Histochemical analysis of pathological alterations in oral lichen planus and oral lichenoid lesions

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Abstract: Lichen planus is a dermatologic disease of unknown etiology characterized by keratotic plaques on the skin. Many patients also harbor white lesions of the oral mucosa. The literature contains numerous reports of lichen planus-like lesions evolving in conjunction with the administration of a variety of pharmacologic agents. It is difficult, if not impossible, to distinguish such lesions from one another. The present study evaluated the epithelial and basement membrane thickness, mast cells (intact cells and degranulated cells subepithelially) and the presence or absence of blood vessels in oral lichen planus and oral lichenoid lesions. The evaluation was done using the periodic acid-schiff (PAS) and toluidine blue staining techniques on 20 cases each of oral lichen planus and oral lichenoid lesions and 5 control specimens of normal buccal mucosa. The results showed an increased number of degranulated mast cells in areas of basement membrane degeneration, increased vascularity and increased PAS-positive basement membrane thickness in oral lichen planus as compared with oral lichenoid lesions. Reduced epithelial thickness was found in oral lichen planus. The present study emphasizes the importance of these parameters in differentiating oral lichen planus from oral lichenoid lesions using special staining techniques. (J. Oral Sci. 48, 185-193, 2006)

Keywords: oral lichen planus; oral lichenoid lesions; mast cells; epithelial thickness; basement membrane thickness; vascularity.

Introduction

Lichen planus is a mucocutaneous disease of unknown etiology characterized by keratotic plaques on the skin. Many patients also harbor white lesions of the oral mucosa. However, lichen planus may be restricted to the oral cavity with no significant cutaneous lesions. Such lesions are referred to as oral lichen planus and should be treated as a precancerous condition.

Microscopically, the disease is characterized by hyperkeratosis, basal cell degeneration, basal lamina thickening and lymphocytic infiltration of the submucosa, which is zonal in nature, lying in juxtaposition to the epithelial layer. This microscopic appearance is not unlike that encountered in cutaneous delayed hypersensitivity reactions. The literature also contains numerous reports of lichen planus-like lesions evolving in conjunction with the administration of a variety of pharmacologic agents. Most of these cases manifested dermal lesions, which evolved in conjunction with the administration of drugs such as thiazides, quinidines and penicillamine. Contacts with various metals have also been associated with lichenoid skin lesions (1). The oral mucosa also manifests these lichen planus-like lesions as hyperkeratotic, white, thickened, inflammatory reactions, which are said to be “lichenoid”. Various terminologies such as oral lichenoid lesions (OLL), oral lichenoid reaction (OLR), oral lichenoid tissue reaction (OLTR), lichenoid contact stomatitis, lichen planus-like lesions and oral lichen planus (OLP) have been used to describe this reaction.

The term OLP is now considered to represent those lesions where no trigger can be identified and are hence “idiopathic”, whereas all other lesions that are associated with drug intake, systemic disease (such as chronic liver disease), food or flavor allergies, hypertension and diabetes...
mellitus are considered as lichenoid lesions (2). Oral lichenoid lesions are similar to those of OLP. They can be distinguished from OLP lesions by their close relationship with resin or other metal restorations, and their tendency to be localized and asymmetrically distributed (3). Drug-induced lichenoid reactions may resolve promptly when the offending drug is eliminated (4). In contrast, OLP appears more commonly as a bilateral lesion (5). This distinction may not hold true in exceptional cases of unilateral presentation of OLP, when no triggering factors can be identified. The distinction becomes even more difficult in the absence of skin lesions. It is difficult, if not impossible, to distinguish such lesions from one another. An incorrect diagnosis may have serious implications in the treatment planning for such patients. So far, research has focused on immunological aspects in order to distinguish the two, and various studies have reported differences between them (6-12). However, precise distinction between them still cannot be made using routine histopathological techniques.

In the present study, we attempted to clarify more precisely the parameters that are useful for making a histopathological distinction between OLP and OLL using facilities normally available in a routine laboratory.

