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Lupus erythematosus: Clinical and histopathological study of oral manifestations and immunohistochemical profile of the inflammatory infiltrate

Background: Lupus erythematosus (LE) is a multifactorial autoimmune disease, which may affect the oral mucosa in either its cutaneous and systemic forms, with varied prevalence.
Methods: Forty-six patients with confirmed diagnosis of LE, presenting oral lesions were included in the study. Oral mucosal lesions were analyzed clinically, their histopathological features were investigated and inflammatory infiltrate constitution was assessed using immunohistochemistry against the following clusters of differentiation: CD3, CD4, CD8, CD20, CD68 and CD1a.
Results: From 46 patients with specific LE oral lesions 34 were females (25 with cutaneous LE and nine with systemic LE) and 12 were males (11 with cutaneous LE and one with systemic LE). Clinical aspects of lesions varied, and lips and buccal mucosa were the most affected sites. Histologically, lesions revealed lichenoid mucositis with perivascular infiltrate and thickening of basement membrane. Inflammatory infiltrate was predominantly composed by T lymphocytes of the CD4 subtype, with a minor prevalence of B lymphocytes, isolated macrophages and rare Langerhans cells.
Conclusions: Oral lesions of lupus erythematosus show a variety of clinical aspects and histologically consist of a lichenoid mucositis with deep inflammatory infiltrate, composed predominantly of T CD4 positive lymphocytes.

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Lupus erythematosus (LE) is a chronic inflammatory condition, considered the prototype of autoimmune human disease. Its cause is still unknown and genetic, immunologic, hormonal and environmental factors have been implicated in its pathogenesis. The disease is more prevalent amongst women of childbearing age, although it can affect both sexes equally at any age. Classically, LE has been subdivided into a systemic and a cutaneous form. While systemic lupus erythematosus (SLE) is a multiorgan disease with variable prognosis, cutaneous lupus erythematosus (CLE) is a more

benign condition – limited to skin and/or mucosal surfaces.[1–3]

The prevalence of mucosal involvement in LE patients is debatable. Some authors suggest that oral lesions are present in 9–45% of patients with the systemic form of the disease and in 3–20% in those with CLE.[4–6]

The present study was undertaken (1) to study the clinical aspects of oral mucosal lesions specific to LE; (2) to study the main histopathological features of these mucosal lesions; and (3) to assess the composition of the inflammatory infiltrate involved in the oral lesions of LE using immunohistochemistry.

Materials and methods

Forty-six patients with confirmed diagnosis of LE and presenting oral lesions were included in the study. Their disease was classified regarding its main involvement, as systemic or cutaneous LE (SLE and CLE, respectively). Diagnosis of SLE was established based on the criteria established by the American College of Rheumatology.[7,8] Cases with drug-induced LE was suspected were excluded from this study.

Demographical data included age, race and sex of all patients. Oral lesions were examined clinically and classified according their morphologic aspects and localization. The oral lesions were then biopsied and analyzed microscopically by two pathologists. Sections of all biopsied lesions were stained with routine hematoxylin–eosin and periodic acid–Schiff (PAS). PAS stain was used to disclose the presence of colloid bodies in the epithelium and basement membrane thickening. Direct immunofluorescence examination (DIF) was also performed in all specimens.

Immunohistochemical analysis to assess the composition of the inflammatory infiltrate was performed in all oral lesions biopsied. Briefly, 3-μm serial sections of paraffin-embedded specimens were subjected to immunohistochemical technique of streptavidin–biotin peroxidase against the proteins CD3, CD4, CD8, CD20, CD68 and CD1a. Antigen retrieval was performed by incubating the specimens with citrate pH 6.0 at boiling temperature for 10 min. All monoclonal antibodies were diluted in Tris–HCl and used at the following concentration – CD3 (1:50) (clone F7.2.38, Dako Cytomation, CA, USA), CD4 (1:20) (clone MT310, Dako Cytomation), CD8 (1:50) (clone C8/144B, Dako Cytomation), CD20 (1:50) (clone L26, Dako Cytomation), CD68 (1:50) (clone KP1, Dako Cytomation) and CD1a (1:40) (clone Sc-5265, Santa Cruz Biotechnology, CA, USA). Staining was completed with the chromogen 3,3′ diaminobenzidin. The specimens were then lightly counterstained with Mayer's hematoxylin, dehydrated and mounted with glass cover slip and xylene-based mountant.

