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Evaluation of Mast Cells in Oral Lichen Planus and Oral Lichenoid Reactions

(Penilaian Sel Mast dalam Lichen Planus Oral dan Tindak Balas Lichenoid Oral)

YET CHING GOH[1], ADRIANA ZULKARNAIN[2], NURUL NADIAH MOHD SHUKOR[2], ANAND RAMANATHAN[1,3], ALIAS ABD AZIZ[4] & ZURAIZA MOHAMAD ZAINI[1,3,*]

[1]*Department of Oral and Maxillofacial Clinical Sciences, Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[3]*Oral Cancer Research and Coordinating Centre, Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[4]*Department of Oral and Craniofacial Sciences, Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

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ABSTRACT

Oral lichen planus (OLP) and oral lichenoid reactions (OLR) including oral lichenoid contact lesion (OLCL) and oral lichenoid drug reaction (OLDR), are clinically and histopathologically similar conditions. Mast cells play a major role in immune responses and have been implicated in the pathogenesis of OLP. This study investigated mast cell counts in OLP, OLCL and OLDR using toluidine blue staining and its association with demographic, clinical and direct immunofluorescence findings. 60 samples (30 OLP, 18 OLDR, 12 OLCL) cases were examined for mast cells using toluidine blue staining. The mean mast cell counts were obtained from five subepithelial fields at 100x magnification and analyzed using Chi square and Kruskal-Wallis tests. OLDR showed significantly elevated mean mast cell counts (p=0.015), followed by OLCL and OLP. Significant associations were found between mean mast cell counts and presence of clinical symptoms in OLP (p=0.013) and number of involved sites in OLCL (p=0.045). Buccal mucosa was the most common affected site, and reticular form was the most frequently encountered clinical presentation in all lesion types. This study demonstrated significant differences in mast cell counts among OLP, OLCL, and OLDR, with the highest mast cell density observed in OLDR, supporting a contributory role of mast cells in the pathogenesis of oral lichenoid reactions. The observed associations with clinical features further suggest that mast cells may influence disease activity and clinical expression. Toluidine blue staining is a practical and reproducible adjunctive method for mast cell assessment and may aid in distinguishing clinically and histologically overlapping OLP and OLR lesions in routine diagnostic practice.

Keywords: mast cell; oral lichen planus; oral lichenoid contact lesion; oral lichenoid drug reaction

ABSTRAK

Liken planus oral (OLP) dan tindak balas likenoid oral (OLR) termasuk lesi sentuhan likenoid oral (OLCL) dan tindak balas ubat likenoid oral (OLDR) merupakan keadaan yang mempunyai persamaan dari segi ciri klinikal dan histopatologi. Sel mast memainkan peranan penting dalam tindak balas imun dan telah dikaitkan dengan patogenesis OLP. Kajian ini menilai bilangan sel mast dalam OLP, OLCL dan OLDR menggunakan pewarnaan toluidine biru serta hubungannya dengan maklumat demografik, ciri klinikal dan penemuan imunofluoresens langsung. Sebanyak 60 sampel (30 OLP, 18 OLDR, 12 OLCL) telah diperiksa untuk bilangan sel mast menggunakan pewarnaan toluidine biru. Purata bilangan sel mast diperoleh daripada lima medan subepitelium pada pembesaran 100x dan dianalisis menggunakan ujian Chi-square dan Kruskal-Wallis. OLDR menunjukkan purata bilangan sel mast yang meningkat secara signifikan (p=0.015), diikuti oleh OLCL dan OLP. Terdapat hubungan signifikan antara purata bilangan sel mast dan kehadiran simptom klinikal dalam OLP (p=0.013) dan bilangan tempat yang terlibat dalam OLCL (p=0.045). Mukosa bukal merupakan tempat paling kerap terlibat, manakala bentuk retikular merupakan persembahan klinikal yang paling biasa ditemui dalam semua jenis lesi. Kajian ini menunjukkan perbezaan yang signifikan dalam bilangan sel mast antara OLP, OLCL dan OLDR dengan ketumpatan sel mast tertinggi diperhatikan dalam OLDR, menyokong peranan sel mast dalam patogenesis tindak balas likenoid oral. Hubungan signifikan antara bilangan sel mast dan ciri klinikal terpilih turut menekankan kaitannya dengan aktiviti penyakit dan manifestasi klinikal. Pewarnaan toluidin biru terbukti sebagai kaedah tambahan yang praktikal dan boleh diulang untuk penilaian sel mast,

serta berpotensi membantu dalam membezakan lesi OLP dan OLR yang bertindih dari segi klinikal dan histologi dalam amalan diagnostik rutin.

