Nuclear and cellular volumetric alterations in oral lichen planus and lichenoid lesions: a histomorphometric study

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Abstract: There is presently no line of distinction between oral lichen planus and other oral lichenoid lesions. The aim of this study is to determine using histomorphometry, the differences between these lesions. Paraffin sections from 7 normal buccal epithelium, 19 oral lichen planus (LP), 14 oral lichenoid lesions (LL) and 7 discoid lupus erythematosus-like lesions (DLE-II) were selected. The nuclear volume (VN) and cellular-volume (VCELL) of the epithelium were assessed using an image analyser. The VN and VCELL derived for both basal and spinal strata in LP and DLE-II were 2.3 times more than that of normal tissues. There was a significant difference between LP and LL (P < 0.005) and between LL and DLE-II (P < 0.001), but not between LP and DLE-II. In conclusion, there appears to be a difference between LP, LL and DLE-II and VN and VCELL may serve as potential discriminators between these groups of lesions. (J. Oral Sci. 43, 151-157, 2001)

Key words: oral lichenoid lesions; nuclear; cellular volumes.

Introduction

Lichen planus (LP) is a chronic inflammatory epidermal and mucosal disease affecting 0.5% to 2.0% of the population (1,2). There are many other lesions encountered on the oral mucosa which are remarkably similar to LP clinically and yet do not possess the histopathological features of LP. These lesions have been variably termed as lichenoid lesions, lichenoid mucositis and lichenoid dysplasia (3). Oral lesions of discoid lupus erythematosus (DLE) can also exhibit clinical features that are strikingly similar to those of LP (4). There is a great deal of overlap of both the clinical and histological features in many lichenoid processes. It is necessary to distinguish oral LP from lichenoid lesions as the former carries a potential risk for malignant transformation (5,6). At present there are no generally accepted criteria which permit the differential diagnosis of oral LP from lichenoid lesions.

Morphometry and stereology are quantitative structural techniques which enable objective transformation to be acquired from sections of cells and tissues. These methods have been applied to studies of normal and abnormal tissues (7,8) including lichen planus lesions (9-11). Their objectivity may find application in differential diagnosis and prognosis (12-13).

In this present preliminary study, histomorphometric methods have been used to determine whether the inflammatory infiltrate found in relation to oral LP produces similar or different effects on the epithelial cellular and nuclear volumes compared to those produced by the infiltrate associated with oral lichenoid lesions (LL). Cellular and nuclear volumes are quantified as they are readily acquired morphometric parameters. In addition there have been several reports which have suggested that cellular and nuclear size parameters may be significantly altered in malignant transformation (9,13-15).
Materials and Methods
In this retrospective study, all materials was obtained from the files of the Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, University of Malaya. Samples of histopathologically normal epithelium were obtained from 7 patients, from oral LP and oral lichenoid lesions (LL); the numbers were 19 and 14 respectively. Seven cases of discoid lupus erythematosus-like lesions (DLE-II) were also included (Table 1).

Normal epithelium were obtained from different patients. All specimens had been fixed in 10% formalin for 24 hours, dehydrated in increasing concentrations of ethanols and cleared in chloroform before being embedded in paraffin wax. Sections were prepared on a microtome at 4 μm thickness and stained with haematoxylin and eosin (HE).

Normal epithelium was obtained from the margins of the buccal gingiva (3 cases) and buccal mucosa (2 cases) during minor oral surgical procedures where a flap had been raised. All specimens were free from inflammatory infiltrate.

Oral LP group comprised clinically diagnosed LP and confirmed histologically as such. Oral LP presented as plaques or in a reticular pattern. Some of the lesions exhibited Wickham’s striae which tended to radiate from the main body of the lesions. These patients did not have any relevant medical history or intake of drugs. Histopathologically many of the lesions had irregular rete processes and a well-defined band of chronic inflammatory cells close to the epithelium, accompanied by liquefaction degeneration (Fig. 1). In some cases atrophic epithelium was present. All lesions had parakeratotic epithelium with no evidence of cellular atypia.

