Evaluation of Mast Cell Density in Oral Lichen Planus

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ABSTRACT

Introduction : Mast cell (MC) density shows considerable variation in oral inflammatory lesions, precancerous lesions, and oral cancer, highlighting the importance of studying MCs to identify in-depth pathogenesis and the treatment aspect of Oral Lichen Planus (OLP).

Objective : The purpose of this study is to evaluate the density of mast cells in Oral Lichen Planus and Inflamed Gingival Mucosa (IGM) and to compare the density between the lesions.

Methodology : A total of 60 cases (30 OLP and 30 IGM) were processed and stained with toluidine blue. MCs were counted using a microscope fitted with an oculometer grid. The intact and degranulated MCs were identified separately. MCs were then counted at two different levels, described as Zone I and Zone II. Zone I, representing the sub-epithelial region within the inflammatory cell infiltrate and Zone II, corresponding to the deeper connective tissue beneath the infiltrate. MC counts were expressed per μm^2 for MC density. The mean values were calculated and expressed as mean and standard deviation. The statistical analysis was done using Statistical Package for Social Sciences version 21.

Result : A statistically significant increase in mast cell density was noted in oral lichen planus compared to controls (IGM). In addition, both intact MCs and degranulated MCs in OLP were also increased compared to controls and were statistically significant. We also observed a significant increase in MC density in Zone I than in Zone II; degranulated being more in both groups. However, it was observed that the mean MC density was not statistically significant among the clinical types of OLP.

Conclusion : The present histochemical study demonstrated a signific antly higher MC density in oral lichen planus compared to inflamed gingival mucosa. Degranulated MCs were found in significantly higher numbers than intact MCs in oral lichen planus as well as in inflamed gingival mucosa.

Keywords : Inflammation; lichen planus; mast cells; stain.

INTRODUCTION

Oral lichen planus (OLP) is a chronic autoimmune mucocutaneous disorder of stratified squamous epithelium and underlying lamina propria that affects oral mucous membranes along with skin,

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genitals, nails, and scalp.[1] A study conducted in a tertiary care dental hospital reported a prevalence of OLP of 7.2%.[2]

According to Li et al.[3] in 2020, the global prevalence of OLP is 0.89% in the general population and 0.98% among clinical patients. OLP is a T-cell-mediated disease in which basal keratinocytes are targeted by lymphocytes, triggering the activation of cytokines and matrix metalloproteinases (MMP), leading to degranulation of mast cells (MCs).[1]

Although MCs are considered a minor component of the cellular infiltrate, they are believed to play a significant role in the pathogenesis of OLP.[4] MC detection is more accurate and clearly observed when using toluidine blue staining. Thus, toluidine blue provides a reliable, cost-effective and practical method for identifying MCs.[5] The present histochemical study employed toluidine blue staining to assess MC density, their different types, and variation in their distribution in OLP. These parameters were also compared with the inflamed gingival mucosa (IGM).

METHODOLOGY

An observational cross-sectional comparative study was conducted over 12 months at Kantipur Dental College and Hospital, Kathmandu, Nepal. The sample size was calculated using data from the study of Pereira et al.[6] using the formula,

$$n = \frac{2(Z\alpha+Z\beta)s^2}{d^2}$$

where, n = sample size required per group Zα= Z deviate corresponding to the α error rate (1.96 for α = 5%), Zβ= Z deviate corresponding to the β error rate (1.28 at 90% power), s= standard deviation of the differences of paired observation, d = difference to be detected (mean difference) = 26.72 (per group). The calculated value of 53.44 was increased by 10% to account for permissible error, yielding 58.78; therefore, a total sample size of 60 was selected to ensure equal distribution. The study protocol was approved by the Institutional Review Committee of the same institute [KDC-IRC (Ref. No. 13/019)], and informed consent was obtained from all the participants.