Kata kunci: Lesi sentuhan likenoid oral; liken planus oral; sel mast; tindak balas ubat likenoid oral

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease of unknown aetiology that is typically manifested as white reticular lesions frequently accompanied by atrophic and erosive lesions with episodic flare-ups (Ismail, Kumar & Zain 2007). The global prevalence of OLP is approximately 1.01% and the risk of developing this disease is higher in individuals over 40 years age (González-Moles et al. 2021). Intraorally, the buccal mucosa, tongue, and gingiva are frequently involved although other sites may be less common. Patients with OLP may present with any five types of clinical appearances including reticular, plaque-like, atrophic, erosive-ulcerative, and bullous either alone or in combinations. Common symptoms include mouth soreness or roughness, sensitivity to hot or spicy food, presence of red or white patches and oral ulcerations.

Oral lichenoid reactions (OLRs) are conditions that cannot be distinguished from OLP clinically and histologically (Carrozzo et al. 2019). The main subtypes of OLRs: (1) Oral lichenoid contact lesion (OLCL) resulted from a direct contact to dental restoration material,cinnamon, or betel quid, and (2) Oral lichenoid drug reaction (OLDR) related to drug exposure that may appear any time, ranging from weeks to months following the commencement of certain medications. OLCL commonly presents unilaterally in topographic relationship to restorations with adjacent mucosal erosion and atrophy. Although the clinical presentations of OLDR is less specific, it tends to occur unilaterally with erosive lesions and is sometimes having temporal relationship with a new drug intake (Carrozzo et al. 2019).

White lichenoid striations can also be seen in other autoimmune conditions. Direct immunofluorescence (DIF), an adjunct test is used to primarily rule out pemphigus and pemphigoid. In OLP, DIF present as a linear or shaggy pattern of fibrinogen deposition at the basal membrane zone associated with or without positive immunoglobulin IgM in apoptotic cells, better known as Civatte bodies. Other patterns may include IgA, IgG, and complement 3 (C3) in the apoptotic cells (Yamanaka et al. 2018).

Pathogenesis of OLP involves a T-cell immune mediated reaction with migration of cytotoxic T-cells (CD8) and helper T-cells (CD4) to the basal epithelial layer leading to destruction of keratinocytes (Kurago 2016). Role of the mast cell has been implicated in OLP and OLR through recent studies that showed presence of mast cells (Aravind et al. 2021; Ramalingam et al. 2018). This finding is linked with antigen exposure and immune reaction arising in OLP and OLR (Adami et al. 2014).

This study aimed to quantify and compare mast cell counts in oral lichen planus (OLP), oral lichenoid contact lesions (OLCL), and oral lichenoid drug reactions (OLDR) using toluidine blue staining. Additionally, we sought to evaluate the association between mean mast cell counts and demographic characteristics, clinical features, and direct immunofluorescence (DIF) findings in these conditions.

MATERIALS AND METHODS

SAMPLE SELECTION AND STUDY APPROVAL

A total of 60 formalin-fixed paraffin embedded blocks were retrieved from Diagnostic Oral Pathology Unit, Faculty of Dentistry, Universiti Malaya after their haematoxylin and eosin (H&E) sections were reviewed for the adequacy and suitability of the lesional tissues. The samples comprised of 30 OLP and 30 OLR (18 OLDR and 12 OLCL) cases. The use of archival materials and data without individual consent was approved by the Faculty of Dentistry Medical Ethics Committee (FDMEC) (reference number DF OS2004/0009(L)).

Demographic and clinical data were obtained from the laboratory request form of each case. The inclusion criteria of selected samples were: (1) Samples with no restoration and no medical contributing factors for OLP cases, (2) Samples with identifiable causative restoration and no medical contributing factors for OLCL cases, (3) Samples with lesions arise following drug intake and no identifiable causative restoration for OLDR cases, (4) Samples with available DIF and H&E histological reports, and (5) Sufficient lesional tissue. Samples with overlapping restoration and medical contributing factors, samples without DIF investigation and insufficient lesional tissue available were excluded from this study.

