patients had been diagnosed by clinical as well as histopathological examinations according to the world health organization (WHO) guidelines and Burket's Oral Medicine criteria (26). Demographic and clinical data were obtained from the medical records. None of the patients had prior OLP treatment experience. Patients with a history of cancer, systemic or inflammatory disease, as well as those with other forms of OLP lesion rather than reticular or atrophic/erosive, were excluded. The control group of this study was 82 age- and gender-matched healthy individuals without any clinical sign of oral disease.

CD105-MVD assessment

To assess the MVD, the formalin-fixed paraffin-embedded tissue blocks were sliced into 4-μm-thick sections, deparaffinized in histology grade xylene, and rehydrated in descending ethanol series. After washing with distilled water, the slides were subjected to an immunohistochemical staining method using the Envision Labeled Peroxides System (DAKO, Carpentaria, CA, USA) according to the manufacturer's recommendations. Antigen retrieval was performed using DakoCytomation target retrieval solution (pH=9) for 20 minutes. Internal peroxidase activity was inhibited by 3% H_2O_2. Slides were then incubated for 30 minutes with the anti-CD105 antibody at 1:10 solution (Monoclonal Mouse Anti Human, Cat#M352701, DAKO, Santa Clara, CA, USA). Complexes were visualized using liquid 3,3′-diaminobenzidine tetrahydrochloride chromogen (DAKO, Glostrup, Denmark) at room temperature. To terminate the color development, the slides were washed three times in distilled water and then lightly counterstained with Harris' hematoxylin, dehydrated, and permanently mounted. Samples of the control group were also stained with the same amount of anti-CD105 antibody used for staining OLP lesions. Appropriate positive and negative controls were also included. The omission of the primary antibody was employed as a negative control, while liver tissue was used as a positive control for

CD105. Specimens were assessed blinded using a light microscope, and brown staining of the cytoplasm was considered as positive. Under 40× magnification, the sites of the greatest vascularity (hotspots) were selected, and then, the number of microvessels were counted under 400× magnification from five fields of view.

Statistical analysis

All statistical analyses were carried out using the statistical software SPSS version 21 (SPSS Inc, Chicago, USA). Variables were tested for normal distribution using the Kolmogorov-Smirnov test. Continuous variables were expressed as the mean±standard deviation (SD). The Chi-square test was used for binary variables, while the comparison of continuous variables was performed using Kruskal-Wallis or Mann-Whitney test. Pearson's correlation coefficient (r) was used to describe relationships between continuous variables. A P value of less than 0.05 was considered to be statistically significant.

Results

The patients' group consisted of 82 patients with clinically and histopathologically proven cases of OLP including 12 (14.6%) males and 70 (85.4%) females. The mean age for the OLP group was 45.3 years, with an age range of 26-63 years. The male-to-female (M/F) ratio was 0.17. The mean age of the control group was 45.9 years (age range 21-61 years). No significant differences were observed between the patient and control groups concerning age and gender (P>0.05).

Analysis of CD105 expression exhibited the significantly higher mean MVD values in the OLP patients (14.61±12.48) compared to the control group (8.67±1.76) (P<0.0001). Of 82 patients, 52 (63.4%) showed reticular OLP, while the remaining patients suffered from atrophic/erosive lesions (n=30, 26.6%). CD105 might be helpful to differentiate the reticular lesions from atrophic/erosive OLPs (Figure 1).

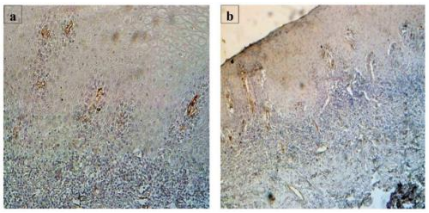

Figure 1. Immunohistochemical staining of CD105 in OLP specimens with reticular (a) and atrophic/erosive (b) forms

There was a significant difference in the mean MVD value between reticular and atrophic/erosive forms (8.19±7.13 *vs.* 25.73±12.06; P=0.001). However, no significant difference was observed between the reticular lesions and normal tissues (P=0.58). Furthermore, no significant difference was observed between the mean of MVD in males and females, neither in the patient nor in the control groups (P>0.05).

