Evaluation of mast cell count in oral and cutaneous lichen planus lesions

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Patients and Methods: The present descriptive-analytical and cross-sectional study was conducted on 125 LP blocks, including 40 OLP samples and 85 CLP samples. The mean number of granulated, degranulated and total MCs were counted under light microscope (×100 and ×400). Data were analyzed by SPSS version 24 software using Mann-Whitney U and Pearson’s chi-square tests.

Results: The mean total MC count was 14.92 ± 3.90 in OLP samples and 14.04 ± 5.60 in CLP samples respectively which was not statistically significant. The mean granulated MC count in CLP (10.57 ± 2.72) was higher than in OLP (4.34 ± 3.46) (P<0.001). The mean degranulated MC count in OLP (10.57 ± 2.99) was higher than in CLP (3.51 ± 2.72) (P<0.001).

Conclusion: No statistically significant difference was observed in the total MC count between OLP and CLP however the difference in the degranulated MC count was detected between OLP and CLP. Despite the homogeneity in the total MC count, the MCs appear to play different pathological roles in OLP and CLP.

Abstract

Introduction: Lichen planus (LP) is a relatively common chronic mucocutaneous disorder diagnosed based on clinical manifestations and histopathological findings. Mast cells (MCs) are multifunctional immune cells that are involved in the pathogenesis of LP by releasing certain chemical mediators.

Objectives: The purpose of this comparative study was to estimate the number of intact (granulated), degranulated and total MCs in oral lichen planus (OLP) and cutaneous lichen planus (CLP) lesions.

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Conclusion: No statistically significant difference was observed in the total MC count between OLP and CLP; however the difference in the degranulated MC count was detected between OLP and CLP. Despite the homogeneity in the total MC count, the MCs appear to play different pathological roles in OLP and CLP.

Key point

In a study on 125 Lichen planus blocks, including 40 OLP samples and 85 CLP samples, we found that the mean degranulated mast cells count in OLP was higher in CLP.

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Histopathological features of the disease confirm the main role of the immune system in the development of LP. Destruction of the basal cell layer of the epithelium (hydropic degeneration) is also evident. This is accompanied by an intense, band-like infiltrate of predominantly T lymphocytes immediately subjacent to the epithelium. This phenomenon is interpreted as a manifestation of the role of the cell-mediated immune system in the pathogenesis of LP (6).

The unknown disease-causing antigenic agent suggests autoimmune pathogenesis. On the other hand, the association of LP with autoimmune diseases, similar manifestations in graft-versus-host disease (GVHD) and loss of tolerance to autoantigen confirm the autoimmune nature of the disease (7). Self-reactive T cells are unable to distinguish between self and non-self antigens and may be important in the development of LP. However, it seems that a single peptide cannot stimulate the immune response, but several peptides do so based on the specificity of the stimulated T lymphocyte. Hence, it is very difficult to introduce a single etiologic factor for LP (8).

Mast cells (MCs) are derived from bone marrow precursors and are widely distributed in tissues. The MCs are located predominantly around arteries, nerves and sub-epithelial sites. The activated cells release a variety of mediators that act on blood vessels, smooth muscle and leukocytes (9). The MCs play an important role in innate immune responses and tissue repair by localized release of many bioactive substances. Some other roles of MCs include participating in antigen-antibody complex formation, autoimmune reactions, and T cell-dependent immune system responses. In other words, these cells can exert both positive and negative effects in the regulation of immune responses (10, 11).

Objectives
So far, studies have been conducted on the presence of inflammatory cells in LP; investigations have also compared the MCs in OLP and oral lichenoid reaction (OLR) lesions. According to the literature review, no comparison has been made so far regarding the MC count between OLP and CLP lesions. Therefore, the aim of this study was to compare the MC frequency, quantitative (total MC count) and qualitative [granulated (intact) and degranulated MC count] between OLP and CLP. The purpose of this comparative study was to estimate the number of intact (granulated), degranulated and total MCs in OLP and CLP lesions.

Patients and Methods
Study design
The present descriptive-analytical and cross-sectional study was conducted on paraffin-embedded tissue blocks of OLP and CLP lesions. The diagnosis of LP was recorded for these samples according to clinical manifestations and histopathological findings. Four-micron cross-sections were prepared from the blocks for hematoxylin-eosin (H&E) staining to confirm the diagnosis and enter the study. After observing H&E-stained slides and confirming the diagnosis of paraffinized blocks, slides with sufficient connective tissue thickness and appropriate epithelial cell diameters were selected and blocks that did not meet these criteria were excluded.

Finally, 125 samples (including 85 CLP samples and 40 OLP samples) were enrolled in the study.

In the next step, 4-µ cross-sections were prepared from paraffinized blocks and stained with toluidine blue (12-14). After staining, 5 densely packed cell regions of the reticular lamina propria were selected under a light microscope (Nikon, Eclipse E200, Tokyo, Japan) at magnifications of ×100 and ×400. The mean numbers of granulated, degranulated and total MCs were counted in these regions.

Microscopic observation and MC count were examined by two separate pathologists (dermatopathologist and oral pathologist), and the mean value would be considered if different results were obtained between the two.

Data analysis
Data were described as percentage, mean and standard deviation. The normal distribution of MC count variable was investigated by Shapiro-Wilk test. Mann-Whitney U test was performed to compare the MC count between the two groups. Gender distribution and age group were compared between the two groups by Pearson’s chi-square test. Additionally, the Kruskal-Wallis test was applied to find the difference in the mean number of granulated, degranulated and total MCs based on age group. Spearman’s correlation coefficient was also applied. Data were analyzed using SPSS version 24 software at a significance level of less than 0.05.