TOLUIDINE BLUE

Toluidine blue was utilized for mast cell identification due to its superior metachromatic properties, which yield distinctive purple-magenta granules against a blue background. The dye is widely employed to visualize acidic mucins and cartilage in routine diagnostic pathology. Its utility extends to the clinical setting as a well-established vital stain for the *in vivo*

detection of oral dysplasia and carcinoma. This proven diagnostic profile and ready availability support its use as an adjunctive stain in distinguishing oral lichen planus from lichenoid reactions, where mast cell density and degranulation patterns serve as critical histological discriminators.

TOLUIDINE BLUE STAINING PROCEDURE

Tissue sections of 4 μm thickness were deparaffinized in two changes of xylene (5 min each) and rehydrated through graded alcohols (absolute, 95%, 70% ethanol; 3 min each). Sections were rinsed in distilled water for 2 min.

Toluidine blue stock solution was prepared by dissolving 1 gm toluidine blue powder in 100 mL of 70% ethanol. Working solution was prepared by mixing toluidine blue stock with 45 mL of 1% sodium chloride to achieve pH of 2.3. The acidic pH (2.3) is critical for metachromatic staining of mast cell granules.

Sections were treated with the working solution for 2-3 min at room temperature, followed by three changes of distilled water wash (1 min each). The slides were rapidly dehydrated in 95% alcohol and two changes of absolute alcohol and cleared in two changes of xylene (2 min each), and mounted using Dibutylphthalate Polystyrene Xylene (DPX) mounting medium.

MAST CELL IDENTIFICATION AND QUANTIFICATION

All samples were digitalized using Panoramic Desk Single Digital Slide Scanner, 3DHistech (Budapest, Hungary). Five representative fields of interest from the subepithelial region were chosen randomly and images were captured at 100× magnification. The selected fields being those with the most intense inflammatory cell infiltration, as mast cell activity is known to be closely associated with inflammatory cell recruitment. Areas of folding or artefact were avoided. 100× magnification was selected to provide a wider field of view compared to higher magnifications, allowing assessment of mast cell distribution patterns across a larger sampling area while maintaining adequate cellular resolution for identification.

Mast cells stained deep purple or magenta (metachromatic staining) against a blue background (orthochromatic staining) as shown in Figure 1, were categorized as degranulated or intact cells. Degranulated mast cells have less intense stainability and have clear outline of the nucleus possibly with free granules close to the cell membrane. Intact mast cells are fully granulated cells with well-defined cell borders. In this study, mast cell count is the total of degranulated and intact mast cells.

Mast cell quantification was performed by three independent examiners: one experienced oral pathologist and two students. Intra-examiner and inter-examiner calibrations were practiced throughout the scoring process, and any disagreements were discussed to reach a consensus. The inter-examiner kappa value was 0.86, indicating strong agreement.

Data obtained were analysed using IBM Statistical Package for Social Sciences (SPSS) version 26.0. Chi-square and Kruskal-Wallis test were performed for comparative analyses between the three groups. A p-value of <0.05 was considered as significant.

RESULTS

DEMOGRAPHIC CHARACTERISTICS

The sociodemographic characteristics of all participants (n=60) are summarised in Table 1. OLPs were seen in younger age group with peak incidence in fourth and sixth decades of life. In contrast, OLCL and OLDR demonstrated higher mean age than OLP. There was marked female predominant in all types of lichenoid lesions. Ethnicity analysis showed Chinese population was more susceptible to have OLP and OLCL whereas OLDR was more commonly encountered in Indian population.