**Materials and Methods**

The protocol was presented to the institutional ethical committee after approval of which archival formalin-fixed paraffin-embedded tissues were obtained from the Department of Oral Pathology, Manipal College of Dental Sciences, Mangalore. Twenty cases each of OLP (Group I) and OLL (Group II) occurring on the buccal mucosa diagnosed both clinically and histologically were included in the study. The control group (Group III) comprised 5 specimens of normal buccal mucosa that were taken from the margins of the archival formalin-fixed paraffin-embedded tissues of benign lesions affecting the buccal mucosa and were free of inflammation on histological examination. Complete case histories and clinical findings of the patients were recorded from the files maintained in the Department of Oral Pathology, Manipal College of Dental Sciences, Mangalore (Table 1).

In this study, the definitive clinical and histopathological criteria used to distinguish OLP and OLL were (6-12):

**OLP:**
- Bilateral presentation
- Well-defined subepithelial band of chronic inflammatory infiltrate composed predominantly of lymphocytes
- Liquefactive degeneration of the basal cell layer
- Absence of eosinophils and neutrophils

**OLL:**
- Unilateral presentation
- Poorly differentiated lower border of the subepithelial inflammatory infiltrate zone
- Presence of a substantial number of plasma cells in the lymphocytic infiltrate
- Perivascular infiltrate
- Increased number of colloid bodies
- Presence of acute inflammatory cells, such as eosinophils and neutrophils.

Based on the above definitive criteria, the diagnoses of OLP (Fig. 1) and OLL (Fig. 2) were made by three independent observers. The use of these criteria also eliminated any intra- and inter-observer bias. Cases of lichenoid dysplasia and erosive-type lichen planus were not included in the study.

Three sections were prepared from each tissue and stained with hematoxylin and eosin (H-E), periodic acid-schiff (PAS), and toluidine blue (TB) individually. Standard procedures for PAS and TB staining were employed (13,14). PAS staining colored the basement membrane magenta, and areas of basal cell layer degeneration showed markedly thickened areas delineating areas of reduplicated basement membrane. None of the histological sections showed *Candida* hyphae (Figs. 5 and 6).

One percent toluidine blue in 1% sodium chloride was used to stain mast cells (14). This stain gave the section a light blue background, with mast cells appearing reddish-purple in color (Figs. 3 and 4). This allowed easy mapping of stained mast cells.

**Examination of sections**

The slides stained with H-E were used to diagnose the cases as lichen planus or lichenoid lesions according to the criteria described above. In addition, they were also used to ascertain the presence or absence of blood vessels present subepithelially in the areas of basal cell degeneration. PAS-stained slides were used to identify areas of basal cell degeneration, areas of reduplicated basement membrane, and to measure epithelial thickness. Areas of basal cell degeneration were correlated with TB sections for counting mast cells.

PAS-positive basement membrane thickness was examined throughout each section, and three specific areas showing the maximum width of the PAS-stained basement membrane were measured using an eyepiece reticle at a total magnification of ×400. The mean of three values was expressed in µm. For measuring epithelial thickness, areas of basal cell degeneration in PAS-stained slides were observed. Three specific areas, showing maximum thickness (perpendicular distance) from the epithelial surface to the deepest part of the basal layer degeneration...
in the epithelium, were determined with a ×10 objective and the average of three measurements was made.

Mast cells were identified and counted at a magnification of ×40 in TB-stained slides. Counting was done in areas of subepithelial inflammatory infiltrate, with separate counting of mast cells in areas of basal cell degeneration and areas with an intact basal cell layer. All the areas showing disrupted basement membrane and intact basement

<table>
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<th>Sex</th>
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<th>Type</th>
<th>Distribution</th>
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<td>Unilateral</td>
<td>Buccal mucosa</td>
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membrane were observed. Three specific areas showing the maximum number of mast cells were used in each case to count the number of intact as well as degranulated mast cells separately. The area of the microscopic field was calibrated with an ocular grid fitted inside the eyepiece and having 49 squares, with a total area of \(0.030625 \times 10^6 \mu m^2\). The mean of three values was calculated and expressed as cells per \(10^6 \mu m^2\). The grid also prevented any overlapping of the areas while counting the mast cells.