Negative controls were treated as above, but a solution of 1% BSA in Tris–HCL pH 7.4 replaced the primary antibody. Normal oral mucosa from healthy patients was used as controls.

Results

All clinical information regarding age and sex, race, involvement of the disease (systemic or cutaneous LE), sites of mucosal lesions and their clinical aspects are summarized in Table 1. All patients examined and included in the study presented with some degree of skin affection – chronic, subacute or acute LE. Clinical examples of the oral lesions of LE are depicted in Fig. 1.

The main histological aspects in all the oral biopsied lesions corresponded to a lichenoid mucositis with a deep and perivascular inflammatory infiltrate associated. The covering epithelium presented areas of acanthosis alternated with areas atrophy. In some specimens a pseudoepitheliomatous proliferation was seen. Variable degree of spongiosis was observed in most cases. On three specimens focal areas of mild to moderate epithelial atypia was detected. Foci with hydropic degeneration of the epithelial basal layer were evident in all biopsies. Widespread or focal basal cell apoptosis, sometimes with the presence of colloid bodies, was frequently observed in the sections examined. These aspects are shown in Fig. 2 (A–E). Thickening of epithelial and vascular basement membranes was clearly demonstrated on PAS-stained sections (Fig 2F).

DIF showed linear deposits of IgG and/or C3 in the basement membrane zone of all cases studied with the exception of two specimens corresponding to bullous lupus erythematosus, which had lost the covering epithelium (Fig 2F). Immunoglobulin (Ig)M fluorescence on cytoid bodies was also observed in all samples.

The histological aspects observed were similar for all clinical lesions of oral lupus, independent of the clinical form of the disease – SLE or CLE.

Immunohistochemistry revealed that the predominant cellular component of the inflammatory infiltrate of LE oral lesions were T lymphocytes (LT), mainly $CD4^+$. $LTCD3^+$ cells presented with a liquenoid distribution and $LTCD4^+$ showed intense diffuse positivity contrasting with the isolated and sparse $LTCD8^+$. B lymphocytes $CD20^+$ cells also composed the infiltrate, although less predominately and distributed in the superficial lamina propria of the oral mucosa.

Macrophages ($CD68^+$) were also detected in all the specimens, but were fewer in number. Langerhans

Table 1. Clinical information including age, sex, type of LE involvement, sites and aspects of oral lesions