CLINICAL CHARACTERISTICS AND DIF FINDINGS

Among the 18 OLDR patients, 10 (55.56%) had more than one comorbidity whereas the remaining had a single comorbidity. Hypertension was the most prevalent medical comorbidity affecting 48.28% of the patients, followed by diabetes mellitus and dyslipidaemia. 83.33% of OLCL cases were associated with amalgam restoration, and the remaining 16.67% were associated with porcelain-fused-metal crowns and porcelain crowns (8.33%). Desquamative gingivitis was clinically identified in patients presenting with concurrent reticular and erosive variants of both OLP and OLDR. Table 2 presents the clinical characteristics and DIF findings for all samples. The majority of the OLP and OLDR patients exhibited a single symptom while most of OLCL cases were asymptomatic. Burning sensation was the most common symptom across all three types of lichenoid lesions. Reticular was the most frequent clinical presentation, although more than half of patients presented with mixed multiple clinical forms. DIF showed fibrinogen was detected in more than two-thirds of cases, while other immunoglobulins (IgG, IgM, IgA) were rarely or not detected.

MAST CELL COUNTS IN OLP, OLCL, AND OLDR

The mean mast cell count was 6.46, 6.71, and 10.32 for OLP, OLCL, and OLDR, respectively, which was significantly associated with types of lichenoid lesions ($p = 0.015$) (Table 3). It is noteworthy to highlight that both OLCL and OLDR showed higher mean

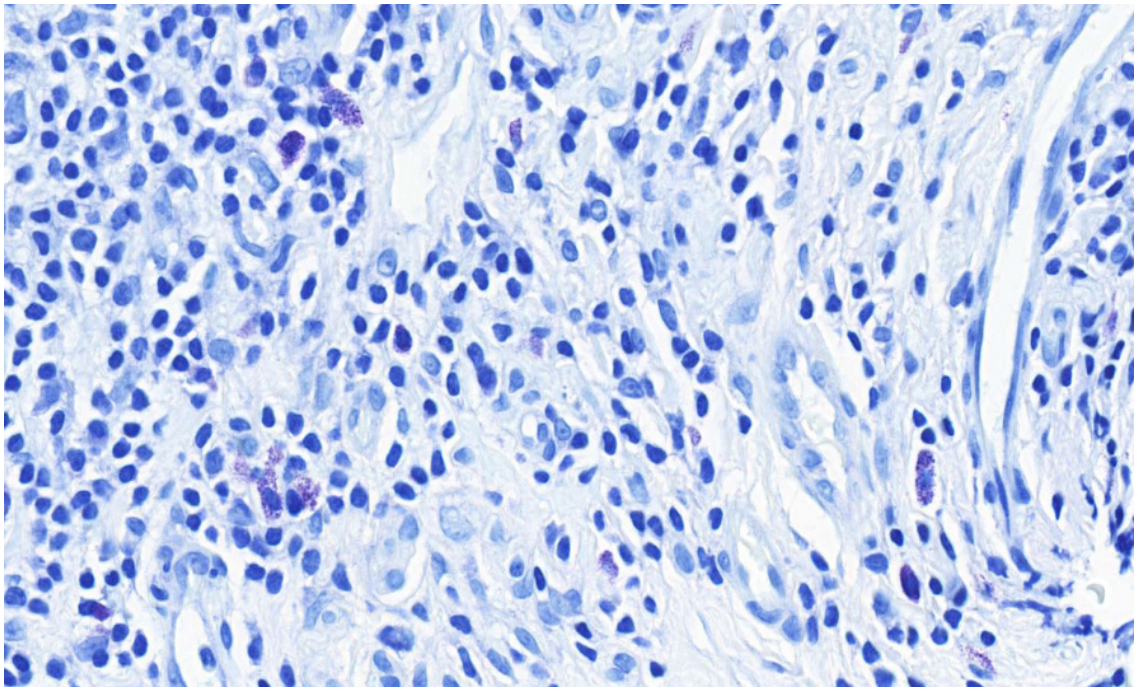

FIGURE 1. Special stain with toluidine blue highlights mast cells in magenta staining. The intact mast cell is indicated by red arrow while degranulated mast cell is indicated with orange arrows (Original magnification: x100)

TABLE 1. Sociodemographic characteristics of OLP, OLCL, and OLDR cases

Variables	OLP		OLCL		OLDR	
	n	%	n	%	n	%
Total	30		12		18	
Age group						
20-39	13	43.34	1	8.33	2	11.11
40-59	14	46.67	4	33.34	6	33.33
60 and above	3	10.00	7	58.33	10	55.56
Mean age	43		57.67		58.5	
Median age	43.5		60.5		61	
Age range	22-68		37-71		32-74	
Gender						
Male	10	33.33	1	8.33	7	38.89
Female	20	66.67	11	91.67	11	61.11
M:F ratio	1:2		1:11		1:1.6	
Ethnicity						
Malay	5	16.67	4	33.33	1	5.56
Chinese	15	50.00	6	50.00	8	44.44
Indian	9	30.00	2	16.67	9	50.00
Others	1	3.33	0	0.00	0	0.00