Based on the intensity of metachromasia, mast cells were categorized into two groups (15):

1) Intact mast cells exhibiting intense metachromasia and dense granules obscuring the nucleus
2) Degranulated mast cells with less intense metachromasia and a clear outline of the nucleus

Aggregations of externalized mast cell granules not obviously associated with mast cells were excluded from the analysis.

### Statistical analysis
Data were analyzed using Mann-Whitney “U” test (Z), Kruskal-Wallis test (H), t-test and chi squared test (\(\chi^2\)). Differences at \(P < 0.001\) were considered to be very highly significant.

### Results and Observations
In the present study, paraffin sections taken from all three groups were analyzed with respect to their clinical data...
and histopathological features. H-E-stained sections were used for diagnosis of the lesions. Mast cells were analyzed quantitatively by counting the total number of mast cells as well as qualitatively by counting the number of intact and degranulated mast cells in TB-stained sections. Epithelial thickness and PAS-positive basement membrane were calculated for the different groups in PAS-stained sections. H-E-stained sections were also used to analyze vascularity in the subepithelial zone.

Group I (oral lichen planus)

In this group of patients, the mean age was 34.05 years (SD = 12.4836 years) with an equal prevalence between the sexes. Of the 20 patients, 5 presented with reticular-type lichen planus, 10 with the plaque type, 2 with the pigmented reticular type and 3 with the pigmented plaque variety.

Quantitative analysis of mast cells showed that the total number (both intact and degranulated) was significantly higher \((P = 0.03)\) in areas of disrupted basement membrane (mean value = 29.95663) than in areas of intact basement membrane (mean value = 14.69365). In areas of basement membrane disruption (Fig. 3), qualitative analysis of mast cells showed an increase in the number of degranulated cells (mean value = 49.57340) as compared with the number of intact cells (mean value = 10.33985). The difference was very highly significant \((P = 0.001)\). Areas of intact basement membrane also showed an increase in the number of degranulated mast cells (mean value = 19.04745) when compared with the number of intact mast cells (mean value = 10.33985). The difference was statistically significant \((P = 0.047)\).

Group II (oral lichenoid lesions)

In this group of patients, the mean age was 38.45 years (SD = 16.9596 years). There was a slight male predominance, 11 patients (55%) being male and 9 (45%) being female. Of the 20 patients, 7 presented with reticular-type lichen planus, 10 with the plaque type, 2 with the pigmented reticular type and 3 with the pigmented plaque variety.

Quantitative analysis of mast cells showed that the total number (both intact and degranulated) was significantly higher \((P = 0.03)\) in areas of disrupted basement membrane (mean value = 29.95663) than in areas of intact basement membrane (mean value = 14.69365). In areas of basement membrane disruption (Fig. 3), qualitative analysis of mast cells showed an increase in the number of degranulated cells (mean value = 49.57340) as compared with the number of intact cells (mean value = 10.33985). The difference was very highly significant \((P = 0.001)\). Areas of intact basement membrane also showed an increase in the number of degranulated mast cells (mean value = 19.04745) when compared with the number of intact mast cells (mean value = 10.33985). The difference was statistically significant \((P = 0.047)\).

Group III (control)

The control group comprising specimens of normal buccal mucosa showed degranulated mast cells (mean value = 6.53040) as well as intact mast cells (mean value = 17.41460). The number of intact mast cells was higher than that of degranulated mast cells, but the difference was not statistically significant \((P = 0.059)\).

Comparison among different groups

Results of comparisons of the various parameters are shown in Table 2.

Comparison of total number of mast cells in different groups

The OLP group showed the highest mean total number of mast cells among the three groups. The OLL group and the control group showed similar mean total numbers of mast cells and the difference was not statistically significant \((P = 0.061)\). However, a significant difference was observed between the OLP group and the OLL group \((P = 0.019)\) (Table 2).

Comparison of total number of degranulated and intact mast cells in areas of basement membrane disruption

The OLP group showed a higher mean total number of degranulated mast cells than the OLL group and the difference was statistically significant \((P = 0.046)\) (Table 2). However, the mean total number of intact mast cells was lower in the OLP group than in the OLL group, although the difference was not statistically significant \((P = 0.897)\) (Table 2).