	Sex/Age	Race	LE	Sites of oral lesions	Clinical aspects of mucosal lesions
1	F/29	C	CLE	Buccal mucosa	Erythemato-atrophic plaques
2	F/59	C	SLE	Lips, buccal mucosa	Erythemato-squamous plaques
3	F/44	M	SLE	Lips	Squamous-atrophic
4	F/57	B	CLE	Lips	Squamous discoid
5	F/49	C	CLE	Lips	Squamous discoid
6	F/20	M	CLE	Buccal mucosa	Enanthematous
7	F/32	B	CLE	Palate	Erythematous
8	F/-	C	CLE	Buccal mucosa	Erythemato-squamous
9	F/41	C	CLE	Buccal mucosa, palate	Enanthematous (buccal mucosa)/white-squamous (palate)
10	F/59	M	CLE	Palate	Erythemato-squamous
11	F/49	C	CLE	Buccal mucosa	Atrophic/hyperchromic
12	F/35	C	CLE	Palate	White-squamous
13	M/55	C	SLE	Lips	Bullous
14	F/55	C	CLE	Palate	Keratotic
15	F/43	B	CLE	Palate	Erythemato-squamous
16	F/51	C	CLE	Lips	Ulcerative plaques
17	F/39	M	SLE	Palate, gingival	Erythemato-ulcerative plaques
18	M/38	C	SLE	Palate, buccal mucosa	Purpuric (palate)/erythemato-ulcerated (buccal mucosa)
19	F/49	C	CLE	Lips	Squamous discoid
20	F/21	C	SLE	Buccal mucosa	White/keratotic
21	M/37	C	CLE	Buccal mucosa	Keratotic-ulcerated lesion
22	M/6	M	CLE	Lips	Squamous discoid
23	F/62	A	SLE	Lips	Erythemato-squamous
24	F/45	C	CLE	Lips	Cicatricial-discoid
25	F/46	M	SLE	Lips, tongue, floor of mouth	Erythemato-squamous-ulcerative (tongue/floor of mouth)/cicatricial discoid (lip)
26	F/23	B	SLE	Lips, palate	Erythemato-purpuric (palate)/erythemato-squamous (lip)
27	F/25	__	SLE	Buccal mucosa, lips, tongue	Bullous
28	F/21	__	SLE	Lips	Atrophic discoid
29	F/27	__	SLE	Palate	Purpuric
30	M/37	__	CLE	Palate	Erythemato-squamous
31	M/35	__	CLE	Lips	Atrophic-discoid
32	M/38	__	CLE	Lips	Atrophic-squamous-discoid
33	F/45	__	CLE	Buccal mucosa, lips	Atrophic-discoid
34	M/25	__	CLE	Buccal mucosa	Ulcero-keratotic
35	F/47	__	CLE	Palate	Purpuric
36	F/41	__	CLE	Lips	Squamous discoid
37	M/42	__	CLE	Lips	Cicatricial-atrophic discoid
38	M/21	__	CLE	Lips	Atrophic-discoid
39	F/39	__	CLE	Lips	Atrophic discoid
40	F/35	M	CLE	Lips, buccal mucosa	Ulcero-erosive
41	F/68	C	CLE	Buccal mucosa	Keratotic
42	F/46	M	SLE	Lips	Squamous discoid
43	F/48	C	CLE	Buccal mucosa	Keratotic-ulcerated
44	F/49	B	CLE	Buccal mucosa	Ulcero-squamous and atrophic
45	M/26	C	CLE	Lips	Ulcerated
46	M/15	M	CLE	Lips, buccal mucosa	Verrucous

__, information unavailable; F, female; M, male; C, Caucasian; M, Mulatto; B, black; A, Asian; CLE, cutaneous lupus erythematosus; SLE, systemic lupus erythematosus.

cells ($CD1a^+$) were rarely detected on the 46 studied samples.

The main immunohistochemical aspects of the inflammatory infiltrate are depicted in Fig. 3.

Discussion

The analysis of 46 patients with LE presenting oral lesions showed that this manifestation occurs more frequently in adult women (73.9%) (female to male ratio 2.83:1), being more common in CLE (73.9%) than in SLE (26.1%). Oral manifestations of lupus erythematosus in both forms of the disease, systemic or exclusively cutaneous, are infrequent.[9] In agreement with our results, literature data revealed that oral lesions were more common in females (female to male ratio 2.7:1), starting at a medium age of 41.8 years. Variable ranges of oral affection are described by several authors – from 9 to 45% in SLE and 3 to 20% in CLE; however, in our review, oral lesions were more prevalent in CLE.[4–6,9,10]

Clinically, most patients examined in our study presented with multiple oral lesions. In a decreasing order, locations more frequently affected were buccal mucosa, hard palate and lower lips. Some patients had lesions affecting simultaneously more than one oral site. These findings agree with previous studies, in which buccal mucosa, palate and vermilion of lips (more the lower than the upper lip) are referred as the commonest sites for lupus oral lesions.[3,9,11]

