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Immunohistochemical Evaluation of Angiogenesis Markers (CD34 and VEGF) in Oral Lichen Planus Lesions with or Without Epithelial Dysplasia

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Abstract

Objectives: The aim of the present article is to evaluate the role of angiogenesis in reticular and erosive oral lichen planus and dysplastic changes of these lesions via the immunohistochemical evaluation of VEGF and CD34 markers. Methods: 40 fixed formalin parfin embedded (FFPE) samples of oral lichen planus were selected and 7 samples of normal tissues were chosen as control samples. The samples were stained by IHC markers including CD34 and VEGF. After IHC staining Mean Vascular Density (MVD) was estimated by the count of endothelial cells. Results: All samples of the study were positive for CD34 and VEGF markers. The range of MVD in control samples were less than lichen planus samples and the maximum values of MVD for CD34 and VEGF markers were seen in atrophic lichen planus with mild dysplasia and atrophic lichen planus without dysplasia respectively. There was a significant difference in lichen planus samples compared to control samples for both CD34 and VEGF markers. For the expression of CD34 marker there was significant difference between the control samples and patients with mild Atrophic dysplasia and Atrophic with no dysplasia. For VEGF marker the significant difference was seen between control samples and samples of atrophic lichen planus with no dysplasia. Conclusion: Results of our study proposed that increased vascular density in oral lichen planus could be the result of the angiogenesis phenomenon and mediators of angiogenesis are potential markers for the progression of lesion toward dysplastic changes.

Key words: Angiogenesis, Dysplasia, Immunohistochemistry, Oral lichen planus.

Introduction

Lichen planus is a skin-mucosal chronic inflammatory autoimmune disease with unknown etiology. It is especially prevalent among women aged 30-60 year old. The prevalence of lichen planus varies between 0.5 to 4 % in the population. Lichen planus is a T-lymphocytes mediated autoimmune disease, in which T- cytotoxic lymphocytes could cause apoptosis of epithelial cells. Epithelial

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dysplasia might be seen in some cases of lichen planus. Some authors considered oral lichen planus as a precancerous lesion that could be converted to oral squamous cell carcinoma (Mollaoglu, 2000; Laeijendecker et al., 2005). Squamous cell carcinoma (SCC) is responsible for 90-95% of malignancies of the oral cavity and is one of the top ten reasons of global mortality (Laeijendecker et al., 2005).The range of malignant changes in OLP is reported as 2-4%. The relation between the enhancement of malignant risk and certain type of OLP lesion is not clear. Some studies, consider the risk of SCC in atrophic and erosive types and some others in plaque-like type. Dysplastic changes of lichen planus could be due to the co-occurrence of two separate lesions or could be the result of the secondary lichenoid reaction in a dysplastic leukoplakia. Early detection of malignant or dysplastic changes in a precancerous lesion is very essential (Lončar Brzak et al., 2012; Mohtasham et al., 2010).

Angiogenesis is a process which leads to the formation of new blood vessels from the adjacent vessels. It has an important role in a range of physiologic or pathologic changes of the oral cavity including chronic inflammatory diseases, tissue repair, neoplasia and metastasis. The angiogenesis phenomenon is formed by various factors such as FGF (Fibroblast growth factor), TGFB (Transforming growth factor B), TNFα and VEGF (vascular endothelial growth factor). Vascularization is a vital phenomenon of malignant processes, so the angiogenesis is considered as an independent predictive marker in malignant processes (Mittal, Shankari and Palaskar, 2012). Since the inflammatory lesions and tumors have a heterogeneous structure, the density of blood vessels is not similar in the different areas of the lesion. Microvascular density is correlated with dysplastic and malignant changes of tumors, therefore, MVD has been used as an index for evaluation of angiogenesis and malignant changes in tumors and a predictive factor of their aggressive behavior (Raica, Cimpean and Ribatti, 2009).

The angiogenesis markers could be used as potential indicators for the prediction of dysplastic changes of oral lesions. Also treatments based on the inhibition of angiogenesis, could be a potential therapeutic option in the management of precancerous lesions. Various indicators such as CD31, CD34, and CD105, BFGF, and VEGF are used for the evaluation of blood vessel density. VEGF is a polypeptide growth factor that serves its mitotic function specifically on endothelial cells and is directly involved in angiogenesis. CD_{34} is a 110-120 dalton glycoprotein and a marker of endothelial cells which acts as a binding ligand. This marker is used to detect the blood vessels as a special indicator in immunohistochemistry method (Shih et al., 2002). The role of angiogenesis in the pathogenesis of OLP lesions, especially those with dysplastic changes, has not been well understood. The present study evaluated the angiogenesis in reticular and erosive lesions with or without dysplastic changes of oral lichen planus through the detection of CD_{34} and VEGF by immunohistochemistry (Varma et al., 2014; Hussein, 2007; Nielsen and McNagny, 2008).