Convenience sampling was used to recruit individuals above 18 years of age attending the outpatient department with a definitive diagnosis of OLP and patients undergoing periodontal surgery. Individuals with a definitive diagnosis of OLP and those undergoing surgical periodontal therapy were included in the study. Only histological sections with adequate epithelium and connective tissue were selected, and MC counts were performed exclusively in the central portion of the grid field. Individuals with similar histological features, like Oral lichenoid reaction, or individuals with periapical abscess, pyogenic granuloma, soft tissue cysts, tumor, drug-related gingival enlargement, etc were excluded. Patients with smoking habit and medications were not included in the study. Additionally, defective slides with inadequate margins and slides with artefacts were also excluded.

The tissue specimens from clinically diagnosed OLP cases constituted the case group, while the inflamed gingival tissue excised during periodontal surgeries served as the control group. The specimens were then fixed with 10% formalin. The working solution of toluidine blue for staining was prepared based on previous work by Janardhanan and Ramesh.[4] The prepared tissue sections were stained with toluidine blue solution for 10 seconds, rinsed with running tap water, immersed in 100% alcohol for 2 minutes, transferred to a clearing solution, dried and mounted with Dibutylphthalate Polystyrene Xylene, DPX (Thermo Fisher Scientific), and covered by coverslips.

For Microscopic evaluation, MCs were counted using an optical microscope (Labomed Lx 400) equipped with an oculometer grid (MUHWA) with 0.5 µm net-shaped eyepiece ruler, the area of 5 x 5 μm^2 scale, each divided into 10 equal parts,

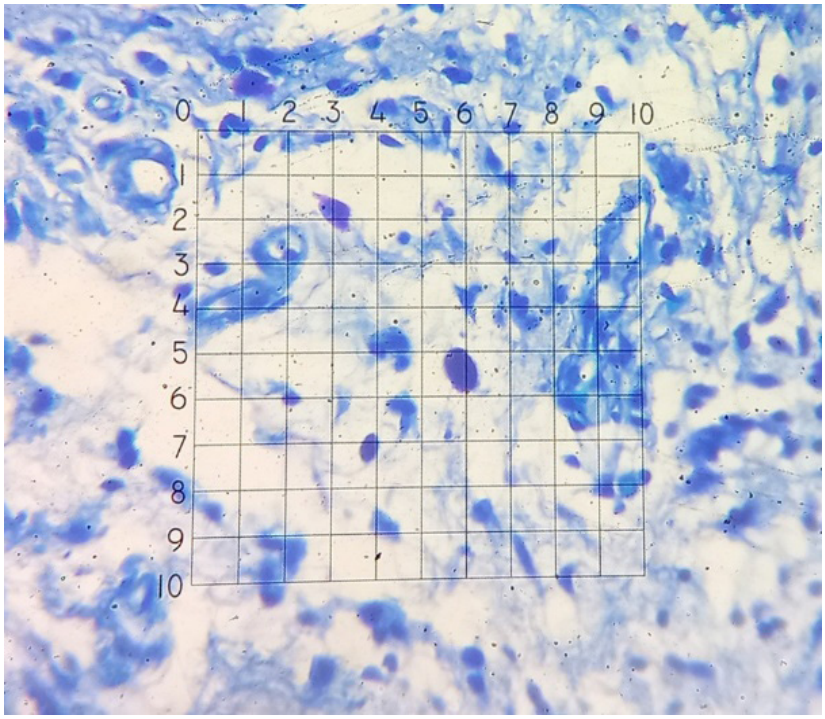

Figure 1: Grid field for counting of mast cells in toluidine blue stained section (x40)

