Histopathological features of oral lichen planus and its response to corticosteroid therapy
A retrospective study
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Abstract
Oral lichen planus (OLP) exhibits variations in severity and response to corticosteroid therapy. This study aims to assess the histopathological features of OLP at the time of diagnosis and their relationship in response to corticosteroid therapy.

In this retrospective study, OLP patients were selected if a histopathological report was available. Data were collected regarding patients’ demographics and medical history. Clinical and histological data were also obtained. The outcomes were histopathological findings, clinical form of OLP, number of exacerbations per year, and the response to corticosteroid therapy.

In this study, 100 OLP patients were enrolled. Basal layer hydropic degeneration and band-like subepithelial lymphocytes infiltrate were observed in all patients. Plasma cells, identified in 62% of OLP patients, were significantly associated with fewer disease exacerbations and better response to corticosteroid treatment.

Identifying histopathological features that may affect the clinical course would be clinically helpful in tailoring patient management.

Abbreviations: BOR = B cell receptor for antigen, HUA = Hospital Universitario Araba, LSD = least significant difference, OLP = oral lichen planus, PCG = plasma cell gingivitis, STROBE = Strengthening the Reporting of Observational Studies in Epidemiology, TLRs = toll-like receptors, WHO = World Health Organization.

Keywords: corticosteroids, oral lichen planus, plasma cells, T lymphocytes

1. Introduction
Oral lichen planus (OLP) is a chronic inflammatory disorder affecting the oral mucosa, most commonly affecting middle-aged adults. It affects both sexes, but with a slight predominance in women (ratio 1.4:1). It is considered an autoimmune disease. Corticosteroids in topical or systemic forms are commonly used for OLP management.

The evolution of the disease may involve 2 important aspects. The first one is the classification of the OLP by the World Health Organization (WHO) as a potentially malignant disease, and the second is the presence of refractory cases for treatment with corticosteroids. Many studies have been focused on the identification of several predictive variables of the dysplastic changes of the disease. Mattila et al have assessed the usefulness of aneuploidy as a prognostic marker. The authors have suggested that the 2.5c excitation rate, the proliferation index and G2/MER in OLP could be prognostic markers for malignancy risk. Molecular biomarker profiling in OLP is of increasing interest for the assessment/prediction of potentially malignant OLP lesions. Shahidi et al has reported salivary predictive markers (microRNA-320a and C-reactive protein) for dysplastic lichenoid lesion. Dilenburg et al found that epigenetic modification and the accumulation of DNA double-stranded breaks in OLP could predict poor response to treatment.

However, there is a paucity of studies that investigate the histopathological features of OLP and their correlation with the clinical course of the disease. Most of the studies regarding the histopathological features of OLP have been focused on the histological variations of the different clinical forms of OLP and on the differential diagnosis between OLP and lichenoid lesions. To our best knowledge, there are no studies that assess the histopathological findings in OLP at the time of diagnosis, OLP response to corticosteroid treatment and the frequency of disease exacerbations in each patient per year. In routine clinical practice, it is not common to harvest biopsies from OLP lesions unless potential malignant/dysplastic changes are suspected or to confirm the differential diagnosis. Finding a relationship between the histopathological features in the diagnostic biopsy and the clinical course of OLP would be clinically helpful.
The aim of this study was to compare the histopathological features of OLP at the time of diagnosis with the aim of identifying distinguishing histological features of the response to corticosteroid treatment and the aggressiveness of the disease (i.e., the frequency of exacerbations per year in each patient).

2. Methods

The manuscript was prepared according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[21] The study was performed following Helsinki Declaration and its later amendments. The ethical committee of research of Hospital Universitario Araba (HUA) (FIBEA-05-ER-17(Expte.2017-064)) approved the study protocol. Patients enrolled in this study signed the informed consents.

Patients who were diagnosed for the first time with oral lichen planus between January 2010 and January 2017 in a single dental clinic (Vitoria, Spain) were screened for this study. All cases were diagnosed following the WHO criteria that were modified in 2003.[22,23] All patients reported to have pain and discomfort on eating.

Only the cases classified as clinically typical (bilaterial and roughly symmetrical lesions, white-grey papules in a reticular pattern, and occasional presence of erosive-ulcerative, vesicular, and/or plaque-like lesions) and as histopathologically typical (basal layer hydropic degeneration, band-like subepithelial chronic lymphocytic inflammatory infiltrate, absence of epithelial dysplasia) were selected.