Materials and Methods:

Study population

40 FFPE samples of oral lichen planus were selected. These samples were reticular lichen planus without dysplasia, reticular lichen planus with dysplasia, erosive OLP without dysplasia, erosive lichen planus with dysplasia and 7 samples of normal tissues were chosen as control samples from Pathology Department of Mashhad dental school. (Table-1)

Laboratory equipment and staining

All the samples were reviewed again by a pathologist to confirm the recorded diagnosis. After that, the samples of paraffin blocks were chosen from the archive. The FFPE samples which had the adequate tissue for immunohistochemistry (IHC) evaluation were selected for this study. Positive control samples included CD34 hemangioma and VEGF colon cancer and negative controls were studied samples which had not been exposed by initial antibodies. The samples were cut into 4-micron slices and then were stained by IHC markers including CD34 (LAR483-5R, ready to use, 6ml) and VEGF marker (AR483-5R, ready to use, 6ml) according to the manufacturer manual instructions (Biogen-USA). The expression of endothelial growth factors was evaluated by IHC staining with After IHC staining MVD (Mean Vascular Density) was estimated by the count of endothelial cells.

Statistical analysis

Statistical analysis was done by SPSS V.16 software. For comparison of data between different groups ANOVA and Kruskal-Wallis tests were used. Also post hoc test (Tukey) was conducted for pairwise comparison of the groups. P values <0.05 was considered significant.

Results:

40 lesions were evaluated in this study including 21 lichen planus without dysplasia (two reticular lichen planus and 19 atrophic lichen planus), 19 samples had dysplastic changes. Among dysplastic samples 14 samples had mild dysplasia (2 were reticular and 12 were atrophic) and 5 samples had moderate dysplasia (all atrophic). 7 normal mucosa tissues were selected from the archive of pathology

department of Mashhad dental school as control samples. Table-1 shows the clinical characteristics of samples according to the type of lichen planus, presence of dysplasia and location of the lesions. 18 males and 18 females were in the group of patients with reticular lichen planus and from the 4 patients with atrophic lichen planus there was only one female. All 47 samples of the study were positive for CD34 and VEGF markers. MVD was evaluated in five various fields, which had the most stained cells and the mean amount of these five areas was recorded for each sample. According to the results, the range of MVD in control samples were less than lichen planus samples and the maximum values of MVD for CD34 and VEGF markers were seen in atrophic lichen planus with mild dysplasia and atrophic lichen planus without dysplasia respectively. ANOVA statistical analysis showed a significant difference in lichen planus samples compared to control samples for both CD_{34} and VEGF markers. Pairwise comparison of the number of observed blood vessels between control samples and samples of patients with reticular OLP with dysplasia, atrophic OLP with mild dysplasia, atrophic OLP with moderate dysplasia, atrophic with no dysplasia, and reticular with no dysplasia was done by Tukey test. According to the results for the expression of CD34 marker there was only significant difference between the control samples and patients with mild atrophic dysplasia and atrophic with no dysplasia. For VEGF marker the significant difference was seen between control samples and samples of atrophic lichen planus with no dysplasia.

Discussion

The prevalence of oral lichen planus is varied from 0.76 to 2.2%. Lichen planus is accompanied by some systemic diseases such as immune diseases, infections and malignancies but the mechanism of co-occurance of lichen planus and these systemic diseases is not well understood. The lesions of lichen planus can affect skin, mucosa or both. The most prevalent site for skin lesions are limbs. Genitals, nails, face and head are some of the other affected sites. Mucosal lesions affect the oral cavity, nose, throat, esophagus, stomach, bladder and genitals. Although genetic and environmental factors such are effective on the pathogenesis, but the exact etiology of the disease is unkown (Sugerman and Sabage, 2002; Sugerman et al., 2002; Gerayli et al., 2015). In our study, the most prevalent clinical form was atrophic lichen planus.

Previous studies suggested that the angiogenesis could be effective in the pathogenesis of oral lichen planus. But the role of angiogenesis in the dysplastic changes of lichen planus lesions has not been yet evaluated (Mittal, Shankari and Palaskar, 2012). Like another inflammatory lesions, angiogenesis and vascular density may be important determinants in the pathogenesis of OLP and its malignant changes. Similar to other chronic inflammatory lesions, OLP has secondary angiogenesis capability in response to hypoxic inflammation site. VEGF is a natural mediator of angiogenesis and its receptors are expressed on endothelial cells. The expression of VEGF increases in both physiologic and pathologic condition (Roopashree et al., 2010). The role of VEGF has been shown in many chronic inflammatory conditions such as rheumatoid arthritis, psoriasis, and arthritis (Afuwape, Kiriakidis and Paleolog, 2002; Marina et al., 2015). It is proposed that this growth factor could play a role in the pathogenesis of OLP and may be useful as a marker for the activity of lesions (Murphy et al., 2010).