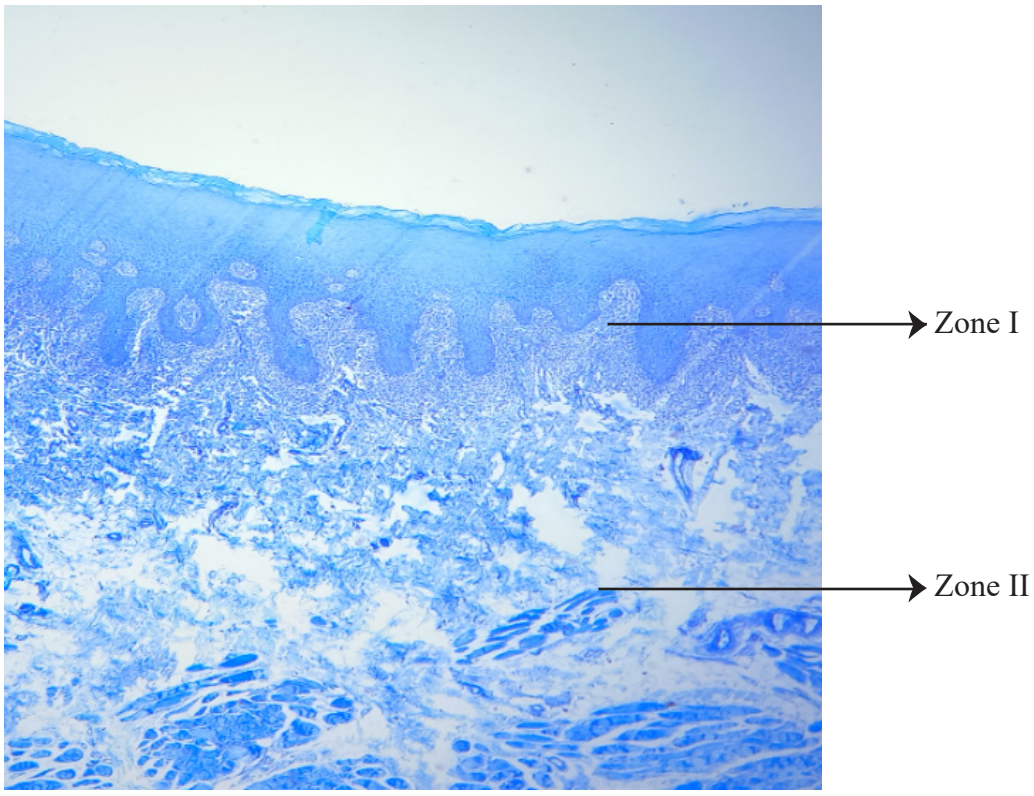

Figure 2: Different zones of toluidine blue stained section (x10)

each small square glass 0.25 μm^2, a diameter of 19 µm (Figure 1). The MCs were counted using 40X across six non-overlapping fields of each slide.[4] MCs were enumerated within two different zones: Zone I, representing the sub-epithelial region within the inflammatory cell infiltrate and Zone II, corresponding to the deeper connective tissue beneath the infiltrate.[7] (Figure 2) The intact

and degranulated MC were identified separately. MC density was expressed as counts per sq. µm. The mean values were calculated and expressed as mean ± standard deviation (SD)/µm.[2,8]

The data were entered, edited, and coded using Microsoft Excel and subsequently analyzed in Statistical Package for the Social Sciences (SPSS) version 21.0. The continuous variables- including age, intact MC density, degranulated MC density and total MC density were summarized using mean and standard deviation. The categorical variables, such as gender and clinical subtypes of OLP, were presented as frequencies and percentages. Mean differences between groups were assessed using the independent t-test, and comparisons among clinical subtypes and MC densities were evaluated using analysis of variance (ANOVA).

RESULT

A total of 60 patients were included, comprising 30 clinically and histopathologically confirmed cases of OLP and 30 cases of inflamed gingival mucosa (IGM) serving as controls. The toluidine blue-stained slides were evaluated for adequacy of staining at magnification (40x). The MCs, including intact and degranulated forms, were evaluated in two different zones, i.e., Zone I and Zone II, in three grid fields.

