Original Article

Degranulated mast cells and TNF-α in oral lichen planus and oral lichenoid reactions diseases

Parichehr Ghalayani, Gholamreza Jahanshahi[1], Zahra Saberi[2]

Department of Oral and Maxillofacial Medicine and Torabinejad Dental Research Center, [1]Department of Oral Pathology, [2]Department of Oral and Maxillofacial Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: The objective of this study was to assess mast cells and TNF-α in oral lichen planus (OLP) and oral lichenoid reactions (OLR) patients as diagnostic marker to the differential diagnosis of OLP and OLR diseases.

Materials and Methods: In this cross-sectional study, samples were obtained from 30 OLP and 30 OLR patients, between June 2010 and March 2011 in Dental clinic of the University of Isfahan, Iran. Mast cells in the reticular layer of the lamina propria for samples were evaluated using toluidine blue method and immunohistochemical technique. The clinical relevant data taken into account were: demographical data, total number and degranulated mast cells, ratio of degranulated mast cells and TNF-α positive degranulated mast cells.

Results: In OLP and OLR, the total mast cells were 21.2 ± 7.9 and 20.3 ± 6.8, degranulated mast cells were 15.5 ± 6.9 and 19.2 ± 6.9, ratio of degranulated mast cells to total mast cells were 0.716 ± 0.067 and 0.946 ± 0.081, and TNF-α positive degranulated mast cells were 13.6 ± 6.3 and 17.1 ± 6.04, respectively. There was no significant difference for the total mast cells. But degranulated mast cells, ratio of degranulated mast cells and TNF-α positive degranulated mast cells in OLR were significantly higher than OLP patients.

Conclusions: Our results showed that the degranulated mast cells, ratio of degranulated mast cells and TNF-α in OLR was significantly more than OLP patients and these may be able to be used as diagnostic markers to the differential diagnosis of OLP and OLR.

Key Words: Lichen planus, lichenoid reactions, mast cell, TNF-α

Address for correspondence:
Dr. Zahra Saberi, Department of Oral Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: saberi_777@yahoo.com
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INTRODUCTION

Lichenoid reactions represent a family of lesions with different etiologies with a common clinical and histological appearance. These reactions include lichen planus, lichenoid contact reactions, lichenoid drug eruptions, and lichenoid reactions of graft-versus–host disease (GVHD). Oral lichen planus (OLP) is a chronic inflammatory oral disease of unknown etiology. But during recent years, some

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factors related to development of this disease have been known. The important role of cell-mediated immune system has become more evident in lichen planus.[1,2]

T cell–mediated immune system has an important role in development processing of the lesion. One of the histological appearances in OLP is epithelial-based cell destruction and a subepithelial band like infiltrate of T lymphocytes. Tumor necrosis factor-α (TNF-α) is a cytokine involved primarily in T- cell mediated immunopathological reactions.[2] Mast cells are multifunctional secretory immune cells which participate in the regulation of immune responses by the release of chemical mediators. These cells are present in mucosa and connective tissue areas.[3]

In oral mucosa and skin, mast cells are distributed around the microvascular bed, near the basement membranes of blood vascular endothelial cells, and can move among tissues as a group of migratory cells.[4,5] The significance of the distribution of mast cells in tissue compartment relates to the potential for mast cell derived mediators to influence nearby cells, with resulting stimulatory, inhibitory, or toxic effects.[6]

Mast cell mediators include histamine, chymase, tryptase, and TNF-α. These mediators are deposited in extracellar environment following degranulation, where they affect on endothelial cells.[7,8] TNF-α can induce endothelial expression of E-selectin CD62E (ELAM-1), an adhesion molecule, that is necessary for rapid adhesion of neutrophils, T hymphocytes, and monocytes to endothelial cells.[9-11] E-selectin is expressed in variety inflammatory lesions of oral mucosa including lichen planus,[12] gingivitis,[11,13] periodontitis,[14] acute pulpitis,[15] and periapical inflammation.[16]

In previous studies, the important role of TNF-α has been proven in incidence of OLP lesions and regarding to this issue that TNF-α in this lesions primarily release from degranulated mast cells.[3,9,17-24] And because of this fact that differentiation between lichen planus and lichenoid eruptions of oral mucosa are problematic for oral and maxillofacial specialists and pathologists, it is visible to compare mast cells count and their inflammatory mediators in diagnosis of these two lesions.