Oral LL presented with almost similar features of oral LP but the distribution was asymmetrical and some of the patients had a medical history of hypertension, diabetes or had been taking drugs for these conditions. Some of these lesions were associated with an adjacent amalgam restoration. Histopathologically, unlike oral LP, oral LL does not have a prominent, well-defined band of lymphocytic infiltrate but a diffused admixture of inflammatory cell infiltrate which tended to extend into the deeper layers of the corium (Fig. 2).

Lesions which comprised the DLE-II group presented as erythematous papules or erosions, usually accompanied by delicate white keratotic striae radiating from the periphery of the lesions. Three of these patients had skin lesions. Histopathologically, basal cell destruction, hyperkeratosis and epithelial atrophy were present. In

Table 1 Age (years) and gender of samples in the normal, oral Lichen planus (LP), lichenoid lesions (LL) and discoid lupus erythematosus-like (DLE-II) groups

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>LP</th>
<th>LL</th>
<th>DLE-II</th>
</tr>
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<tbody>
<tr>
<td>Number of cases</td>
<td>7</td>
<td>19</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>39.2</td>
<td>52.3</td>
<td>50.2</td>
<td>43.3</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>5</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>8</td>
<td>8</td>
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Fig. 1 Section showing oral lichen planus exhibiting a keratotic, acanthotic stratified squamous epithelium with a prominent band of lymphocytic infiltrate in the subjacent connective tissue. Liquefaction degeneration is evident here. HE (×100)

Fig. 2 Sections showing oral lichenoid reaction. The lymphocytic infiltrate is diffuse and sparse. Liquefaction degeneration is not evident. HE (×100)
addition, the lymphocytic infiltrate was in a perivascular distribution together with vascular dilation with edema of the upper dermis or submucosa. Immunofluorescence had not been carried out on any of these cases to prove a definitive diagnosis of DLE.

A strictly standardized sampling procedure was used. Measurements of cellular and nuclear areas of basal and spinous cells were made on HE stained sections using a Leica Q500MC image analyser interfaced with a Leica microscope to which was attached a video camera. Basal cells were defined as those cells in contact with the underlying lamina propria. Spinous cells were suprabasal cells which showed no signs of flattening in the plane perpendicular to the epithelial surface and which did not contain keratohyaline granules.

Five fields from each representative section from each patient were analyzed. The fields were randomly selected, commencing with the first representative field on the left hand side of the section being measured, moving the stage to the next field, and then continuing with measurement of alternate fields. A cursor was used to outline basal and spinous cells and their nuclei at a magnification of x400.

Primary parameters were nuclear areas \( (A_N) \) and diameters \( (D_n) \) and cellular areas \( (A_{CELL}) \). From these data, the secondary parameter of the nuclear/cytoplasmic ratio \( (N/C) \) was generated and was used to calculate nuclear \( (V_N) \) and cellular \( (V_{CELL}) \) volumes using the relationship described \((16,17)\).

\[
\text{Nuclear-cytoplasmic ratio (N/C)} = \frac{\sum A_N}{\sum A_{CELL} - \sum A_N}
\]

\[
\text{Mean corrected nuclear diameter (D)} = \sqrt{\frac{(a.b)^{4}}{\pi}}
\]

\[
\text{Mean nuclear volume (V}_N\text{)} = D^3 \pi / 6
\]

\[
\text{Mean cytoplasmic volume (V}_{CYT}\text{)} = V_N \times N / C
\]

\[
\text{Mean cellular volume (V}_{CELL}\text{)} = V_N + V_{CYT}
\]

Data was calculated on an individual patient basis and means and standard deviations of each parameter were obtained for each of the groups investigated. Statistical analysis was performed using one-way analysis of variance (ANOVA).

Results

The results of the quantitative analysis are shown in Tables 2 and 3. Table 4 shows the results of the statistical analysis. Nuclear and cellular volumes were invariably lower in the basal cells when compared to spinous cells, irrespective of the group from which they were derived. The increase in cellular volume during epithelial differentiation between basal and spinous strata was greatest in the DLE-II and oral LP groups. This was also true for increase in nuclear volumes during epithelial differentiation.
differentiation process.

