

Assessment of microvessels density and inflammatory status in oral lichen planus

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ABSTRACT

Background: Oral lichen planus (OLP) is a chronic inflammatory disease with an autoimmune inflammatory pathogenesis. The purpose of this study was to evaluate the role of angiogenesis in the pathogenesis of OLP, using CD34 stain to highlight the blood vessels for measuring the microvessel density (MVD) as well as to evaluate the relation of this marker with the degree of inflammation

Materials and Methods: Immunohistochemical (IHC) staining technique was used to evaluate angiogenesis using CD34 in 46 paraffin blocks 10 of them obtained from normal mucosa and 36 from cases diagnosed as lichen planus, 20 of them diagnosed as Reticular type while 16 as erosive type. Severity of inflammation was divided into mild, moderate and severe according to the number of mononuclear cell infiltrate.

Results: The mean MVD as determined by the mean number of CD34 positive vessels in hot areas for OLP group was higher 39.2 (31.9 for reticular group & 48.375 for erosive group), whereas the mean MVD for control group 19.5 so that there was statistically significant difference between OLP group and the control group (p value<0.001). Regarding the severity of inflammation mean MVD was significantly increased as the degree of inflammation increased (p value<0.001).

Conclusion: Angiogenesis may be an integral component associated with the development of the OLP

Key words: Oral lichen planus, angiogenesis, CD34, microvessels density. (J Bagh Coll Dentistry 2013; 25(Special Issue 1):43-47).

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease with unknown etiology appears in different clinical forms and Neville et al. Recognized essentially two types, the reticular and the erosive ⁽¹⁾. OLP affects approximately 2% of the population ^(2,3).

The reticular lesions appear as a network of connecting and overlapping lines, papules or plaques. The erosive and ulcerative forms are more destructive forms and cause enormous oral discomfort ⁽⁴⁾, while reticular forms are associated with fewer symptoms and therefore might reflect an intermediate phase ⁽⁵⁾.

LP most commonly affects middle-aged people, although childhood-onset LP has also been well described. Women are affected as frequently as men. OLP is a self-limited condition that, according to one epidemiologic study, may resolve after 1 month to 7 years. ⁽⁶⁾ A range of topical and systemic medications have been shown to improve the symptoms associated with LP and to hasten the resolution of LP ⁽⁷⁾.

The pathogenesis of OLP is not entirely understood. In general, activated T lymphocytes are recruited to the dermal-epidermal junction and induce apoptosis in basal keratinocytes. Both CD4⁺ and CD8⁺ T lymphocytes are found in the lichenoid infiltrate of LP, with a predominance of the latter cell type being present in established lesions ⁽⁸⁻¹⁰⁾.

The interaction between pathogenic T lymphocytes and basal keratinocytes is enhanced by increased expression of intercellular adhesion molecule-1 (ICAM-1) by basal keratinocytes ^(11,12).

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IHC studies showed that the infiltrating cells in OLP are predominantly T lymphocytes with very few B lymphocytes. Several cytokines ⁽¹³⁻¹⁵⁾, adhesion molecules ⁽¹⁶⁾, and apoptosis-related molecules ⁽¹⁷⁾ are involved in its pathogenesis. Neo-angiogenesis, together with a rich vascular proliferation, were reported to be essential factors in the pathogenesis of different types of OLP ^(18,19).

Angiogenesis, which is the formation of new blood vessels, is an important component in many biological processes, whether physiological as in proliferating endometrium, corpus luteum formation and embryogenesis, or pathological including neoplastic, inflammatory, and degenerative conditions ⁽²⁰⁾. In normal tissues, new vessel formation is dependent on a delicate balance between several different stimulatory and inhibitory factors; any change in this balance, whether physiological or pathological can result in acquisition of an angiogenic phenotype.