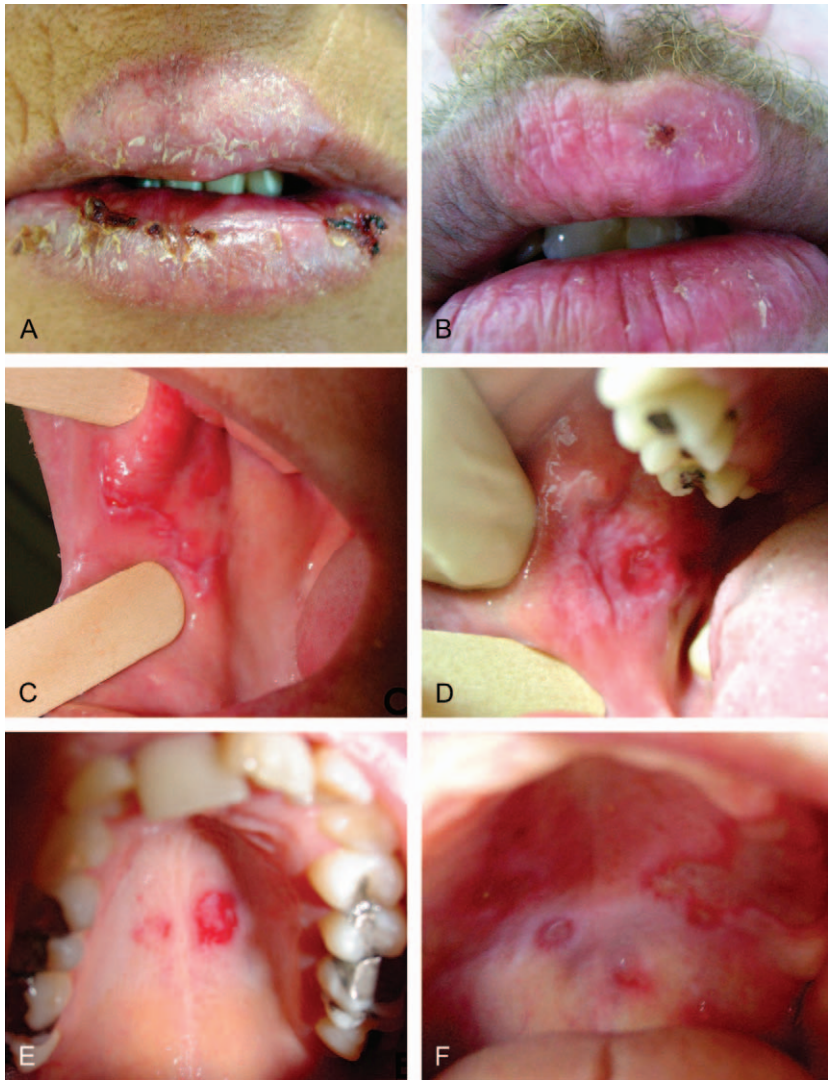

Fig. 1. Clinical aspects of lupus erythematosus in the oral mucosa/lips. Discoid lesions in (A) superior and inferior lips involved and (B) on superior lips. (C) Erythematous lesion with central a fissure surrounded by a delicate keratotic border on buccal mucosa. (D) Erythemato-keratotic lesion on buccal mucosa. (E) Erythemato-purpuric lesion on hard palate present in SLE. (F) Bullous lesions and erosions on palate and alveolar border.

Considering morphologic aspects, the oral lesions examined presented varied clinical aspects, ranging from the classic plaques with central erythema surrounded by a white rim with radiating keratotic striae and occasionally telangiectasias described by several workers[9,12] to bullous lesions. In our study classic lesions were present in less than half of the patients. This reflects the importance of considering other clinical hypotheses when examining oral lesions suggestive of lupus erythematosus and shows that in many circumstances their diagnosis is challenging. In our experience and according to other workers the main clinical differential diagnoses are lichen planus, leukoplakia, squamous cell carcinoma and even vesico-bullous diseases.[10] Histopathological and DIF examinations are, therefore, mandatory for determining the final diagnosis.

Histopathological features of LE oral lesions are mainly of a lichenoid mucositis associated with deep and frequently perivascular inflammatory infiltrate. In the 46 specimens included in this work, the key findings were epithelial hyperkeratosis with atrophy of the rete pegs, superficial and deep mononuclear inflammatory infiltrate, edema in the lamina propria, liquefative degeneration of basal epithelial cells and predominantly patchy PAS-positive sub-epithelial deposits. These results are coincident with previous reports.[3,6,13,14]

Histopathological diagnosis of oral LE should also be confirmed with DIF exam, which is a useful tool to rule out other oral lesions such as lichen planus and non-specific white lesions.[15–17] The three major classes of immunoglobulins IgA, IgM and IgG as well as different complement components may be found in the basement membrane zone deposits of LE, in a linear and/or granular pattern.[18] DIF in oral LE lesions is frequently positive and the most commonly immunoreactants identified are IgM and C3. In the current study all the specimens showed a positive DIF test, but in contrast to literature

