Histopathologic evaluation of mast cells in reticular and erosive oral lichen planus using toluidine blue staining

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Original Article

Abstract

Introduction: Oral Lichen Planus (OLP) is one of the most common mucocutaneous lesion, and its etiologic factors have not been elucidated yet. OLP has two different clinical forms including reticular and erosive. Mast cells function in non-specific immunology is an etiologic factor involved in the formation of lichen planus. This study aimed at determining the pattern of mast cells presence in clinical forms of reticular and erosive oral lichen-planus in basal, epithelial, and superficial connective tissue layers.

Methods: The study population in this retrospective research included archival histopathological files in Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Tehran University of Medical Sciences. A total number of 53 paraffin-embedded blocks consisting of 18 reticular and 17 erosive OLP samples were included in the study. Four-micron sections were prepared for toluidine blue staining. Mast cells were counted in ten fields of light microscopy at magnification of 400 in epithelial, basal, and superficial connective tissue layers, and the mean number of mast cells in each layer was calculated. Mann-Whitney and Friedman tests were used to analyze the data while p-values less than 0.05 were considered significant.

Results: The mean number of mast cells was 1.85±2.9 in reticular and 2.02±3.18 in erosive groups. The mean count of mast cells in epithelial, basal, and connective tissue layers were 0.23±0.31, 0.34±0.45, and 4.98±3.28, respectively, in reticular lichen planus, and 0.21±0.25, 0.23±0.38, and 5.53±3.31, respectively, in erosive lichen planus. There was no significant difference between reticular and erosive forms of OLP and between epithelium and basal layers in terms of mast cells count ($P=0.474$, $P=0.148$, respectively), whereas a significant difference existed between epithelial and connective tissue layers ($P<0.001$).

Conclusion: The number of mast cells in reticular and erosive forms of OLP was higher in connective tissue than epithelium. Regardless of tissue layer, mast cells count was higher in erosive than reticular form of lichen planus.

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Introduction:

Lichen planus is a common chronic mucocutaneous disease which affects the skin and mucosa in 40%, only skin in 35%, and only mucosa in 25% of the cases (1). The disease was first described in 1869 by Erasmus Wilson as lesions semblable to lichens growing on rocks (2). Middle-aged women are more affected with a female to male ratio of 3 to 2. The infection is rare in children but may occur occasionally. Its incidence rate is 0.1-2.2% (3). The etiologic factor of lichen planus is unknown. Research on the pathogenesis of lichen planus is essentially concentrated on antigen-presenting macrophages in epithelium (Langerhans cells) and its interaction with T lymphocytes accumulated in the connective tissue of submucosa (4). Oral lichen planus is mainly seen in two clinical forms, reticular and erosive. Although reticular form is more prevalent than erosive, treatment of the erosive form is more requested by patients due to its symptoms such as atrophy and more damage (3).

Two specific and non-specific mechanisms are currently known to be involved in the occurrence of lichen planus. Specific mechanism refers to the presence of specific antigens such as basal keratinocytes and cytotoxic T cells, while non-specific mechanism includes the function of mast cells and activation of matrix metalloproteinases (5). In non-specific mechanism, the basement membrane seems fractured, branched, and duplicated (6). Studies have shown that in OLP, the number of mast cells is significantly higher in sub-epithelium than normal mucosa (7). Recently, studies have shown an increased density of mast cells in various types of lichen planus (5,8). In addition, it has shown that in oral lesions, mast cells are commonly seen in areas with damaged keratinocytes (9).

No study has been performed so far regarding the quantitative difference of mast cells in epithelial and connective tissue layers in reticular and erosive lichen planus. This study aimed to histopathologically evaluate mast cells in reticular and erosive clinical types of oral lichen planus in basal, epithelial, and superficial connective tissue layers (connective tissue beneath the epithelium).

Methods:

The study population in this retrospective research included archival files of Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Tehran University of Medical Sciences, from 1999 to 2006.

The simple sampling method was used to select the samples of oral lichen planus from the archive.