Several scientific studies ^(21,22) have verified the presence of neo-angiogenesis and its importance in a number of inflammatory pathologies such as rheumatoid arthritis, psoriasis, bronchial asthma, diabetic retinopathy, atherosclerosis, and Alzheimer's disease. The importance of angiogenesis in the pathogenesis of chronic inflammatory illnesses is through its allowing of better oxygenation and a greater contribution of metabolites to the proliferating tissue by the formation of new vessels, and through an increase in the turnover of the cells involved in the inflammatory process. ⁽²³⁾

Angiogenesis has been recognized as an important feature in chronic inflammation accompanies many autoimmune and

inflammatory conditions including OLP. Moreover, investigations have also shown a close relationship between angiogenesis and the activity of these diseases. Therefore, it is necessary to elucidate the role of angiogenesis in the pathogenesis of OLP to better understand its mechanisms and, better understanding of the etiopathological mechanism underlying OLP will help in the development of new treatment strategies, as well as to manage persistent inflammation in patients showing poor response to conventional immunosuppressive drug regimes.⁽¹⁹⁾

Microvessel density (MVD, the number of micro vessels per mm²) is a commonly applied estimate of tumor angiogenesis and is widely accepted to play a role in the pathogenesis of some inflammatory conditions. CD34 monoclonal antibody is considered to be an appropriate marker to investigate the vascular endothelium^(24, 25) and to quantify microvessel density (MVD) in inflammatory or neoplastic disorders because of its capability of staining the vascular endothelial cells. Few studies have reported the importance of angiogenesis in OLP^(18, 19).

The purpose of this study was to evaluate the role of angiogenesis in the pathogenesis of OLP, using CD34 stain to highlight the blood vessels in both normal and lichen planus affected oral mucosa for measuring the MVD as well as to evaluate the relation of this marker with the degree of inflammation

MATERIALS AND METHODS

This is a retrospective study conducted at the oral pathology department, college of dentistry, Baghdad University. A total number of 46 cases involved in this study. They were categorized into 2 groups as follow;

Group 1 (control group); consisted of 10 cases of apparently normal mucosa taking from subjects underwent tooth extraction or other dental procedures, those were subjected to classical 4-6 mm punch biopsy of normal looking oral mucosa(buccal or gingival)

Group 2 those with OLP lesion 36 cases, which were collected either from the blocks of previously diagnosed cases from the archives of the department of oral and maxillofacial pathology and from surgical specialist hospital. Those were asserted as;

Group 2 a; those with histopathological diagnosis of reticular type OLP 20 cases

Group 2b; those with histopathological diagnosis of erosive type OLP 16 cases

The files of pts with OLP were reviewed

Biopsies were fixed in formalin and embedded in paraffin wax. Four micron thick sections from the paraffin embedded biopsies were stained by haematoxylin and eosin to verify the clinical diagnosis of LP for those with a clinical diagnosis of OLP and to confirm the diagnosis of normal oral mucosa for the control group. The histopathological features of OLP shows varying degrees of orthokeratosis and parakeratosis, the rete ridges may be absent or hyperplastic, but classically have seen tooth appearance. Hydropic degeneration of basal cell layer and band like infiltration of T lymphocyte. Civatte bodies may be seen⁽¹⁾

IHC staining was carried out using CD34 antibody. All blocks that collected from the archives of the department of pathology were cut at a thickness of 4 micron & were mounted on glass slides. Sections were deparaffinized in xylene, & subsequently rehydrated with ethanol & water. The section then incubated with the primary monoclonal antibody at 4c overnight for CD34 (diluted 1:40). The bounded antibodies were detected by streptavidin-biotin complex. Washing by PBS (phosphate-buffered saline). The sections were then counterstained with hematoxylin and the slides were observed under Olympus research microscope

Identification of microvessels was indicated by cytoplasmic immunostaining of the endothelial cells with anti-CD34 monoclonal antibody. The entire section was scanned systematically by light microscopy at low magnification to identify the area with the highest number of stained microvessels(hot spots). Then the vessels were counted in the 5 areas of hot spots at X400 magnification MVD was expressed as the average number of vessels in these areas⁽¹⁹⁾.