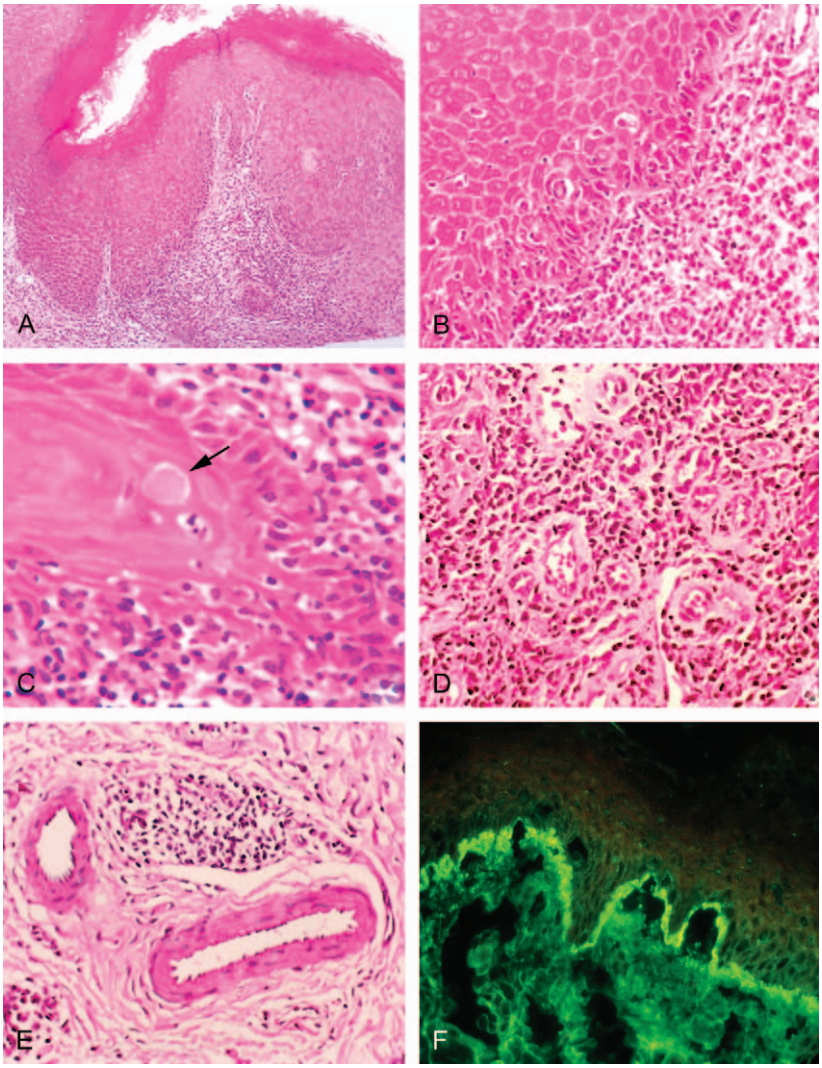

Fig. 2. Lupus erythematosus: histopathological aspects and immunofluorescence of oral lesions. (A) Fragment of oral mucosa with hyperkeratosis, acanthosis and intense lichenoid infiltrate (hematoxylin–eosin original magnification ×40). (B) Spongiosis, lymphocyte exocystosis and basal layer destruction by the lichenoid infiltrate (HE, original magnification ×250). (C) Colloid body (arrow) (HE, original magnification ×400). (D) Intense perivascular chronic inflammatory infiltrate (HE, original magnification ×250). (E) Blood vessels showing basement membrane thickening and perivascular infiltrate (periodic acid–Schiff, original magnification ×250). (F) Direct immunofluorescence showing thickening of epithelial basement membrane (Immunoglobulin G/fluorescein, original magnification ×250).

data,[15–17] IgG with or without C3 in a linear pattern was the most conspicuous finding.