Comparisons between normal and oral LP, LL and DLE-II groups respectively showed significant differences in nuclear and cellular volumetric parameters in basal and spinous strata. When comparisons were made between oral LP and LL and also LL and DLE-II, significant increases were invariably detected with all comparisons having P values of <0.001 (Table 4).

Comparisons between normal epithelium and LL groups revealed no significant alterations in either nuclear or cellular volumetric parameters for either basal or spinous strata. However in contrast, when comparisons were made between normal and LP or normal and DLE-II, significant increases were invariably detected with all comparisons having P values of <0.005.

Discussion

There are lesions occurring in the oral mucosa which have the clinical presentation of oral LP. These have been given various terms by various clinicians e.g. lichenoid drug reaction (18,19) nonspecific lichenoid stomatitis (3), lichenoid dysplasia (3). Another specific entity, discoid lupus erythematosus, have often been included under this category.

Lichenoid disorders of the oral mucosal have been often diagnosed as oral LP (3). The reason is that oral LP share common histopathological features with several diverse entities. For instance, hyperkeratosis and discontinuity of the basal cell region can be seen not only in oral LP but also in nonspecific stomatitis, allergic phenomena, lichenoid drug reactions, dysplasia and also in association with betel-tobacco chewers (20). In addition, the intimate relationship of lymphocytes with surface epithelium reflects merely local immune interaction, another finding shared by various mucocutaneous disorders. In DLE for example, although the distribution of the lesions coupled with the clinical appearance of the lesion may be helpful in obtaining a diagnosis, it is still subjective. Therefore the diagnosis of oral LP and other lichenoid disorders is fraught with inconsistencies due largely to overlapping, nonspecific features seen in a number of different diseases (3,21).

Morphometric and stereological methods can be used to generate objective data on previously unsuspected alterations or to provide information on the extent of a structural difference which is already apparent. The results presented in this study have shown unequivocally that epithelial volumetric alterations in normal tissues were significantly different from those in oral LP and DLE-II. However no significant differences were evident between normal epithelium and oral LL. These results suggest that oral LL may represent a nonspecific inflammatory stomatitis which possess a lichenoid appearance, and hence do not behave the same way as oral LPs. White et al. (8) when comparing normal tissues with nonspecific inflammatory mucosal lesions noted no differences between these two groups. In contrast, they showed that significant differences were present when normal tissues were compared to oral LP and when nonspecific inflammatory lesions were compared to oral LP.

The results of this study also demonstrated differences in the epithelial volumetric alterations between oral LP, LL and DLE-II themselves. Nuclear and cellular volumes were significantly higher in DLE-II and oral LP compared to oral LL. However, there were no significant differences between oral LP and DLE-II. Therefore, since cellular and nuclear volumes appear to be greater in DLE-II and oral LP compared to oral LL or normal tissues, the actions and effects of the inflammatory infiltrate may also differ between these lesions. That there were no significant differences between oral LP and DLE-II suggests that these lesions are very similar, although they may be different entities. Alternatively, these lesions may be similar and that factors such as drug intake and a positive

<table>
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<tr>
<th>Table 2 Nuclear volumes in normal tissues, lichenoid lesions and discoid lupus erythematosus-like lesions</th>
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<tr>
<td><strong>Normal</strong></td>
</tr>
<tr>
<td>Spinous cells x (µm³)</td>
</tr>
<tr>
<td><strong>sd</strong></td>
</tr>
<tr>
<td>Basal cells x (µm³)</td>
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<td><strong>sd</strong></td>
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<tr>
<th>Table 3 Cellular volumes in normal tissues, lichen planus, lichenoid lesions and discoid lupus erythematosus-like lesions</th>
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<tr>
<td><strong>Normal</strong></td>
</tr>
<tr>
<td>Spinous cells x (µm³)</td>
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<tr>
<td><strong>sd</strong></td>
</tr>
<tr>
<td>Basal cells x (µm³)</td>
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<tr>
<td><strong>sd</strong></td>
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<th>Table 4 Results of statistical analysis using ANOVA: comparisons between lesions</th>
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<tr>
<td><strong>Basal cells Vₙ</strong></td>
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<td><strong>V₉₉</strong></td>
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<td><strong>Spinous cells Vₙ</strong></td>
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medical history may have altered some of its clinicopathological features. Histomorphometry hence possess the potential to discriminate between these lesions and may be of value where diagnosis is ambiguous.