TABLE 2. Clinical characteristics and DIF findings for OLP, OLCL, and OLDR cases

Variables	OLP		OLCL		OLDR	
	n	%	n	%	n	%
Total	30	100	12	100	18	100
Presence of symptom(s)						
Absent	7	23.33	6	50.00	7	38.89
Single symptom	17	56.67	4	33.33	9	50.00
More than one symptoms	6	20.00	2	16.6	2	11.11
Types of symptom present						
Burning	13	43.33	4	33.33	11	61.11
Discomfort	6	20.00	1	8.33	0	0.00
Pain	10	33.33	3	25.00	2	11.11
Site of lesions						
Buccal mucosa	29	96.67	12	100.00	18	100.00
Palate	0	0.00	0	0.00	0	0.00
Tongue	8	26.67	2	16.67	3	16.67
Lip	2	6.60	2	16.67	1	5.56
Retromolar	2	6.67	2	16.67	0	0.00
Gingiva	4	13.33	4	33.33	1	5.56
Floor of the mouth	2	6.67	0	0.00	1	5.56
Clinical presentation						
Single type	11	36.67	3	25.00	6	33.33
More than one type	19	63.33	9	75.00	12	66.67
Types of clinical forms						
Reticular	28	93.33	11	91.67	16	88.89
Erosive	5	16.67	0	0.00	5	27.78
Atrophic	14	46.67	8	66.67	11	61.11
Papular	4	13.33	1	8.33	0	0.00
Plaque-like	4	13.33	3	25.00	2	11.11
Types of medication						
Statin					5	27.78
Anti-hypertensive					13	72.22
Oral anti-diabetes					7	38.89
Thyroxine					1	5.56
Insulin (Injection)					1	5.56
Types of restoration						
Amalgam			10	83.33		
Porcelain			1	8.33		
Porcelain fused metal			2	16.67		
Desquamative gingivitis						
Absent	25	83.33	12	100.00	17	94.44
Present	5	16.67	0	0.00	1	5.56

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DIF findings						
Fibrinogen	23	76.67	9	75.00	16	88.89
Complement 3	3	10.11	1	8.33	0	-
Ig G	0	-	0	-	0	-
Ig M	0	-	0	-	0	-
Ig A	1	3.33	0	-	0	-

TABLE 3. The mean count for intact and degranulated mast cells in OLP, OLCL and OLDR

Variables	OLP	OLCL	OLDR
Mean mast cell count	6.46	6.71	10.32
Mean intact mast cell	3.87 (59.90%)	1.91 (28.46%)	3.44 (33.33%)
Mean degranulated mast cell	2.59 (40.1%)	4.80 (71.54%)	6.88 (66.67%)

degranulated mast cell count whereas mean intact mast cell count was highest in OLP. Table 4 depicts the associations between the mean mast cell count and demographic and clinical factors. Although not significant, an increase of the mean mast cell count in relation to the age groups can be observed in OLCL and OLDR. Conversely, the mean mast cell count was relatively higher in younger OLP patients. In OLP cases, a statistically significant association (p=0.013) was observed between mean mast cell counts and the presence of symptoms. Patients with a single symptom had the highest mean mast cell count, compared to asymptomatic patients and those with multiple symptoms. For OLCL cases, mast cell count was significantly associated with number of involved sites (p=0.045), with the higher counts observed in cases involving multiple sites. These findings suggest that certain clinical features, such as symptom presence in OLP and site involvement in OLCL, may be associated with variations in mast cell counts, potentially indicating their role in the pathogenesis of these conditions.