The characterization of the inflammatory infiltrate by immunhistochemistry showed that the population of inflammatory cells in all specific lesions of LE (systemic or cutaneous) is mainly composed of T lymphocytes, while B lymphocytes CD20 positive, macrophages and Langerhans cells are a minor component of the infiltrate, regardless the clinical aspect of the lesion. These findings are in accordance with studies that analyzed the quality of the inflammatory component in biopsies of cutaneous and mucosal lesions of LE, which report the predominance of T cells (about 75%).[19–21] Among the subsets of T lymphocytes CD3 and CD4 were the main subsets, followed by CD8. This phenotype is probably contributory for the local physiopathology of the disease and is concurrent with literature data – predominantly T-helper/inducer phenotype.[19,22,23] Additionally this provides more evidence on the role of T lymphocytes as inducers of autoantibodies production by hyperactive B cells as is already well established in the literature.[24] Macrophages scattered throughout the inflammatory infiltrate and Langerhans cells were only rarely detected in the specimens and are possibly only adjuvant in the disease process. These immunohistochemical findings contribute to the evidence that the immunopathology background of LE manifestations share common features, regardless the subtype of LE or distribution/clinical aspect of the lesions.

The study presented herein showed that oral lesions of LE present varied aspects. However, regardless of these aspects, i.e. morphology, distribution of lesions or LE classification, the inflammatory infiltrate is predominately composed of T lymphocytes. These finds are contributory for the understanding of the pathological process of LE with mucosal involvement.

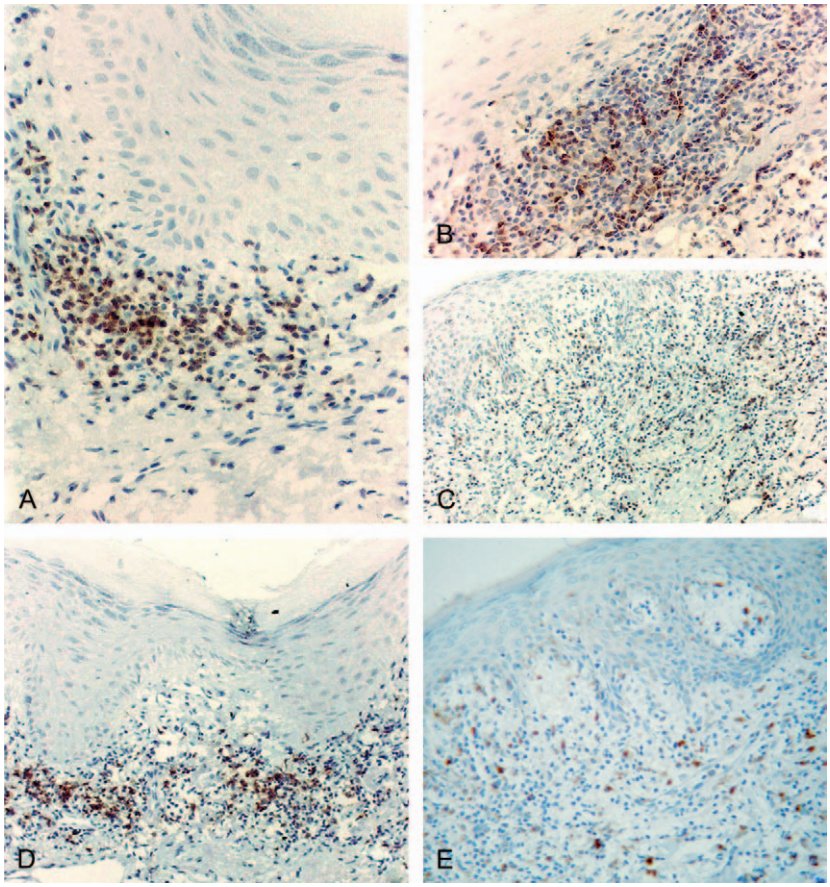

Fig. 3. Oral lesions of lupus erythematosus: immunohistochemical aspects of the inflammatory infiltrate (A) Intense presence of T lymphocytes $CD3^+$in the lichenoid infiltrate (streptavidin–biotin peroxidase, original magnification ×250). (B) T lymphocytes $CD4^+$in the lichenoid infiltrate (streptavidin–biotin peroxidase, original magnification ×150). (C) Scattered T lymphocytes $CD8^+$in the lichenoid infiltrate (streptavidin–biotin peroxidase, original magnification ×100). (D) Aggregates of B lymphocytes $CD20^+$in the lichenoid infiltrate (streptavidin–biotin peroxidase, original magnification ×150). (E) Few macrophages $CD68^+$permeating the lymphocytic inflammatory infiltrate (streptavidin–biotin peroxidase, original magnification ×150).

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