The IHC evaluation of angiogenesis in the cutaneous lichen planus lesions proposed the probable role of angiogenesis in the progression of lichen planus. This finding was the results of the localized proliferation of capillaries at the inflammation site (Al‐Hassiny et al., 2018). Tao *et al.* showed the increased expression of VEGF marker in different forms of lichen planus including atrophic-erosive and reticular lesions. But there was no significant difference between these two groups of lesions (Tao et al., 2006). Raspollini *et al.* evaluated the role of vascular markers (MVD, VEGF, and COX-2 expression) in malignant changes of lichen sclerosus to squamous cell carcinoma. The expression of VEGF and COX-2 was statistically different between these two groups of samples but the difference of MVD was not statistically significant (Raspollini, Asirelli and Taddei, 2007). In our study, the expression of VEGF in lichen planus samples was higher than controlled samples, but MVD was not statistically different among the different forms of lichen planus. These findings, could propose the potential role of IHC in the diagnosis of lichen planus lesion in initial stage. Results of our study proposed that the inflammation is not the exclusive cause of the increment of vascular density in oral lichen planus samples and to some extent it could be attributed to the angiogenesis. Detection of angiogenesis in in-vivo environment proposed that it could be used as a marker of progression of the lesion and the initiation of dysplastic changes. Different studies suggested that angiogenesis and VEGF expression are related to different clinical forms of OLP lesions, which shows a potential of new approach to therapeutic strategies based on the inhibition of vascularization.

Conclusion

The majority of samples in our study were the atrophic lichen planus samples. According to the results of our study, MVD in normal oral mucosa was less than lichen planus samples. The mean scores of MVD in the atrophic lichen planus samples with mild dysplasia was more than the other samples. The mean number of VEGF and CD34 positive cells in normal mucosa was less than the lichen planus samples. The maximum values of MVD for CD34 and VEGF markers were seen in atrophic lichen planus with mild dysplasia and atrophic lichen planus without dysplasia respectively. Also the number of stained cells for VEGF and CD34 positive cells between the study groups was significantly different.

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Table 1: Distribution of lichen planus samples in different locations.

	Atrophic without dysplasia	Atrophic with slight dysplasia	Atrophic with moderate dysplasia	Reticular without dysplasia	Reticular with mild dysplasia
Lip	4	1	1	0	0
Buccal mucosa	9	5	3	2	2
Tongue	6	4	0	0	0
Gum	0	1	1	0	0
Palatal	0	1	0	0	0
Total	19	12	5	2	2
	36			4	

Table 2: The MVD values of different types of lichen planus after IHC staining for CD34.

	Histologic diagnosis			Numbers	Mean MVD	Range of values
CD34	Normal tissue			7	47	40-54
	With dysplasia	Slight	Reticular	2	52	48-57
			Atrophic	12	67	48-83
		Moderate	Atrophic	5	59	50-70
	Without dysplasia		Atrophic	19	65	55-87
			Reticular	2	62	40-87

Table 3: The MVD values of different types of lichen planus after IHC staining for VEGF.

	Histologic diagnosis			Numbers	Mean MVD	Range of values
VEGF	Normal tissue			7	51	38-72
	With dysplasia	Slight	68	2	68	56-80
			66	12	66	46-85
		Moderate	68	5	68	52-94
	Without dysplasia		Atrophic	76	76	49-94
			Reticular	68	68	58-79