The age of OLP patients ranged from 23 to 68 years (mean 48.00 ±13.54). The control group had a mean age of 28.37±11.36 years. Among OLP cases, females predominated 20 (66.7%), while the control group exhibited an equal gender distribution. The atrophic subtype was the most frequent clinical variant of OLP 12 (40.0%), followed by the reticular 7 (23.3%), papular 5 (16.7%), bullous 4 (13.3%), and erosive 2 (6.7%) forms. (Table 1)

The mean total MC density was significantly higher in OLP (3.77 ± 0.88) compared with controls (1.31 ± 0.89). The mean number of intact MC was less than that of the degranulated ones (1.05±0.66 and 2.72 ± 0.76, respectively) in OLP. Also, the result showed a decreased density of intact MCs in the control group (0.33 ± 0.41 and 0.98 ± 0.66, respectively) compared to degranulated MCs. The degranulated MCs were higher in both the groups (2.72 ± 0.76 in OLP and 0.98 ± 0.66 in IGM). The mean density of intact, degranulated and total MC were greater in OLP than that of the control group (Table 2).

Across all grid fields, Zone I demonstrated higher MC densities than Zone II in both case and control groups. In Zone I, the mean total MC density was 5.20 ± 1.36 in OLP and 1.78 ± 1.06 in IGM; in Zone II, the corresponding values were 2.34 ± 0.92 and 0.84 ± 0.95. Also, intact and degranulated MC were found to be more in Zone I compared to Zone II in both groups. The mean density of intact MC of OLP in Zone I was 1.34 ± 0.92, whereas that of IGM was 0.42 ± 0.47. The degranulated MC density in Zone I was 3.86 ±1.23 in OLP and 1.36 ± 0.85 in IGM. In Zone II, the mean degranulated MC were found to be higher than intact MC in both groups. (Table 3, 4)

The mean total MC density was found to be greater in OLP than in control. The MC density showed a statistically significant increase in OLP compared to IGM ($p<0.001$). In addition, both intact MCs and degranulated MCs in OLP were also increased compared to controls (IGM). All of the findings were statistically significant ($p<0.001$). (Table 2) In Zone I, the mean total MC density, intact MC and degranulated MC were observed in 3 grid fields for both groups. All the parameters were found to be greater in OLP than in the IGM and were statistically significant ($p<0.001$). (Table 3) Similarly, the mean total MC density, intact and degranulated MC in OLP, was found to be greater than in the IGM in Zone II. All the findings were statistically significant ($p<0.001$). (Table 4)

One-way ANOVA demonstrated no significant differences in MC density among the different clinical subtypes of OLP, indicating that MC distribution did not vary by clinical presentation $p=0.363$.

Table 1: Frequency distribution of different subtypes of OLP

Clinical types of OLP	Frequency (n)	Percentage (%)
Reticular	7	23.3
Papular	5	16.7
Atrophic	12	40.0
Erosive	2	6.7
Bullous	4	13.3
Total	30	100.0

Table 2: Independent t-test comparing total, intact, and degranulated mast cells in OLP and IGM

		Mean ± SD	Std. Error Mean	t	95% CI of the Difference		Sig. (2-tailed)
					Lower	Upper	
Total	**OLP**	3.77±0.88	0.16	10.74	2.00	2.92	<0.001*
	IGM	1.31±0.89	0.16				
Intact	**OLP**	1.05±0.66	0.12	5.076	0.43	0.99	<0.001*
	IGM	0.33±0.41	0.08				
Degranulated	**OLP**	2.72±0.76	0.14	9.527	1.38	2.11	<0.001*
	IGM	0.98±0.66	0.12				

* Statistically significant (p<0.001)

S.D.= Standard Deviation; CI= Confidence Interval

Table 3: Independent t-test comparing mast cell total, intact and degranulated among OLP and IGM in Zone I

		Mean ±SD	Std. Error Mean	t	95% CI of the Difference		Sig. (2-tailed)
					Lower	Upper	
Total	**OLP**	5.20±1.36	0.25	10.86	2.79	4.05	<0.001*
	IGM	1.78±1.06	0.19				
Intact	**OLP**	1.34±.92	0.17	4.89	0.54	1.30	<0.001*
	IGM	.42±.47	0.09				
Degranulated	**OLP**	3.86±1.23	0.22	9.18	1.95	3.05	<0.001*
	IGM	1.36±.85	0.16				