Data were collected on patient age, sex, medical history, location of OLP lesions, type of OLP, clinical features of OLP (erosion, ulceration, plaque, papular), date of diagnosis, histopathological findings, number of exacerbations per year, treatment in each exacerbation, and treatment effectiveness.

The treatment protocol in all cases of OLP with treatment-need criteria was as follows: triamcinolone acetonide 0.5% + nystatin 100,000 IU per millilitre in aqueous solution (rinsing once a day for 10 minutes) over 30 days. After this regimen, the patient was evaluated. The treatment was stopped when complete remission was achieved (the lesions and symptoms had completely disappeared). Otherwise, patient was prescribed “triamcinolone acetonide 1% + nystatin 100,000 IU per millilitre in aqueous solution for another 30 days.” If the treatment was
still insufficient to achieve a complete remission, then patient continued to systemic corticosteroid (prednisone at a dose of 1 mg/kg per day) was prescribed.

All the patients included in this study who received treatment achieved a complete remission of the disease. The response to corticosteroid treatment had been classified into:

- Complete remission achieved by 1 cycle of topical corticosteroids.
- Complete remission achieved by 2 cycles of topical corticosteroids.
- Complete remission achieved by 2 cycles of topical corticosteroids + systemic corticosteroids.

In all patients, incisional biopsy (size of 3–4 mm) was harvested by a surgical blade for diagnosis. The samples were fixed in 10% neutral formalin solution and were processed for inclusion in paraffin. The histological sections were stained in Haematoxylin and eosin.

2.1. **Statistical analysis**

The qualitative variables were described by frequency and compared by chi-squared test. Mean and standard deviation were calculated for quantitative variables. Normal distribution of the quantitative variables was checked by Shapiro-Wilk test. The students t test was then selected.

The analysis of the differences in the frequency of exacerbations between the different forms of OLP was performed by the univariate analysis of variance and the post-hoc test of the least significant difference (LSD).

| Table 1 | Histological findings for the different clinical forms of oral lichen planus. |
|-----------------|-----------------------------------------------------------------|
| Variable                  | Clinical form of oral lichen planus | P value |
|-----------------|-----------------|--------|
| Number of patients | 45 | 46 | 8 | 1 |
| Hydropic degeneration of basal layer (frequency) | 100.00% | 100.00% | 100.00% | 100.00% | P=1.00 |
| Plasma cells in the connective tissue (frequency) | 68.89% | 54.35% | 62.50% | 100.00% | P=.446 |
| Band-like subepithelial lymphocytic infiltration (frequency) | 100.00% | 100.00% | 100.00% | 100.00% | P=1.00 |
| Fibrin deposit in the epithelium (frequency) | 6.67% | 4.35% | 0.00 | 0.00 | P=.855 |
| Epithelial hyperkeratosis (frequency) | 73.33% | 82.61% | 75.00% | 100.00% | P=.491 |
| Acanthosis (frequency) | 53.33% | 26.09% | 37.50% | 0.00 | P=.051 |
| Civatte bodies (frequency) | 11.11% | 2.27% | 0.00 | 0.00 | P=.278 |
| Necrotic keratinocytes in the epithelium (frequency) | 20.00% | 34.78% | 37.50% | 0.00 | P=.529 |
| Epithelial hyperplasia (frequency) | 24.44% | 15.22% | 25.00% | 0.00 | P=.67 |
| Flattening of epithelial crest (frequency) | 20.00% | 17.39% | 37.50% | 0.00 | P=.577 |
| Hypergranulosis (frequency) | 44.44% | 50.00% | 37.50% | 100.00% | P=.637 |

Frequency is represented by the percentage of cases with a histological finding in each clinical form of oral lichen planus.
Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc, Chicago, IL) was used. The results obtained were considered significant when \( P < .05 \).

### 3. Results

In this study, 100 OLP patients were enrolled. No patient was excluded. There were 18 men and 82 women. The patients’ mean age was 58 ± 11 years (range: 30–89 years), and 93 patients were non-smokers. The following systematic conditions were also identified: hepatitis C (9 patients), hypertension (4 patients), atrial fibrillation (2 patients), diabetes mellitus type 2 (2 patients), hypothyroidism (1 patient), and Crohn disease (2 patients).