Degree of inflammation was subjectively graded into mild, moderate, or severe according to density of mononuclear infiltrate

Data processing & Statistical analysis was done by excel 2003(Microsoft,seattle WA,USA) .The T student test was used to test the difference. Chi square test for the association of MVD and inflammation

RESULTS

A total number of 46 subjects were included in this study. The age range of control group was 25-50 years with a mean of 39.4 years, while the range for OLP group was 28-60 years with a mean of 38.6 years with no statistically difference between OLP cases and controls as regards the mean age

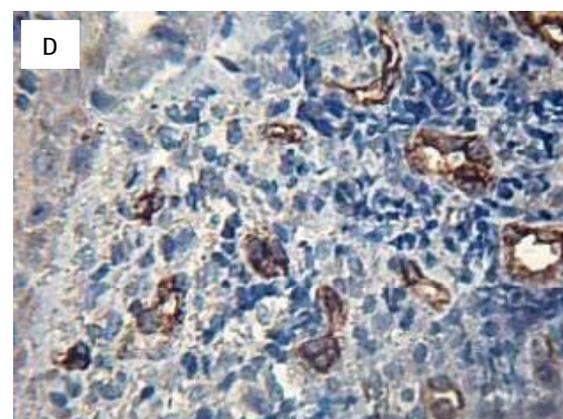
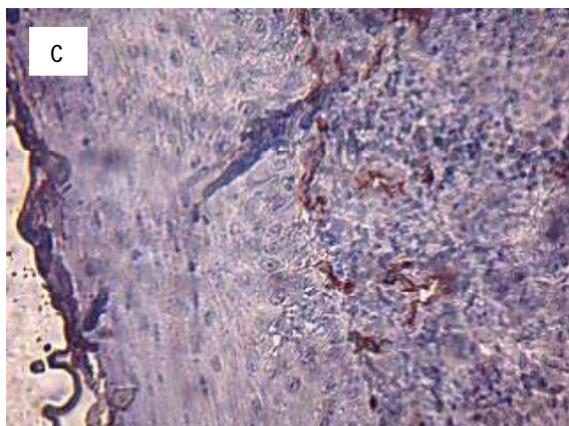
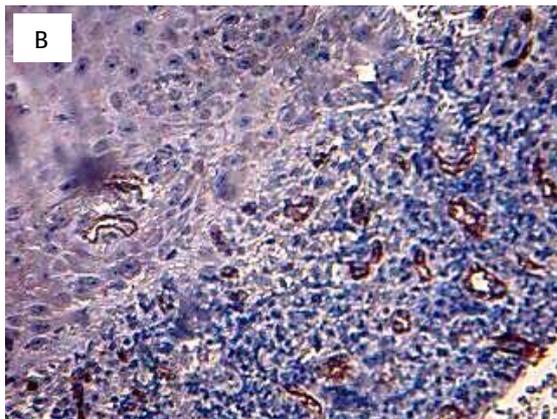
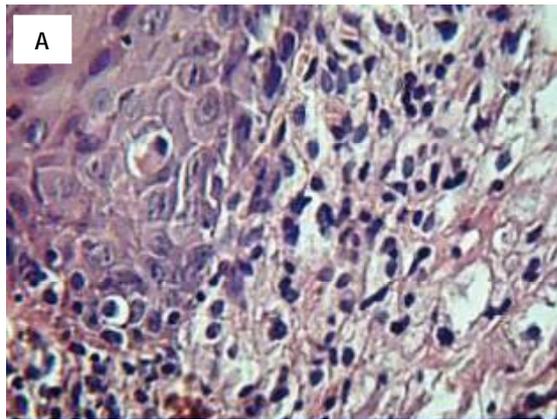


Figure 1: Oral Lichen Planus: A- H & E X 400, B & C- marked newangiogenesis X200 D-X400

Table1: MVD in different group

Groups	Minimum MVD	Maximum MVD	Mean +SD	P value
Group1 (control)	15	24	19.5	P<0.001
Group 2 A (reticular OLP)	28	41	31.9	
Group 2 B (erosive OLP)	36	65	48.375	

Table 2: mean MVD in different grades of inflammation in the lichen planus group

	Mild (9)	Moderate (16)	Severe (11)
MVD	26.33 ± 4.1	37.37 ± 3.94	52.45 ± 7.7
P<0.001			

Regarding sex distributions in the OLP group, 24 patients (66.6%) were females & 12 patients (33.3%) were males while in the control group, there were 5 females (50%) & 5 males (50%) with significantly higher rates of females in patients of OLP group compared with control of OLP group when all OLP patients were regarded as a whole.