DISCUSSION

To the best of our knowledge, this is the first study in literature that investigate differential expression of mast cell count in OLP, OLCL, and OLDR as separate entities. In contrast to reports by Ramalingam et al. (2018) who found higher mast cell count in OLP compared to oral lichenoid reactions, we demonstrated that the highest mast cell count was found in OLDR followed by OLCL and OLP (p = 0.015). Removal of causative restoration materials or initiation of treatment prior to biopsy may cause discrepancy of the mast cell count in OLCL (Tsushima et al. 2022). In the present study, the biopsy was performed before initiation of treatment and removal of causative restoration material. Hence, it may represent the actual mast cell activities for OLCL. Larger sample size is needed to establish firm conclusion for mast cell involvement in the pathogenesis of OLCL.

The most common OLDR causative drugs are non-steroidal anti-inflammatory and angiotensin-converting enzyme inhibitors, followed by antihypertensive and antimalarial drugs (Yuan & Woo 2020). It has been suggested that patients who developed lichenoid drugs reactions are those who had cytochrome P450 enzymes polymorphisms resulting in altered metabolism of certain medications (Kragelund et al. 2009). In our study, hypertension followed by diabetes are the most common systemic diseases among OLDR cases with 55.56% had more than one co-morbidity. Grinspan syndrome, where the use of multiple drugs to treat diabetes mellitus and arterial hypertension are believed leading to OLDR (Yuan & Woo 2020). The synergistic effects of several drugs may increase the incidence of OLDR as mast cells can undergo degranulation or can be directly affected by local neuropeptides release due to drugs (Agha-Hosseini et al. 2019). In line with previous reports (Suter & Warnakulasuriya 2016; Thornhill et al. 2006) three-quarter of OLCL cases in this study were associated with amalgam restoration. Immunological mediated reaction is elicited following the contact of oral mucosa and the restoration material leading to basal layer destruction (Kamath, Setlur & Yerlagudda 2015).

The differences in mast cell counts observed among OLP, OLCL, and OLDR in this study suggest that mast cells are not merely incidental findings but may actively contribute to the inflammatory processes underlying lichenoid lesions. Mast cells are known to interact closely with T lymphocytes, endothelial cells, and fibroblasts

TABLE 4. Comparison of demographic and clinical variables to the mean mast cell count in OLP, OLCL, and OLDR

Variable	OLP		OLCL		OLDR	
	n	Mean±SD	n	Mean±SD	n	Mean±SD
Gender						
Male	10	7.08±3.41	1	2.20	7	7.80±4.37
Female	20	6.15±3.41	11	7.13±3.23	11	11.92±4.92
p-value		0.344		0.103		0.110
Age Group						
20-39	13	7.11±2.97	1	2.20	2	9.10±1.84
40-59	14	6.17±1.98	4	5.90±2.29	6	10.30±5.92
60 and above	3	5.00±1.04	7	7.83±3.63	10	10.58±5.24
p-value		0.346		0.938		0.273
Ethnicity						
Malay	5	5.12±2.97	4	5.50±2.85	1	11.40
Chinese	15	6.52±2.55	6	7.47±3.84	8	9.18±5.10
Indian	9	6.87±1.96	2	6.90±4.38	9	11.48±5.32
Others	1	8.60	0	-	0	-
p-value		0.430		0.722		0.103
Presence of symptom(s)						
Absent	7	4.77±1.04	6	6.73±3.79	7	10.37±5.50
Single symptom	17	7.37±2.79	4	6.65±4.27	9	10.44±5.38
More than one symptom	6	5.87±1.02	2	6.80±0.28	2	9.60±3.96
p-value		**0.013***		0.936		0.525
Type of symptom(s) present						
Burning	12	7.11±2.15	3	5.33±4.12	9	10.44±5.38
Discomfort	1	7.20		-		-
Pain	4	8.15±4.82	1	10.60±		
Pain + Other symptoms	6	5.87±1.01	2	6.80±0.28	2	9.60±3.95
p-value		0.310		0.637		0.304
Site involvement						
Single site	17	6.60±2.24	6	4.77±2.51	13	11.00±4.79
More than one site	13	6.28±2.75	6	8.67±3.15	5	8.56±5.74
p-value		0.182		**0.045***		0.805
Site distribution						
Buccal mucosa	16	6.67±2.29	6	6.70±2.77	13	11.00±4.79
Gingiva	1	5.40		-		-
Buccal mucosa + Other sites	13	6.27±2.75	6	6.74±4.19	5	8.56±5.74
p-value		0.677		0.810		0.805
Clinical presentation						
Single type	11	7.44±2.77	3	7.80±3.27	6	9.90±7.29
More than one type	19	5.89±2.09	9	6.36±3.55	12	10.53±3.84
p-value		0.182		0.405		0.673
Types of clinical form						
Reticular	9	7.42±3.04	3	7.80±3.27	5	10.52±7.98