According to clinical evidence, the lichen planus lesions were classified into two groups of reticular and erosive. Histopathological specimens stained with hematoxylin-eosin method were observed and samples without tissue artifact, proper fixation, adequate tissue volume, and well-defined junction of epithelium and connective tissue were isolated from the other cases. A total of 18 reticular and 17 erosive lichen planus samples were included in the study. Two 4-micron sections were prepared from each block for staining with toluidine blue. Cells were counted in 10 visual fields with an optical microscope (Olympus, Japan) at 400-fold magnification. Mast cells were counted in 3 layers of basal, intraepithelial, and superficial connective tissue (connective tissue adjacent to the epithelium). The mean number of cells was calculated for each layer, and 3 means were obtained for reticular and erosive lichen planus at three different tissues (epithelial, basal, and superficial connective layers). Statistical analysis was performed through Mann-Whitney and Friedman tests using SPSS 11.5 with regard to the first type of error as 0.05.

Results:

In microscopic examination of the samples, reticular form had a thick layer of hyperkeratosis with acanthosis and short and sharp serrated ridges. Epithelium was thin in the erosive type and no serrated ridges were seen in most layers. Accumulation of lymphocytes in the superficial connective layer was found in the form of strip (band like). After staining with toluidine blue, purple-colored mast cells were seen as almost elongated and oval-shaped cells with abundant granules often in the superficial connective layer and with less amount amidst basement membrane and in epithelium adjacent to basement membrane (Figure 1).

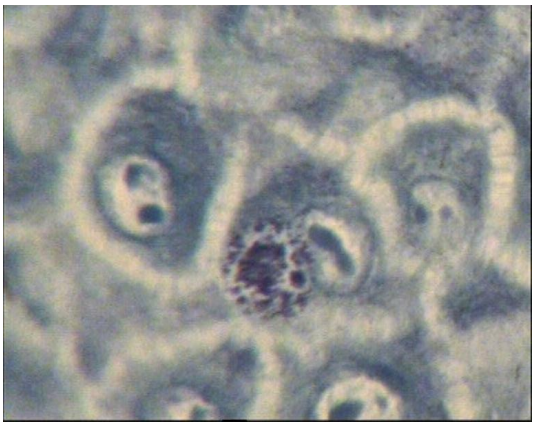

Figure 1. A Mast Cell among Epithelial Cells (toluidine blue staining, 400-fold magnification)

The mean number of mast cells in terms of clinical forms of oral lichen planus and tissue layers is shown in Tables 1 and 2, respectively.

Table 1. The Number of Mast Cells Based on Lichen Planus Type

Lichen planus	Mean number of mast cells	Coefficient of variation
Reticular (n=18)	1.85 ± 2.9	157
Erosive (n=17)	2.02 ± 3.18	157

Out of the three tissue layers, the highest number of cells (4.89±3.1) was seen in the connective layer compared with the basal and epithelium layers, regardless of the clinical type (P=0.001).

The number of mast cells in the connective tissue in the erosive type was 1.1 to 3.1 times of the reticular type, but this difference was not statistically significant (P=0.4).

The number of mast cells in the connective layer was averagely 26 times and 11.6 times higher than the epithelial and the basal layers, respectively (P=0.001).

There was a significant difference in the number of mast cells between the connective layer and the epithelial and the basal layers (P=0.001), but the difference between epithelial and basal layers in terms of the number of mast cells was not statistically significant.

Table 2. Number of Mast Cells Based on Tissue Layer

Layer	Lichen planus	Mean number of mast cells	Coefficient of variation
Epithelial	Reticular	0.23 ± 0.31	130
	Erosive	0.21 ± 0.25	119
Basal	Reticular	0.34 ± 0.45	136
	Erosive	0.23 ± 0.38	165
Connective tissue	Reticular	4.98 ± 3.28	66
	Erosive	5.53 ± 3.31	60

Conclusion:

The findings of this study showed that in both reticular and erosive types of oral lichen planus, the connective layer had the highest number of mast cells. Pairwise comparison of the layers showed a significant difference between epithelial and connective tissue layers.