The present study aimed at comparing the frequency of degranulated mast cells in patients with reticular form oral lichen planus or oral lichenoid reactions (OLR) and its relation to TNF-α with immunohistochemical method and toluidine blue staining.

MATERIALS AND METHODS

In this descriptive analytical cross-sectional study, 60 patients (30 OLP patients and 30 OLR patients) who referred to Dental Clinic of the University of Isfahan, Iran, between Jun 2010 and March 2011, were enrolled. Patients of any age and gender with clinically and histopathologically confirmed diagnosis of OLP and OLR were eligible if they had no history of smoking, diabetes, hepatitis, and any systemic or infection diseases. OLP patients with history of any systemic or topical medication for their oral disease two months prior to study were not eligible for the trial. Written informed consent was obtained from the participants and this study was approved by the Ethics Committee of the Isfahan University School of Dental Medicine. The definitive clinical and histopathological criteria used to distinguish and categorize the lesions were based on the WHO criteria.[25,26] The differential diagnosis between lichen planus and lichenoid reaction was determined by a combination of clinical and histological criteria. Cases of lichen planus must be had all of the clinical and histological criteria. Whereas, lichenoid reaction includes: patients with typical lichen planus clinically but not histologically, patients with typical lichen planus histologically but not clinically, and patients who are both clinically and histologically only compatible with lichen planus.

Biopsies were taken from reticular lesions on buccal mucosa [Figure 1]. Specimens were rapidly placed in a 10% formalin-buffered solution to avoid autolysis and subsequent mast cell degranulation. A minimum of 24 hours, specimens were processed and embedded in paraffin using standard procedures. Then, from each block, a section was stained with hematoxylin and eosin (H&E) to allow histopathology examination.

For toluidine blue staining for evaluation of mast cells, sections (3 to 5 μ thickness) from paraffin blocks were prepared and stained with toluidine blue (Merck, 15930). For immunohistochemical staining for evaluation of TNF-α in degranulated mast cells we

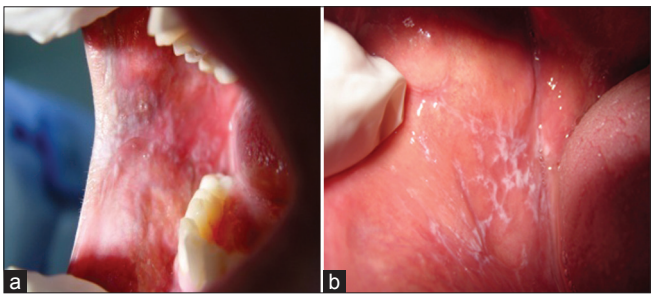

Figure 1: Oral lichen reticular lesions. (a); Oral lichenoid reaction, reticular lesions of buccal mucosa in patient with systemic medication. (b); Oral lichen planus, reticular lesions on buccal mucosa

used B 154.2 antibody (Santa Cruz company, United states, E-7011).

Mast cells were categorized into two groups, according to intensity of metachromasia or stainability and/or granule extrusion:

1. Degranulated: less intense metachromasia or stainability and obvious clear outline of the nucleus and/or free granules in close proximity to the cell membrane.
2. Intact: dense metachromasia or stainability in which the nucleus was not apparent and/or no granule extrusion around the cell was present.

The pathologist was blinded to the assigned groups of stained sections and used light microscopy to evaluate mast cells in five sequential high-power fields of the reticular zone of the lamina propria. Mast cells were evaluated in term of total number, the number of degranulated cells, the ratio of degranulated mast cells to the total mast cells population, and TNF-α positive degranulating mast cells.

The data were analyzed using SPSS-20 and are presented as means ± 1SD or number (%) based on the variables. Variables were comparing between study groups by Independent-Samples T-test and Chi-square test. We considered a *P* value of <0.05 to be statistically significant.

RESULTS

All 60 subjects enrolled in this study were included in the analysis. TNF-α positive degranulated mast cells in the reticular layer of the lamina propria lesion of patients with OLP and OLR detected using IHC and toluidine blue method (Figure 2, magnification 40 × 10).