Regarding the clinical type of OLP, 46% of the cases were reticular, 45% were atrophic-erosive, 8% were plaque, and 1% were papular. Figure 1 shows the locations of the OLP lesions. The most frequent location was the buccal mucosa (29.7%).

Histological features at time of diagnosis: all patients showed basal layer hydropic degeneration and band-like subepithelial chronic lymphocytic inflammatory infiltrate. Plasma cells in the connective tissue (in the band-like infiltrate of T lymphocytes) were present in 62% of the patients (Fig. 2). Other findings were: epithelial hyperkeratosis (78% of cases), epithelial hyperplasia (20%), acanthosis (39%), hypergranulosis (47%), civatte bodies (6%), flattening of the epithelial crest (20%), and fibrin deposits in the epithelium (5%).

It is important to mention that 22 patients (12 reticular form, 2 atrophic-erosive form, 7 plaque form, and 1 papular form) were symptomless during the follow-up and thus no treatment was prescribed. For that, their data had been included in the description of the histopathological features (Table 1) but not in the assessment of the relationship between histopathological features, number of exacerbations, and response to corticosteroid therapy (Tables 2 and 3).

| Table 2 |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Treatment response | Number of exacerbations in atrophic-erosive OLP | Number of exacerbations in reticular OLP | Number of exacerbations in plaque OLP | Number of exacerbations in papular OLP |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| A                 | 26                | 22                | 0                 | 0                 |
| B                 | 14                | 7                 | 0                 | 0                 |
| C                 | 4                 | 4                 | 1                 | 0                 |
| Total number of exacerbations | 44                | 33                | 1                 | 0                 |

Table 2 shows the patient responses to treatment, rated as A, B, or C according to the treatment that achieved the complete remission of the disease. Most of the cases rated as B and C were atrophic-erosive and reticular OLP.

Clinical and histopathological relationship: From all the analyzed histopathological variables of OLP, only the presence of plasma cells showed statistically significant differences in the annual rate of exacerbations and the response to corticosteroids.

The mean annual numbers of exacerbations were 1.60 ± 1.4 in patients with plasma cells and 2.50 ± 1.22 in patients without plasma cells (\( P = .00 \)). Moreover, the presence of plasma cells was associated with better response to treatment. Complete resolution was achieved by 1 cycle of topical corticosteroid in 69% of the patients, by 2 cycles of topical corticosteroid in 23.8% and by 1 cycle of topical corticosteroid + systemic corticosteroid in 7.1%. However, these percentages in patients without plasma cells were 52.8%, 30.6%, and 16.7%, respectively, and these differences were statistically significant (Table 3). It is important to mention that 22 patients (12 reticular form, 2 atrophic-erosive form, 7 plaque form, and 1 papular form) were symptomless during the follow-up and thus no treatment was prescribed. For that, their data had been included in the description of the histopathological features (Table 1) but not in the assessment of the relationship between histopathological features, number of exacerbations, and response to corticosteroid therapy (Tables 2 and 3).

| Table 3 |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Form of oral lichen planus | Number of exacerbations | Atrophic-erosive form | Reticular form | Plaque form | Papular form |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| 0                 | 2                 | 12                | 7                 | 1                 | 22              |
| 1                 | 11                | 8                 | 1                 | 0                 | 20              |
| 2                 | 11                | 6                 | 0                 | 0                 | 17              |
| 3                 | 11                | 13                | 0                 | 0                 | 24              |
| 4                 | 11                | 6                 | 0                 | 0                 | 17              |
| Total             | 46                | 45                | 8                 | 1                 | 100             |

Table 3 shows the histopathological features according to the type of OLP. The lesion locations and the clinical forms of oral lichen planus are shown in Fig. 1.

Clinical course: Table 4 shows the number of exacerbations per the OLP type. Most of the exacerbations were observed in patients with erosive-atrophic and reticular OLP (91% of all recurrences in this cohort). The statistical analysis showed a significant effect of the form of OLP on the number of exacerbations (\( P = .015 \) (Table 4). The post-hoc analysis showed that the differences were statistically significant between the erosive-atrophic form and both the plaque (\( P = .009 \)) and the papular (0.008) forms of OLP but not with the reticular form (\( P = .471 \)). The same trend of statistical significance of the differences was observed when the reticular form was compared with the plaque (\( P = .039 \)) and the papular forms (\( P = .037 \)). The annual rate of exacerbations was between 17 and 24 exacerbations/yr.