* Statistically significant (p<0.001),

S.D.= Standard Deviation; CI= Confidence Interval

Table 4: Independent t-test comparing mast cell total, intact and degranulated among OLP and IGM in Zone II

		Mean± SD	Std. Error Mean	t	95% CI of the Difference		Sig. (2-tailed)
					Lower	Upper	
Total	**OLP**	2.34±0.92	0.17	6.23	1.02	1.98	<0.001*
	IGM	0.84±0.95	0.17				
Intact	**OLP**	0.76±0.66	0.12	3.60	0.23	0.8	<0.001*
	IGM	0.24±0.42	0.08				
Degranulated	**OLP**	1.59±0.81	0.15	5.12	0.60	1.38	<0.001*
	IGM	0.60±0.68	0.12				

* Statistically significant (p<0.001),
S.D.= Standard Deviation; CI= Confidence Interval

DISCUSSION

Mast cells are found in low numbers in normal oral mucosa; however, studies have shown increased MC count in inflamed gingival tissues.[6,9] Since the gingiva is rarely free from inflammation, IGM was selected as a control in this study, thereby avoiding the ethical concerns, using normal oral mucosa.

The age of onset for oral lichen planus typically ranges between 30 and 60 years, with the prevalence increasing significantly and progressively after the fourth decade.[10] This trend may be related to long-standing oral habits, age-related changes, and metabolic changes.[3] In our study, OLP patients ranged from 23 to 68 years (mean 48.00 ± 13.54), consistent with previous reports.[11,12] A female predominance (2:1) was observed, aligning with the earlier study on the Nepalese population.[13] The hormone levels, stress, and susceptibility to immune-mediated inflammatory diseases have been proposed as the contributing factors.[14]

Among clinical subtypes, the atrophic type was most prevalent 12 (40.0%), while the erosive type was least common. However, a study by Mollaoglu N showed reticular type as the predominant type, with the prevalence of 46.15% and bullous as the least common type of OLP, with 0.26%. The atrophic type can cause a burning sensation, particularly when in contact with certain foods.[15] The higher prevalence of the symptomatic atrophic subtype in this study may reflect increased visits to the dental office due to discomfort, such as burning sensations during food intake.

The mean total MC density in OLP was higher than in the IGM (3.77± 0.88 and 1.31± 0.89, respectively). Similar studies with toluidine blue also revealed increased MC density, which confirms the vital role of MCs in the pathogenesis of OLP.[4,7] A study by Sudhakar et al. showed an inverse relationship between MC, vascularity, and inflammation, probably suggesting a role for MC in oral inflammatory lesions.[16] MCs interact with the endothelial adhesion molecules and their surrounding basement membrane components during inflammation, releasing chemical mediators, and contribute various roles in the inflammation as well as immunosuppression.

The mean number of intact MC was less than that of the degranulated ones (1.05 ±0.66 and 2.72 ± 0.76, respectively) in OLP. Also, the result showed a decreased density of intact MC among the control group (0.33 ± 0.41and 0.98 ± 0.66, respectively). The mean density of intact, degranulated and total MC were greater in OLP than that of the control

group. The degranulated MC were higher in both the groups (2.72 ± 0.76 in OLP and 0.98 ± 0.66 in IGM). Another study by Natesan et al. showed an increase in total degranulated MC (65.4 ± 9.71) than intact (51.8 ± 19.63) in OLP.[8] They also observed an increase in degranulated MC in other lesions like Oral pyogenic granuloma, Inflammatory fibrous hyperplasia, and Oral squamous cell carcinoma. A study conducted by Ghalayani et al. also showed increased total MC in OLP when compared to Oral lichenoid reaction however the degranulated MC was less in OLP.[11] The study by Reddy et al. also observed an increase in degranulated MC as compared to intact MC in OLP (6.691 ± 2.977 and 5.866 ± 3.027, respectively).[17] In the study by Pereira et al., the mean degranulated MC (8.04 ± 2.49) was higher in OLP when compared with degranulated MC in the IGM group (0.16 ± 0.18).[6]

In our study, evaluation of MC in two zones revealed that MC density was higher in Zone I than in Zone II in both cases and the controls. A similar result was observed by Basavraj et al. where MC were found to be increased immediately below the inflammatory infiltrate than deeper connective tissue.[18] However, these findings were in contrast with the other studies, where the distribution of total MC was greater in Zone II than in Zone I.[7,17] The result of our study showed that intact and degranulated MC were found to be more in Zone I compared to Zone II in both groups. A study by Reddy et al.[17] found the distribution of degranulated MC was more in Zone I, while the intact MC showed opposing results.