The mean age of the subjects was 42.13 ± 9.79, 27 subjects (45%) were male and 33 subjects (55%) were female. Table 1 showed characteristics and clinical findings in studied groups. Age (41.6 ± 9.12 versus 43.71 ± 9.17 in OLP and OLR patients, respectively, *P* value = 0.38) and sex (19 (73.4%) female, versus 14 (46.7%) female in OLP and OLR patients, respectively, *P* value = 0.23) were not statistically significant among groups. The total number of mast cells in OLP was more than in OLR [Figure 3a]. There were no significant differences in OLP and OLR patients in the total number mast cells (*P* value > 0.05), the number of degranulated mast cells in OLR was more than in OLP [Figure 3b], the ratio of degranulated to the total mast cells in OLR was more than in OLP [Figure 3c] and TNF-α positive degranulated mast cells in OLR was more than OLP patients [Figure 3d]. Increased in the number of degranulated mast cells, the ratio of degranulated mast cells, and TNF-α in OLR patients were statistically significant compared with OLP patients (*P* value < 0.05).

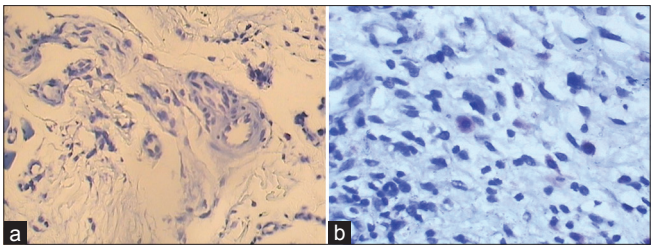

Figure 2: TNF-α positive degranulated mast cells in the reticular layer of the lamina propria in OLP and OLR. (a); IHC (original magnification 40 × 10). (b); toluidine blue staining (original magnification 40 × 10)

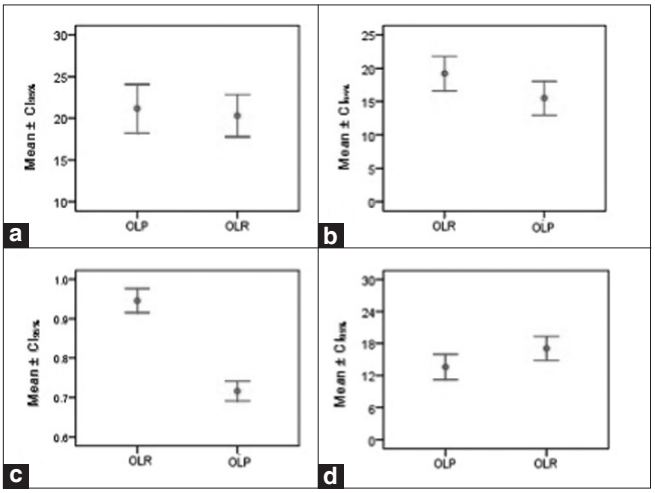

Figure 3: Comprison of variabls between study groups.data are mean with $CI_{95\%}$, (a); the total number mast cells in OLP and OLR (*P* value = 0.54), (b); the number of degranulated mast cells in OLP and OLR (P value = 0.041), (d); the ratio of degranulated to the total mastcell in OLP and OLR (*P* value < 0.0001), (d); TNF-α positive degranulated mast cells in OLP and OLR patients (*P* value = 0.035)

Table 1: Characteristics, mast cells, and TNF-α in study population

	Lichen planus (*n* = 30)	Lichenoid reaction (*n* = 30)	*P* value
Age (year)	41.6 ± 9.12	43.71 ± 9.17	0.38*
Sex			
Male	11 (26.6)	16 (53.3)	0.23†
Female	19 (73.4)	14 (46.7)	
Total mast cells	21.2 ± 7.9	20.3 ± 6.8	0.65*
Degranulated mast cells	15.5 ± 6.9	19.2 ± 6.9	0.041*
Ratio of degranulated to total mast cells	0.716 ± 0.067	0.946 ± 0.081	<0.0001*
TNF-α	13.6 ± 6.3	17.1 ± 6.04	0.035*

Data presented as mean ± 1SD and number (Percent). *P* values calculated with *independent sample *t*-test and †Chi Square test