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Erosive	1	6.40		-	1	6.80
Plaque-like	1	8.60		-		-
Reticular + Other forms	19	5.89±2.09	9	6.36±3.55	12	10.53±3.83
p-value		0.324		0.405		0.999
Desquamative gingivitis						
Absent	25	6.14±2.44	12	6.72±3.39	17	10.47±5.14
Present	5	8.04±1.91	0	-	1	7.80
p-value		0.051				0.699
Fibrinogen						
Negative	7	8.09±3.11	3	9.07±2.16	2	14.70±10.32
Positive	23	5.97±2.02	9	5.93±3.45	16	9.07±4.32
p-value		0.134		0.165		0.574
Complement 3 (C3)						
Negative	27	6.42±2.47	11	6.98±3.43	18	10.32±5.03
Positive	3	6.800±2.62	1	3.80	0	-
p-value		0.653		0.310		
IgG						
Negative	30	6.46±2.44	12	6.72±3.39	18	10.32±5.03
Positive	0	-	0	-	0	-
IgM						
Negative	30	6.46±2.45	12	6.72±3.39	18	10.32±5.03
Positive	0	-	0	-	0	-
IgA						
Negative	29	6.552	12	6.717	18	10.322
Positive	1	3.800	0	-	0	-
p-value		0.148				

*Bold values indicate significant association

through the release of histamine, proteases, cytokines, and chemokines, thereby amplifying epithelial basal cell damage characteristic of lichenoid mucositis (Suter & Warnakulasuriya 2016; Thornhill et al. 2006). The higher mast cell count observed in OLDR may reflect a more pronounced systemic hypersensitivity response, in which drug metabolites act as haptens and trigger mast cell activation and degranulation (Agha-Hosseini et al. 2019; Kragelund et al. 2009). In contrast, mast cell involvement in OLCL appears to be more localised and contact-dependent, consistent with delayed-type hypersensitivity reactions induced by restorative materials (Kamath, Setlur & Yerlagudda 2015). These findings support the notion that mast cell activity may differ according to the underlying aetiology of lichenoid lesions, contributing to their biological heterogeneity.

A critical finding in this study is the significant shift in the ratio of intact versus degranulated mast cells across the types of lichenoid lesions. Our data showed that oral lichenoid reactions (OLDR and OLCL) exhibited a markedly higher count of degranulated mast cells compared to intact ones. In contrast, OLP displayed a higher mean of intact mast cells. This disparity suggests that lichenoid reactions are characterized by a more acute or intense trigger-response mechanism. The presence of exogenous triggers, such as systemic medications in OLDR or local contact dental material in OLCL are likely to induce a more vigorous and immediate mast cell degranulation compared to the more chronic, T-cell-mediated autoimmune environment of OLP. This heightened degranulation in lichenoid reactions may contribute to a more pronounced breakdown of the basement membrane and increased vascular permeability, further distinguishing the clinical behaviour of these reactions from idiopathic OLP (Noronha et al. 2024).

Consistent with an earlier Malaysian study, all three types of lichenoid lesions were predominant in female (Ismail, Kumar & Zain 2007). In addition, we found

a strong female inclination of mean mast cell count in OLDR and OLCL whereas for OLP, the mean mast cell count was higher in male. This may be attributable to higher immune response in females in relation to drug or restorations exposure. Females have been reportedly demonstrating higher TLR7 expression than males leading to increased cytokine production and stronger immune response (Klein & Flanagan 2016).

Age group analysis showed OLDR and OLCL were more prevalent in elder age groups in comparison to OLP. This could be due to the physiological change of the oral mucosa owing to the aging process. As the oral mucosa gets more permeable with increasing age, exposure to drug and restoration materials are more likely to elicit inflammatory responses compared to normal mucosa lining (Lamster et al. 2016). Higher mean mast cell count with increasing age may be due to dysregulated immune responses in the presence of triggering factors (El-Howati et al. 2023).