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| Table 4 |
|-------------------|-------------------|-------------------|-------------------|
| Treatment response | Plasma cells positive | Plasma cells negative | \( P \) value |
|-------------------|-------------------|-------------------|-------------------|
| A (n%)             | 29 (69%)          | 19 (52.8%)        | .03*             |
| B (n%)             | 10 (22.8%)        | 11 (20.6%)        |                  |
| C (n%)             | 3 (7.1%)          | 6 (16.7%)         |                  |
| Mean number of exacerbations per year | 1.60 (±1.4) | 2.50 (±1.22) | .00† |

* Chi-square test. † t student test.
to mention that the clinical form of the OLP did not show a significant relationship with the presence/absence of plasma cells.

4. Discussion

Oral lichen planus has been diagnosed according to the clinical and histological definitions of OLP as described by the WHO criteria that were modified in 2003.[22,23] The identification of substantial numbers of plasma cells has been described as one of the distinctive feature of oral lichenoid lesions.[12] Thus, the presence of plasma cells may call the diagnosis of OLP into question. Moreover, the diagnoses were made according to the established criteria for OLP. Fernández-González et al.[25] identified plasma cells in 26% of OLP patients. Collectively, the cases assessed in this study can be safely categorized as OLP patients. The presence of plasma cells was not related to the clinical behavior of OLP prior to this study. Previous studies have identified plasma cells in OLP but have not investigated their relationship with its clinical course.[12–27] Herein, the presence of plasma cells in the band-like infiltrate of T lymphocytes has been associated with a lower OLP exacerbations and a better response to corticosteroids.

B cells and self-reactive plasma cells participate in the onset and sustainment of autoimmune diseases.[13–32] For example, plasma cell gingivitis (PCG) is mediated by plasma cells and is difficult to treat by corticosteroids (topical or systemic).[33–36] This contraindicates the outcomes of better clinical behavior when plasma cells are identified in OLP. This controversy is a reflection of a topic of much interest in immunology. However, there are B cell subpopulations that may provoke or suppress autoimmunity.

Indeed, B cells producing IL-10 have suppressed autoimmune encephalomyelitis (EAE), transplantation, infection, cancer, and allergy.[31,37–41] This immunosuppressive effect are mediated by T helper (Th)1 cells, Th2 cells, Th17 cells, IL-6-producing B cells, or autoantibodies.[41] CD40 (the B cell-activating receptor) and the Toll-like receptors (TLRs) seem to contribute to the suppression function of B cells. In fact, a 2-step model has been suggested for the initiation and propagation of this suppressive function. In this model, the initiation of suppressive IL-10-producing B cells is controlled by a TLR. The amplification of this subpopulation is then mediated by the stimulation of TLR-primed B cells with CD40 and B cell receptor (BCR) for antigen.[26,42] It has also been reported that the response of B cells to TLR and CD40 agonists might be modulated by a basal level of BCR signaling.[43] In light of these reports and the outcomes of this study, it could be speculated that B cells, through their suppressive cytokines, might provide a mechanism that may control the dynamics of an autoimmune disease.

More research is needed to assess the presence of and the subpopulation of plasma cells in the T lymphocyte infiltrate in the prognosis of OLP. In the interim, it is important to follow the established diagnostic criteria for OLP. As limitations, this study has a retrospective design, does not utilize advanced immunohistopathological techniques due to the lack of availability of samples for processing, and lacks statistical power calculations (due to its retrospective nature). For that, differential diagnosis against chronic ulcerative stomatitis could not be performed. Moreover, the number of plasma cells has not been quantified by an objective method although the presence or not of these cells has been assessed independently.

5. Conclusion

The presence of plasma cells in OLP could be associated with fewer exacerbations and a better response to conventional treatment with topical corticosteroids. More prospective studies are needed to assess this association.

Acknowledgments

The authors would like to thank Dr José Manuel Aguirre Urizar and the Diagnostic Service of Oral and Maxillofacial Pathology (SPOMF) at the University of the Basque Country/EHU for the histopathological analysis of the samples.

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