In view of the relationship between oral LP and malignant transformation, we believe that further histomorphometric studies may help in distinguishing benign from potentially malignant oral LP. There is some controversy regarding the potential of LP to undergo malignant transformation (5,22,23). Other studies however have disputed this (24) and suggest that existing dysplastic lesions mimic oral LP; these are the lichenoid dysplasias (3,25). A number of reports have described increases in nuclear size in malignant lesions (15,17,26). Increases in cellular and nuclear volume have been described in dysplastic lesions of hamster cheek pouch induced by carcinogen DMBA (17). Shabana et al. (9,26) have detected increases in nuclear and cellular size parameters in leukoplasias when compared with normal oral epithelium and traumatic keratotic lesions.

Except for a few studies which we compare our findings with (8,9,26) there is little morphometric information in the literature. Previous quantitative studies have dealt with different aspects of the disease and have utilized other parameters. Shabana et al. (9,26) have described histomorphometricallydetectable increases in basal and spinous cells of oral LP when compared with normal epithelium and with non-neoplastic oral mucosal conditions. The parameters which they used were all related to cellular and nuclear size and included nuclear and cellular diameter, area and perimeter. White et al. (8) compared nuclear and cellular volumetric alterations in normal tissues, inflammatory lesions, and compared to normal tissues and inflammatory lesions.

Ultrastructural stereological methods have been used to investigate differences in epithelial differentiation patterns in reticular oral LP with either distinct or faint Wickham's striae of the human buccal mucosa (27). The study showed that differences in density gradients of a number of intracellular components were more dramatically altered in lesions with distinct striae than those with faint striae. Histomorphometry has been used to quantify the nature of the inflammatory infiltrate (10). Keszler and Cabrini (28) have compared features of oral LP with those of carcinoma in situ and leukoplakia. Oral LP lesions had a lower nuclear density than either carcinoma in situ or leukoplakia, whereas the total basal cellular and nuclear areas were similar in leukoplakia and oral LP but were substantially increased in carcinoma in situ (28).

Despite considerable efforts, the pathogenesis of oral LP remains unclear with more workers in favor of the cell-mediated immune attack rather than humoral immunity (29). A significant positive correlation between the severity of basal cell liquefaction degeneration and the density of mononuclear infiltrate has been described (30). Antigenic changes in the epithelial cells have been suggested as having a role to play in the pathogenesis (1,31), with mast cells and Langerhans being major elements in the evolving lesion (31). However, since experimental models for this disease have not been found, the exact sequence of cellular, let alone molecular events involved in the development of lesions, has yet to be established.

The accuracy and objectivity of histomorphometric methods might be applied usefully to discriminate between purely benign, potentially malignant and malignant lesions of the oral cavity. Since oral LP appears to be a premalignant condition there may be a role for histomorphometry in distinguishing the benign lesions from its counterpart which have a malignant potential. Further histomorphometric studies to compare the various clinical types of oral LP, those lesions with and without dysplasia (32,33) and those of lichenoid drug reactions and oral lichenoid dysplasias are likely to contribute to a better understanding of the behaviour of these conditions.

**Conclusion**

In conclusion, this study has demonstrated the use of histomorphometry as a helpful tool in the differential diagnosis of oral lichenoid disorders where diagnosis is ambiguous. It indicated that there is a difference between oral LP, LL and DLE-II and that oral LP and DLE-II have similar, if not the same, features. Lichenoid lesions represent a benign nonspecific inflammatory stomatitis, behaving like one and not like oral LP.

**References**

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