Our study showed higher mean mast cell count in the Indian population for OLP and OLDR. This finding may be explained by Wesley and Maibach (2003) who reported larger mast cell granules and different structural characteristics and enzymes of mast cells in Black. Based on the study, Black was more likely to experience pruritus in comparison with other races. Due to the disproportionate ethnicity distribution of our samples and no similar study done in Asian population, we are unable to draw any conclusions in relation to varied mean mast cell counts in different ethnicities.

Cases with presence of symptoms demonstrated higher mean mast cell counts in comparison with asymptomatic cases. This may reflect the influence of histamine and inflammatory mediators released following mast cell activation. OLP and OLDR cases with single site of involvement demonstrated higher mean mast cell count. As OLCL's site of involvement is dependent on the location of restoration material, hence the mean mast cell count is probably dependent on the number of restorations presence and more intense mast cell response is expected with increased restoration.

Tongue scored highest mean mast cell count in OLP cases whereas highest mean mast cell count was seen in OLCL and OLDR involving lip. Both the tongue and lips are highly specialized head and neck structures characterized by rich vascularity. Elevated mast cell populations are frequently documented adjacent to the capillary plexus, where they modulate local blood flow and facilitate the recruitment of other immune cells (Balci & Ercin 2016). Beyond vascularity, the high concentration of mast cells in these sites may relate to the distribution of these cells within skeletal muscle regions. The tongue and lips are largely composed of skeletal muscle fibres. Mast cells are naturally distributed within the connective tissue compartments that support these muscles (Pereira & Sequeira 2021). Mast cells in these muscular regions are positioned to respond rapidly to mechanical stress or local injury, contributing to tissue remodelling and inflammatory signalling. Therefore, the significant mast cell response observed in the tongue and lips likely reflects a synergistic relationship between high vascular supply and the inherent density of mast cells within the underlying skeletal musculature of the oral cavity.

Highest mean mast cell count was observed in reticular OLP and OLDR cases relative to other clinical presentations. In contrast, for OLCL, the highest mean cell count was seen in papular clinical form. To date, there is no available study investigating the relationship between differential expression of mast cells and the clinical types of presentation for comparison. Desquamative gingivitis is considered as part of the clinical manifestations for oral lichen planus due to the mucosal changes. We observed elevated mean mast cell count in OLP cases presented with desquamative gingivitis. This might be related to the nature of the disease that involves immune dysregulation (El-Howati et al. 2023).

In concordance to numerous reports on direct immunofluorescence, majority of all cases in this study were positive for fibrinogen and less frequently against complement factor C3. The observed association between mast cells count and fibrinogen may be explained by integrin aIIb3 which is expressed in human mast cells. The interaction of integrin aIIb3 with fibrinogen improves mast cell activity leads to fibrinogen uptake into the mast cells (Oki et al. 2006). Thus, fibrinogen positivity may be associated with increased mast cell count. Complement factor C3 mRNA and proteins were shown to be expressed constitutively by human cutaneous mast cells. Mast cell synthesis of C3 possibly through mast cell tryptase, could further influence inflammation and immunity (Fukuoka et al. 2013). Larger sample size to study expression of C3 and its association with mast cell expression is needed to establish a hypothesis.

CONCLUSION

Our study demonstrated a significant elevation of mean mast cell count in OLDR compared to OLCL and OLP. The mean mast cell counts also showed a significant association with clinical symptoms in OLP and the number of sites involved in OLCL. These findings highlight the potential role of mast cells in modulating disease activity. Mast cells assessment using toluidine blue staining may serve as a useful adjunctive histopathological parameter in differentiating between oral lichen planus and oral lichenoid reactions when clinical and histological features overlap. The use of toluidine blue staining for mast cell identification could enhance diagnostic accuracy and provide additional

insights into the pathogenesis of these conditions. While immunohistochemical markers may provide greater specificity, toluidine blue staining remains a valuable initial tool that can enhance diagnostic confidence and contribute additional insight into the underlying immunopathogenesis of lichenoid lesions. Further studies are warranted to validate for the utility of toluidine blue as adjunct diagnostic staining for diagnosis of oral lichen planus and oral lichenoid reactions.

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*Corresponding author; email: zuraiza@um.edu.my