The mean total MC density was found to be greater in OLP than in control and was statistically significant ($p<0.001$). It was also observed that both intact MC and degranulated MC in OLP were significantly increased ($p<0.001$). While a comparison between degranulated and intact MC showed that degranulated ones were increased in both cases and controls. These results were inconsistent with the other studies.[6,8]

The result of our study showed that the intact and degranulated MCs were found to be more in Zone I compared to Zone II in both groups. Comparing the two different MC (intact and degranulated) in Zone I revealed that these were found to be greater in OLP than in the IGM and were statistically significant ($p<0.001$). Degranulated MCs were increased in both OLP and IGM in Zone I as compared with the intact type. Zone I is subepithelial connective tissue.

A study found that the degranulated MC in OLP may or may not alter the quality of the basement membrane (BM); however, they seem to influence the thickness of the BM both directly and indirectly.[19] The result of our study showed the mean total MC density was significantly less in Zone II (deeper connective tissue). It was also observed that the total MC density was significantly greater in OLP compared to IGM. In this zone, both the intact MCs and degranulated MCs were found to be significantly greater in OLP than in control, degranulated being more than intact in both. But the number of degranulated MC was greater in OLP than in IGM and was statistically significant. Jose et al. stated that MC migrated from blood vessels in the deeper connective tissue to the extravascular compartment, subsequently moved towards the subepithelial zone, where they exerted their biological effect on the blood vessels and helped in the recruitment of inflammatory cells to the lesional area.[20] Other studies also found a significant increase in MCs in deeper layers of the connective tissue in OLP.[17,20] This increase of MCs in the deeper layer suggests that mast cell adhesion and migration occur predominantly in the deeper layer with subsequent migration to the transitional area of the submucosa. Then, they move towards the subepithelial zone, where they exert their biologic effects and help in the recruitment of inflammatory cells to the lesional area.[17] In cases of moderate/severe vascularity, the distribution of MC was predominantly low. The degranulated MC in OLP may or may not alter the quality of the BM; however, they seem to influence the thickness of the BM both directly and indirectly.[19]

Natesan et al. showed that OLP exhibited the maximum amount of MCs when compared to the

other pathologies.[7] It has been shown that MCs promote tumor progression through upregulation of angiogenesis. MC can be an indicator of increased angiogenesis and hence can help in the prediction of carcinogenesis, its progression, and also the prognosis of the malignant lesions.[21] MC count and the degree of angiogenesis can be potentially used as an indicator of the evolution of squamous cell carcinoma from epithelial dysplasia.[5]

This study was limited to a single oral lesion and did not include other oral lesions of the oral cavity. The variations of mast cell distribution among different clinical types of OLP were not evaluated. The oral hygiene of the patients, as well as systemic diseases that may influence inflammation and mast cell count were not considered in the study. Incorporating modern computer-based analysis and additional metachromatic stains may further improve the accuracy of mast cell assessment. Additionally, evaluating intraobserver and interobserver variability may help strengthen the consistency and reliability of future findings.

CONCLUSION

The present histochemical study demonstrated a significantly higher MC density in oral lichen planus compared to inflamed gingival mucosa. Among oral lichen planus cases, the atrophic variant was the most common clinical type observed. MC density was significantly greater in Zone I than in Zone II in both study groups. Degranulated MCs were found in significantly higher numbers than intact MCs in oral lichen planus as well as in inflamed gingival mucosa. All observed differences in MC density, distribution, and type between the groups and zones were statistically significant.

Conflict of interest: None.

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