Results
This study evaluated 125 paraffin-embedded blocks collected from 24 men (19.2%) and 101 women (80.8%), including 40 OLP samples (32%) and 85 CLP samples (68%). The mean age of research units was 43.02 ± 13.55 years.

Table 1 shows the frequency distribution of OLP and CLP by gender and age groups, while Pearson’s chi-square test was applied to compare the two groups. As shown in this table, more cases of CLP occurred in the age group under 43 years and more cases of OLP were above 42 years of age, which is significantly different (P = 0.002).

The frequency of LP in women was higher than in men. OLP and CLP cases were found in 82.5% and 80% of female patients, respectively; since no statistically significant difference was observed between the two groups (P = 0.741).

Table 2 shows the mean granulated and degranulated MC counts in the study regions by LP type. According to the Mann-Whitney U test, no statistical difference...
was observed in total MC count between OLP and CLP. However, the differences in the mean of granulated and degranulated MC counts were statistically significant between OLP and CLP ($P < 0.001$) and the mean granulated MC count was higher in CLP, while the mean degranulated MC count was greater in OLP.

**Table 3** presents the mean number of granulated, degranulated and total MCs based on gender distribution. According to this table and using the Mann-Whitney U test, the mean number of granulated ($P = 0.256$), degranulated ($P = 0.385$) and total ($P = 0.064$) MCs was not statistically significant between men and women.

**Table 4** shows the mean number of granulated, degranulated and total MCs based on age group. According to the Kruskal-Wallis test, the difference in the mean number of granulated, degranulated and total MCs based on age group is not significant between CLP and OLP.

**Table 5** shows the correlation between age and number of MCs as well as between granulated and degranulated MCs based on CLP and OLP using Spearman’s correlation coefficient. The results indicated no correlation between total MC count as well as granulated and degranulated MCs and age in CLP and also in OLP.

**Discussion**

LP is a chronic mucocutaneous disease with an unknown cause that is diagnosed according to its clinical manifestations and histopathological findings (5).

In the present study, 80% of CLP cases and 82.5% of OLP cases were observed in women. The mean age of patients was 43.02 years while the mean age was below 43 years in OLP. Various studies compared MC counts in OLP and OLR, which compared in this study. We found that the mean MC count in OLP was 14.92 ± 3.9, in line with the results of the study by Ramalingam et al (14.98 ± 5.16). In their study, the mean MC count in OLR and in normal mucosa was less than this value, 7.96 and 2.02, respectively (13).

Reddy et al (12) observed an increase in MC count in LP and OLR compared to normal mucosa. Consistent results reported in the studies of Sharma et al, Zhao et al and Juneja et al emphasize the important role of MCs in the pathogenesis of these lesions (24-26). In the study of Reddy et al (12), the mean numbers of total MCs and CLP was detected, however the differences in the mean number of granulated and degranulated MCs were statistically significant between OLP and CLP. Additionally, the mean granulated MC count was higher in CLP and the mean degranulated MC count was greater in OLP. The role of MCs has been demonstrated in the pathology of OLP and CLP lesions (22, 23). Mucosal MCs are linked to nerve fibers and vessels, and chronic stimulation of these nerves and secretion of neuropeptides induces MC degranulation. The MC degranulation is induced by IgE receptor or interaction with a variety of agonists that act directly on the cell surface. Both T cell-derived cytokines and MC-T cell adhesion may cause MC activation and degranulation (4).

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degranulated MCs in OLP were lower than our study, 12.56 ± 3.30 and 6.69 ± 2.97, respectively, nevertheless the mean granulated MC count (5.86 ± 3.02) was higher than our study. In this study, the density of MCs and degranulated MCs in LP was higher than in control group. Moreover, the basement membrane thickness was lower in LP and a clear fragmentation was observed in the basement membrane of LP compared to IgM.

The total MC count in the studies of Zhao et al (25), Juneja et al (26) and Balci & Ercin (28) was not significantly different between OLP and OLR lesions. Furthermore in the studies of Jose et al (29) and Reddy et al (12), the number of degranulated MCs stained with toluidine blue in OLR was significantly higher than in OLP. The studies suggested that these findings may be considered as a diagnostic marker in the differential diagnosis of OLP and OLR. In contrast, Juneja et al (26) observed that the mean total MC count and the mean degranulated MC count in OLR were higher than in OLP. Analysing the results of the present study and increasing the degranulated MC count raised the following questions. Can a different role be imagined for MCs in OLP? Does the underlying difference between mucus and skin cause this change in function? Is an increase in degranulated MC count in OLP related to an increase in malignancy compared to CLP? Can it be concluded that MC stabilizers may show better performance in the mucosa? Further research in this area may answer these questions, and it is also suggested to conduct studies with larger sample size.

**Conclusion**

There was no statistically significant difference in the total MC count between OLP and CLP, however the difference in the degranulated MC count was significant between OLP and CLP. Despite the homogeneity in the total MC count, the MCs appear to play different pathological roles in OLP and CLP.

**Limitations of the study**

The limitation of the study was small size of oral samples compared with cutaneous blocks.

**Authors’ contribution**

MZ, MKD, FM and AS were the principal investigators of the study. MZ, MKD, FM, AS and HA were included in preparing the concept.
and design. MM managed the analyses of the study, MZ and MBA revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest
The authors declare that they have no competing interests.

Ethical issues
The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Mazandaran University of Medical Sciences approved this study (IR.MAZUMS.REC.1398.482). Informed consent was obtained from participants. This study was extracted from DDs thesis of Hadi Alidoust at this university (Thesis#2062). Moreover, Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References