In the present study, anti-CD34 antibodies were used for the determination of MVD. High CD34 staining was observed in the cytoplasm of the vascular endothelium of all specimens. Most of the microvessel hot spots were located in the lamina propria (Fig. 1)

The mean MVD as determined by the mean number of CD34 positive vessels in hot areas for OLP group was 39.2 (31.9 for reticular group & 48.375 for erosive group), whereas the mean MVD for control group 19.5 so that there was statistically significant difference between OLP group and the control group (pvalue<0.001) as shown in table 1

Regarding the severity of inflammation, the mean of MVD was significantly increased as the degree of inflammation increased (P value<0.001) as shown in table 2

DISCUSSION

Angiogenesis is an important part in the inflammatory response that explains the persistence and chronicity of different autoimmune and inflammatory disorders including OLP

Angiogenesis is a complex process characterized by the formation of new capillaries from the preexisting vascular network.

This study revealed that females were more often affected than males was, this is in agreement with the study done by yas L.S in her

histopathological observation of 194 cases of lichen planus⁽²⁶⁾ and other previous studies^(27,28) but it disagrees with the finding of Sugerma et al. Which showed that the females and males were affected equally.

The current study showed that angiogenesis, as estimated by MVD using the endothelial cell markers CD34 is significantly increased in OLP lesion group compared to the control group which agrees with many studies conducted by different authors^(18,19) and also agrees with study done by Mittal et al⁽²⁹⁾ this finding may be explained by the concept that angiogenesis could be an important step in the etiology and pathogenesis of OLP

Furthermore in this study the MVD was significantly higher in OLP lesion with erosive type compared to the reticular type and this finding is in accordance with similar observations in several different studies^(18,19,29) The current study revealed that angiogenesis is significantly increased in OLP as compared to normal oral mucosa (table 1), also in erosive OLP as compared to reticular OLP, this suggests that angiogenesis is one of the main contributing factors in the progression of OLP

It is still possible that the activity of angiogenic factors might be especially elevated at OLP lesion sites. One hypothesis, to be tested for increased angiogenesis in these lesions is that some of the lesional cells switch to an angiogenic phenotype. The molecular basis of the angiogenic switch is not entirely clear but may involve an increase in angiogenesis stimulators, a decrease in angiogenesis inhibitors, or a combination of the two.

Although angiogenesis may not be the primary step in the pathogenesis of LP, understanding the pathways leading to angio-proliferation may help in finding novel therapeutic modalities for this common disease.

The current results, demonstrated that there is a direct relationship between angiogenesis and different clinical presentations of OLP and different degree of inflammation. Since the increase of MVD offered the favorable environment and indispensable condition for the proliferation and transformation of epithelia in the lesions, these findings will be helpful in some extent to explain several important clinical observations.

In summary, this study revealed that the aberrant angiogenesis and CD34 expression occurred in OLP lesions, closely correlated to its clinical forms. The complex regulatory mechanisms of angiogenesis existing in different clinical forms of OLP require further study to

validate. Our result supports the view that angiogenesis may be a future target for the management of different forms of OLP

Angiogenesis has long been known to be closely linked to chronic inflammation, and it is a component of various chronic inflammatory diseases. However the exact pathological mechanism of OLP is still not clear. Most of the studies have not been able to demonstrate a direct relation between angiogenesis and OLP. Antiangiogenic drug is not commonly used in OLP patients; it would reduce the dependency on corticosteroid drugs

The proven role of angiogenesis in the pathogenesis OLP and the resistant of some OLP lesions to the conventional immunosuppressive therapy may govern the attention to new treatment strategies targeted angiogenesis, so that angiogenesis suppressor drug may play a role in the treatment of OLP

In conclusion, it suggests that angiogenesis may be an integral component associated with the development of the OLP

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