The significant role of mast cells in the pathogenesis of oral lichen planus has been demonstrated in many studies (9-12). Tuominen, Walsh, Zhao, and Theoharides have mentioned that mast cells are an important cellular factor in inflammatory pathological conditions such as oral lichen planus (8,10,13,14). Ankle compared the number of mast cells in patients with oral lichen planus, leukoplakia, squamous cell carcinoma, submucosal fibrosis, and normal oral tissue. The largest number of mast cells was seen in this study in oral lichen planus (59.75 per mm^2) and the lowest in normal oral tissue (25.5 per mm^2) (15). In a study by Mosharraf and Jahanbani (1996), the number of mast cells had a 20-fold increase in oral lichen planus compared with oral normal tissue (16). Jontell (1986) and Zhao (1997) also reported results similar to the mentioned study (9,17). Although mast cells around epithelial basement membrane in oral lichen planus in the Zhao's study (1998) had no significant difference with normal tissue, the distribution was higher in basement membrane of blood vessels compared with normal tissue (8). Jose (2001) compared mast cells in reticular lichen planus and lichenoid reaction with

those of normal tissue, and reported a significant increase in both pathological conditions than normal mucosa (12).

The role of mast cells in lichen planus and oral lichenoid reaction has also been investigated. Juneja et al. (2006) studied 20 cases of lichen planus and lichenoid reaction lesions and showed that the number of degranulated mast cells in the degeneration area of basement membrane is more in lichen planus than lichenoid reaction (18).

In a study by Jahanshahi and Amin Zadeh in 2010, mast cells count was used to differentiate oral lichen planus and lichenoid reactions. The statistical difference in the number of degranulated mast cells in this study was significant between these two lesions. They concluded that the ratio of degranulated mast cells to the total number of these cells in the reticular lamina propria can serve as a good standard for differentiation of oral lichen planus from oral lichenoid reactions (19).

In line with other reports, these two reports demonstrate the importance of mast cell count in lichen planus. Difference in the number of these cells in the injuries is also consistent with the findings of this study.

Based on our review, the present study is the first research to compare the number of mast cells in the clinical forms of lichen planus at epithelial, basal, and connective tissue layers.

In this study, a significant difference was observed in the number of mast cells between connective tissue with epithelial and basal layers in reticular and erosive lichen planus. The number of mast cells was increased in the basal than epithelial layer.

These results suggest that the more the distance from the connective layer (the source), the lower the number of mast cells.

Zhang et al. (2002) found that the number of mature and immature mast cells is different in various types of lichen planus such as papular, erosive, and reticular. A difference also existed in the size of these cells (20).

The findings of this research are consistent with the results of the present study. In both studies, the pattern of mast cells presence was different in various patterns of lichen planus. These two studies differed in the dyes used; toluidine blue in this study and allicin blue/Safranin in Zhang's study.

In this study, it was observed that the density of mast cells is higher in the erosive form than the reticular type. In addition, these cells were significantly increased in the connective layer of the erosive type compared with the connective tissue in the reticular type. This can be proportional to the intensity of connective tissue excitation against epithelium or can be considered as a reason for activation of all destructive mechanisms of mast cells in this type of lichen planus. This finding corresponds with the results of the study by Barnett in 1975. He reported that the number of mast cells is higher in areas where keratinocytes are seriously disrupted (7).

It seems that increased mast cells in erosive type can justify the tendency of this type toward malignancy. In other words, increased mast cells in connective tissue of erosive form may result in epithelial dysplasia and atrophy in this specific clinical type, because mast cells cytokine influences the cells life, growth, and proliferation, and along with other inflammatory cells, result in the onset and progression of tumor invasion especially in squamous cell carcinoma (21).

On the other hand, epithelial dysplasia in erosive lichen planus may increase the number of mast cells, because these cells increase during development of squamous cell carcinoma, which can be a reason that mast cells can be considered a direct inhibitor of cell proliferation in squamous cell carcinoma through manipulating the process of apoptosis and controlling cell cycle (22).

In reticular and erosive lichen planus, the average number of mast cells is higher in connective tissue than epithelium. Mast cells count is higher in erosive lichen planus than the reticular type, regardless of tissue layer. Although a difference exists in the mean number of mast cells in different layers, it is not significant and cannot be considered as a specific pattern for reticular and erosive types.

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