Comparison of the total number of degranulated and intact mast cells in areas of intact basement membrane

The OLP group showed the highest mean number of degranulated mast cells, followed in order by the control group and the OLL group. The differences among the groups were all statistically significant \((P = 0.018)\) (Table 2). The total number of intact mast cells was highest in the control group, followed in order by the OLP group and the OLL group, the differences being non-significant \((P = 0.159)\) (Table 2).
Comparison of epithelial thickness in the three groups

Comparison of epithelial thickness in the disrupted basement membrane zone in the OLP and OLL groups (Figs. 5 and 6) and epithelial thickness in the control group showed the lowest values for the OLP group, followed in order by the OLL group and the control group. The differences were very highly significant ($P = 0.001$) (Table 2).

Comparison of PAS-positive basement membrane thickness

The OLP group showed an increased thickness of the PAS-positive basement membrane as compared with the OLL group (Figs. 7 and 8), and the difference was very highly significant ($P = 0.001$) (Table 2).

Comparison of vascularity in the subepithelial inflammatory infiltrate in areas of basement membrane disruption

A higher number of OLP cases showed vascularity in the subepithelial inflammatory infiltrate as compared with the OLL group (Figs. 9 and 10) and the difference was very highly significant ($P = 0.001$) (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Number</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Statistical tests</th>
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<td></td>
<td>OLL</td>
<td>20</td>
<td>7.6755</td>
<td>2.8285</td>
<td>$P = 0.001$ vhs</td>
</tr>
</tbody>
</table>

Z: Mann-Whitney “U” test
X²: Chi Square test
H: Kruskal-Wallis test
ns: not significant
vhs: very highly significant

Discussion

OLP and OLL are chronic inflammatory lesions of the oral cavity that have been a diagnostic challenge for clinicians as well as pathologists. However, despite the difference between classic OLP and OLL (8), the World Health Organization’s criteria for lichen planus do not distinguish between the two conditions, and several reports have concurred about the lack of distinguishing features (6-12).

The role of mast cells in OLP and OLL has also been studied (15). A number of investigations have demonstrated an increased mast cell density and mast cell degranulation in OLP. It has been shown that, on degranulating, these mast cells release a range of both preformed and newly synthesized cytokines and chemokines, including TNF-α, which regulate immune responses and activate T cells. In OLL, TNF-α upregulates the secretion of RANTES and other mediators from T cells, which in turn triggers the secretion and activation of matrix metalloproteinases by lesional T cells. The activation of matrix metalloproteinases, along with mast cell-derived chymase and tryptase, degrades the basement membrane structural proteins, resulting in basement membrane breaks (16).

Also, TNF-α activates the endothelium and induces expression of adhesion molecules, which bind leukocytes
and might be responsible for the increased subepithelial vascularity. Some studies have also referred to arrested growth or necrosis of epithelial cells by TNF-α, leading to reduced epithelial thickness (17). An ultrastructural study by Jungell (1987) showed that alterations of the basement membrane in oral lichen planus were of three different kinds: breaks, branches, or patch-like thickenings (18). Up to now, however, no studies have evaluated these features in OLL. The aim of the present study was to histochemically analyze mast cells, both qualitatively as well as quantitatively, and also to assess the vascularity, alterations in the basement membrane, and epithelial thickness in OLP and compare the results with those in OLL.

We found that the number of mast cells was significantly higher in OLP and OLL than in the control group, with a clear disposition around/adjacent to blood vessels occurring subepithelially near the basement membrane zone, in concordance with the study by Maji et al. (15). However, in our study we observed a significantly higher total mast cell count, with an increased number of degranulated mast cells, in OLP than in OLL, contrary to the findings of Maji et al. (15).

The exact mechanism by which mast cells or their precursors are recruited to these sites is not fully understood. In a study of normal human gastrointestinal mucosa, Stead et al. (19) observed that mast cells had a close relationship to nerve fibers. Niissalo et al. (20), in a study of OLP and OLL, observed that the pattern of innervation differed between the two. The nerve fibers were found to be relatively evenly distributed in OLL, whereas in OLP they were concentrated in the superficial subepithelial tissue. This might explain the increased concentration of mast cells in the subepithelial zone in OLL observed in our study. They also suggested that neuropeptides released from the nerve fibers could cause degranulation of mast cells in OLP (20-22). The increased number of degranulated mast cells we observed could be explained on the basis of the dense innervation seen subepithelially, exerting an influence on mast cells, and thus leading to degranulation.