Discussion

A significant increase in neoplastic transformation risk was noted among patients suffering from OLP lesions. Angiogenesis, a cycle of physiological processes that lead to the development of new vessels from the preexisting vasculature, is a critical requirement for tumor progression; hence, it has an important prognostic value. CD105 has been introduced as a powerful marker of angiogenesis, which is overexpressed in tumor-associated tissues and stains vessels that are in the proliferating stage. Therefore, it is important to investigate angiogenesis in OLP patients to reduce the burden of this devastating disease (14-16). Currently, tissue biopsy and histological assessment are a gold standard method for evaluating angiogenesis; therefore, we examined the angiogenic potential of OLP compared with normal oral mucosa through the CD105-MVC assessment.

Our results showed that the mean MVD increased significantly in the OLP group in comparison with the controls. Few studies have investigated the angiogenic potential of biomarkers in OLP lesions (12,13,16); however, data on CD105 are too limited. To the best of our knowledge, only two studies have explored the expression of CD105 in OLP. Khalili *et al.*, (27) and Eshghyar *et al.*, (28) were reported the higher expressions of CD105 in OLPs compared to normal mucosa; however, both studies were conducted on a limited number of patients. Several immunohistochemical markers have also been recommended for the evaluation of aberrant angiogenesis in OLP lesions. A significantly higher mean MVD was observed in OLP patients compared with the controls using the CD34 antibody (11,13,16,17). The expression of CD54 and CD106 (13), as well as VEGF (10-14) and activin receptor-like kinase 1 (ALK1) (29), were also able to differentiate patients with OLP lesions from healthy subjects. Our study represents a direct assessment of angiogenesis in OLP and confirms its vital role in the pathogenesis and progression of OLP lesions.

This study included patients with atrophic/erosive or

reticular OLPs to establish a comparison between the rate of angiogenesis in OLP lesions and normal mucosa. We found that CD105 has a significant association with different forms of OLP lesions and might be helpful to differentiate the reticular lesions from the atrophic/erosive forms. The mean MVD in atrophic/erosive lesions was significantly higher than that observed in reticular ones, which show different degrees of aggressiveness between these clinical forms. Furthermore, patients with atrophic/erosive OLPs exhibited a significantly higher mean MVD compared with the control group; however, this was not the case for reticular lesions. In line with our findings, intergroup analysis in previous studies also showed significant differences in the CD105 expressions between the reticular and erosive OLPs compared to normal mucosa (27,28). Besides, CD34, VEGF and ALK1 were closely correlated to different clinical forms of OLP, as reticular/papular forms were showed relatively lower angiogenesis compared with atrophic/erosive OLPs (10,14,16,29). However, the immunodetection of angiopoietin (ANG), which is another growth factor essential for vascular formation could not differentiate the OLP groups (12).

The risk of malignant transformation into oral squamous cell carcinoma (OSCC) is one of the most serious complications of the OLP disease; however, clinical and histopathological factors favorable for malignant change have not been introduced (3,30). Angiogenesis has a vital role in many pathological and physiological processes, including inflammation, repair and tumor growth. Therefore, several studies have conducted to examine the potential of CD105 to quantify angiogenesis in such conditions as well as early monitoring of metastasis and cancer relapse. CD105 up-regulation has revealed in a wide range of tumor endothelia such as OSCC (31), laryngeal SCC (32), head and neck SCC (33), and juvenile nasopharyngeal angiofibroma (34).

Higher expressions of CD105 in atrophic/erosive lesions compared to reticular form exhibited a key role of angiogenesis in the pathogenesis and progression of OLP lesions. Our findings could be helpful for formulating new treatment strategies using antiangiogenic medications. However, further studies are warranted to assess the potential role of CD105 for the early detection of OLP and monitoring patients.

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