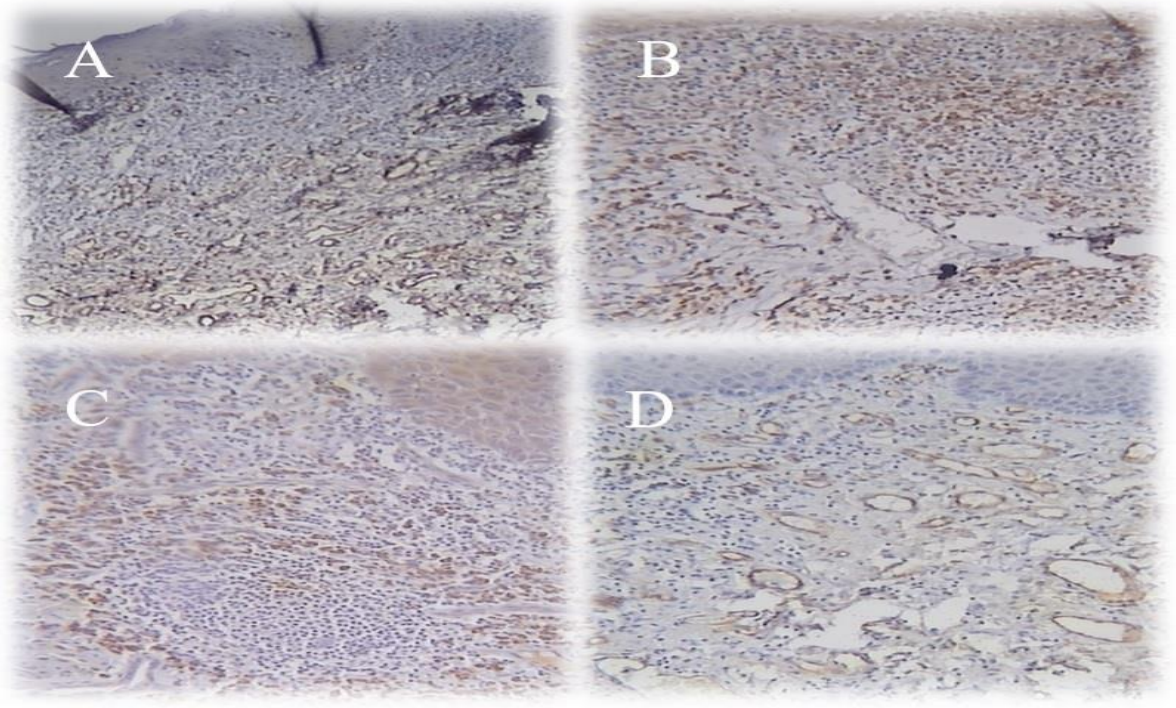

Figure 1: Images of lichen planus samples staining with CD34 markers
A: Lichen planus without dysplasia in 40X magnification
B: Lichenplanus with mild dysplasia in 100X magnification
C: Lichenplanus with mild dysplasia in 100X magnification
D: Lichenplanus with moderate dysplasia in 100X magnification

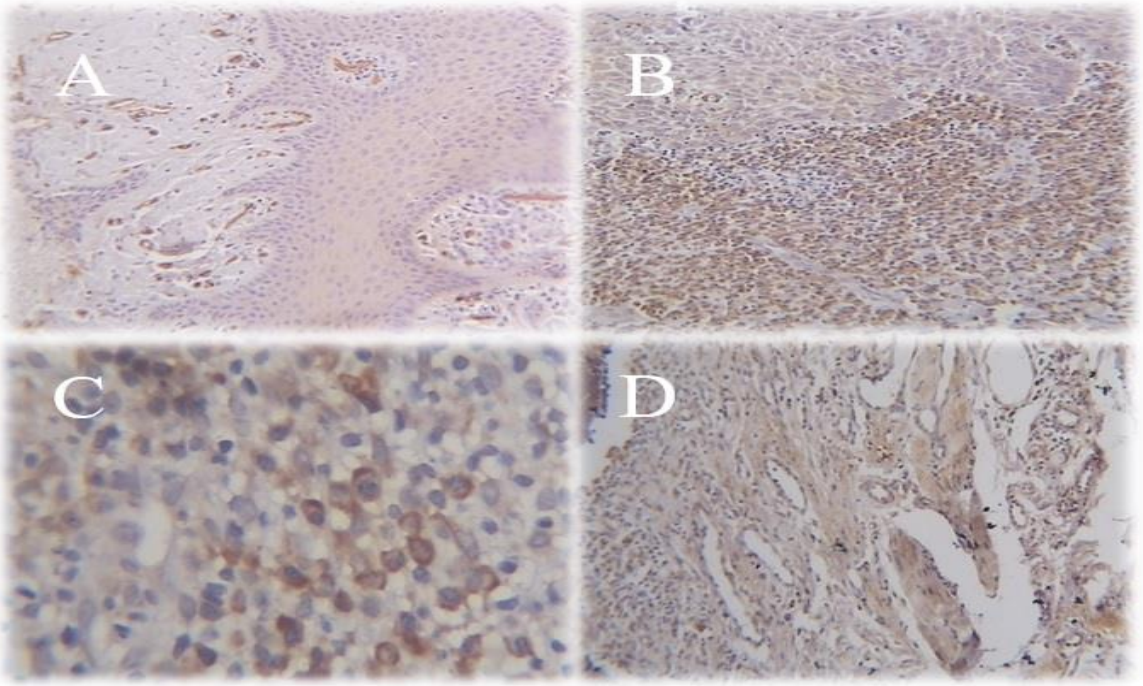

Figure 2: Images of lichen planus samples staining with CD34 and VEGF markers.
A: CD34 staining of lichen planus with moderate dysplasia in 40X magnification.
B: VEGF staining of lichenplanus with mild dysplasia in 100X magnification.
C: Cytoplasmic VEGF staining of lichenplanus in 400X magnification.
D: VEGF staining of lichenplanus without dysplasia in 100X magnification