DISCUSSION

OLR which are considered variants of OLP may be regarded as a disease by itself or as an exacerbation of an existing OLP, by the presence of medication or dental materials. Since that OLP should be more carefully followed because of the possibility of malignant transformation, the definitive diagnosis should be recognized as early as possible. The diagnosis of OLP is difficult and the pathognomonic features of OLR are yet to be identified.[27] Mast cells are well known as effector cells of IgE-mediated allergic reactions. Innate immunity and the induction and regulation of adaptive immune responses have been reported as the important functions of mast cells in different diseases; whereas, their role in pathogenesis of the dermatological diseases is not completely understood.[28,29] Increase in the number of mast cells with degranulation in contact dermatitis was observed.[24] In the present study, the frequency of degranulated mast cells and its relation to TNF-α in patients with OLP or OLR were assessed and our findings showed that the total mast cell in patients with OLP was higher than patients with OLR, but these differences were not statistically significant. Also, we have observed a significant increase when compared with OLP patients, in the number of degranulated mast cells, the ratio of degranulated mast cells to the total mast cell population and the total TNF-α positive degranulating mast cells count in OLR patients.

Previous study reported greater number of mast cells in OLP and OLR patients compared to healthy subjects. Sharma *et al*.,[30] Zhao *et al*.,[9] and Juneja *et al*.,[31] in studied of human buccal mucosa, compared total number of mast cells in healthy subjects, OLP and OLR patients. They showed significant increase in the total number of mast cells count in OLP and OLR patients contrast to healthy subjects. Also, Jahanshahi *et al*.[32] Zhao *et al*.,[18] and Juneja *et al*.,[31] in concordance with present study reported that the total number of mast cells between OLP and OLR patients were similar. Degranulated mast cells stained with toluidine blue were significantly different between OLP and OLR lesions based on Jahanshahi *et al*.[32] and Juneja *et al*.,[31] studies. They reported that degranulated mast cells in OLR lesions are higher than OLP lesions. This finding is, similar to our result which shows that degranulated mast cells count in OLR lesions are significantly higher than OLP lesions.

Because of naturally differences in different locations of oral mucosa and among individuals, in the number of mast cells, a ratio of degranulated to total number of mast cells is suggested to be used as a histopathological marker rather than number of degranulated mast cells for differentiating between OLP and OLR. We find that ratio of degranulated to total mast cells between study groups was statistically significant, whereas this value is higher in OLR in compare to OLP patients. Similar to our results, Jahanshahi *et al*.[32] study reported that ratio of degranulated to total mast cells significantly higher in OLR in compare to OLP patients. Zhao *et al*.[18] showed that in OLP approximately 60 % of mast cells were degranulated, in the present study approximately 71.6 % of mast cells were degranulated in OLP.

TNF-α is a cytokine that plays the key role in host defense, including immunoregulatory responses implicated in the pathogenesis of many autoimmune and inflammatory diseases[19] such as OLP lesions. TNF-α in this lesions primarily release from degranulated mast cells. The differentiation between lichen planus and lichenoid eruptions of oral mucosa are problematic and it is noticeable to compare mast cells count and their inflammatory mediators in diagnosis of these two lesions. Previous studies focused on detecting TNF-α from degranulated mast cells in OLP compare with healthy subjects. Sklavounou *et al*.[21] reported a TNF-α throughout the OLP epithelium in 19 of 22 cases, in contrast to all controls in which TNF-α was undetectable. Also others showed significant increase in TNF-α in OLP compare to healthy subjects. To the best of our knowledge this is the first study to compare TNF-α from degranulated mast cells in OLP and OLR and showed significant higher number of TNF-α in OLR compared with OLP patients. However, further studies at biochemical and molecular levels should aid understanding of the exact role of TNF-α in OLP and OLR to clarify whether this cytokine can be detect as a marker in differentiation between OLP and OLR.

In conclusion, our results showed that the total number mast cell was similar in both OLP and OLR patients. But the number of degranulated mast cells, the ratio of degranulated to the total mast cells and TNF-α positive degranulated mast cells in OLR were significantly higher than OLP patients and it may be able to be used as diagnostic markers to the differential diagnosis of OLP and OLR. However, further studies are necessary.

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