In normal buccal mucosa, the intact mast cells were located closer to the basement membrane than those in OLL. Ruokonen et al. (23), in a study of human buccal mucosa, showed that mast cells differed in their spatial relationship to peripheral nerves in different locations. Studies have also suggested two different populations of mast cells, some with mucosal phenotypes (chymase-, tryptase+) and others with connective tissue phenotypes (chymase+, tryptase+). It was also observed that the oral mucosa showed a mixed population of mast cells with predominance of the connective tissue phenotype (21,24). The findings of our study probably reflect a difference in mast cell populations and their responses to secretory stimuli in the deeper lamina propria. Degranulated mast...
cells were present mainly in areas of basement membrane
disruption as compared with intact areas in the OLP and
OLL groups, indicating that degranulation of mast cells
might have caused degradation of the basement membrane,
as suggested by Zhao et al. (16). We also observed a
higher concentration of degranulated mast cells in OLP
than in OLL.

Reduced epithelial thickness is a common characteristic
of OLP (17). The OLP group showed reduced epithelial
thickness in comparison with the control group and the OLL
group. The reduced epithelial thickness in OLP might be
the result of abnormal or premature terminal differentiation
of keratinocytes (25). Walsh et al. (24,26) reported that mast
cell mediators such as TNF-α and the serine proteases,
tryptase and chymase, were acutely released upon
degranulation and had a potent effect on nearby cells.
TNF-α in particular, induces either arrested growth or
necrosis of epithelial cells (21,27,28). Thus, an increased
number of degranulated mast cells might also be correlated
with the reduced epithelial thickness seen in OLP. In
contrast, the OLL group demonstrated a two-fold increase
in epithelial thickness. This could be due to the release of
inflammatory mediators from the cellular infiltrate, inducing
the basal keratinocytes to proliferate (29). This change was
in accordance with the observation made by Yamamoto
et al., (30) who suggested that the pathological changes
in the epithelium were initiated by inflammatory cells
that produce an array of different cytokines and growth
factors capable of affecting epithelial cell growth and
differentiation.

PAS staining demonstrated basement membrane areas
with clarity. The OLP group showed a continuous thin,
linear band of basement membrane with occasional faults
and with numerous strands extending into the connective
tissue in a few cases, whereas other cases showed focal
thickening or discontinuity of the basement membrane.
These changes were also observed in a few cases of OLL,
but were located mainly in focal areas. These findings were
in accord with those of Zhou et al. (16,31). Zhou et al. (31)
concluded that the discontinuities of the basement
membrane in OLP were associated mainly with matrix
metalloproteinases (MMPs) secreted by T cells and
macrophages, and were further enhanced by mast cell
chymase and tryptase together with T cell-derived MMPs
that degraded basement membrane structural proteins,
resulting in the observed breaks (16). Cases of OLL showed
focal PAS staining irregularities adjacent to areas with
abundant neutrophils and macrophages. Goetzl et al. (32)
observed release of MMPs by neutrophils and eosinophils
in oral lichenoid reactions. The focal PAS staining
irregularities we observed in the OLL group supported their
finding.

An increased number of dilated blood vessels were also
observed adjacent to basement membrane disruption zones
in OLP as compared with OLL. This might be attributable
to the release of vasoactive amines and enzymes by mast
cells for cellular trafficking, promoting the development
of OLP and OLL, and exerting an immunoregulatory
effect on the established change through the release of
cytokines and mediators (21).

Thus, our present observations indicate that four
histopathological parameters play an important role in
the etiopathogenesis and progression of OLP and OLL.
These parameters can be considered useful criteria for
histopathological distinction between OLP and OLL.
However, further studies using a larger number of sample
sizes and definitive immunological markers for
identification of these histopathological parameters may
allow more objective distinction between OLP and OLL.

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