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Research Article

Oral Mucosa Deposits of Immunoglobulins and Complement Component 3 in Patients with Lupus Nephritis

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Received: June 25, 2020; **Published:** September 19, 2020

Abstract

Introduction: Lupus nephritis (LN) is one of the most frequent and serious complications in systemic lupus erythematosus (SLE), in which antibodies affect kidney filtration. In most cases, LN diagnosis is mainly based on the renal biopsy.

Objective: The aim of this study was to evaluate the deposits of immunoglobulins and C3 in oral mucosa as a reflect marker of LN in patients with SLE.

Methodology: Incisional biopsies of oral mucosa of 20 patients were included, 17 patients with diagnosis of SLE (7 diagnosed with LN), and 3 patients with cutaneous lupus erythematosus (with no renal disease). Samples were stained using H&E and PAS, and a portion was processed by direct immunofluorescence (DIF) for the detection of IgG, M, A, and C3. Variables were analyzed and compared using the non-parametric test of Mann-Whitney. P values < 0.05 were considered statistically significant.

Results: All patients in the study were female. Oral mucosa proved the presence of immunoglobulins and C3 in 71.4% of patients with LN. LN group demonstrate higher expression of IgG, M and C3 compared to other groups (p = 0.04). A band of inflammatory infiltrate and increase of basal membrane thickness were observed in oral mucosa, although absence of lesion.

Conclusion: Oral deposits of immunoglobulin G, M, and C3 are present in most patients with LN, suggesting presence of autoantibodies in oral mucosa are potential predictors of renal disease.

Keywords: *Systemic Lupus Erythematosus; Lupus Nephritis; Oral Mucosa*

Introduction

Systemic lupus erythematosus (SLE) is a multisystemic inflammatory autoimmune disease of chronic evolution with a variable clinical course and prognosis, with periods of remission and exacerbations. It may compromise any organ or system in different combinations [1]. Lupus nephritis (LN) is a renal insufficiency that affects more than half of patients with SLE. It occurs when antibodies affect the renal structures leading to kidney inflammation and presence of blood in the urine (hematuria), protein in the urine (proteinuria), and eventually alteration of renal function as renal insufficiency [1,2]. LN is the most frequent and serious complication of SLE, affecting around 50% of the patients, triggering severe renal insufficiency in 30 to 70% of the cases [3].

Currently, in about half of SLE patients, renal involvement first manifests with proteinuria and/or microhematuria on urinalysis, a harbinger of eventual progression to kidney function reduction [4]. Conversely, first signs of SLE are shown with non-renal organ involve-

ment, such as malar rash, arthritis, and oral ulcers. After a diagnosis of SLE is confirmed, evidence of kidney disease, if present, usually emerges within 3 years after diagnosis [5].

Renal biopsy provides complementary information of diagnostic, prognostic and therapeutic interest [5]. Since clinical-pathological correlation is not optimal, the authors agree on the need of doing a renal biopsy, both at the initial diagnosis and in the presence of new activity outbreaks [1,2]. All patients with clinical evidence of active LN undergo renal biopsy so that glomerular disease can be classified [1]. It has been used since many decades ago and some aspects about indications, techniques for obtaining the renal tissue, sample processing, and balance risk-benefit are still valid.

At the histopathological level, feautures of LN are characteristic but not pathognomonic. Glomerular deposits of IgG and C3 are common, with IgM, IgA, and C1q (less frequent). The presence of three immunoglobulins with C3 and C1q is known as "full house" and is very characteristic of the LN and very rare in other diseases, however an exclusive diagnostic criteria [6].

Autoantibodies that characterize SLE are present systemically years before patients develop disease. Oral immune processes occur at epithelial surface, for instance tobacco exposure response, which potentially initiates localized autoimmunity [7]. Attempts have been made to correlate oral mucosa changes with disease activity, but conclusions vary. According to Del Barrio-Díaz., *et al*. [8] oral lesions were correlated with specific organ involvement. For instance, gingival brown macules were statistically associated with proteinuria, positive anti-DNA antibodies and elevated erythrocyte sedimentation rate [8].

The term "lupus band" refers to the immunoglobulin and complement deposits observed at the basement membrane of skin and oral mucosa in patients with SLE [9]. Deposits of immunoglobulins have been found in both oral lesions and in uninvolved oral mucosa [9-11]. Moreover, presence of immunoglobulins has been observed in normal appearing skin of patients with LN suggesting a relation among presence of cutaneous immune complex and disease activity in other organs [12].

Aim of the Study

The aim of this study was to determine the presence of immunoglobulin and C3 deposits in clinically normal oral mucosa of patients with LN.

Methodology

20 patients were included in the study. 17 of them with diagnosis of SLE according to the criteria of The American College of Rheumatology [1], 7 out of 17 had confirmed diagnosis of LN. Three (3) patients with diagnosis of subacute cutaneous lupus erythematosus (CLE) without any renal compromise were also included.

Clinical evaluation

After signing an informed consent, a detailed clinical history of all patients was completed and an extensive intraoral exam was performed. None of the classic forms of SLE related lesions [13] were found among patients; therefore, biopsies were taken from an apparently normal oral mucosa. An incisional biopsy of approximately 0.8 mm in diameter of the interior surface of the lips was taken under local anesthesia. Material was imbibed in OCT for preservation.

Direct immunofluorescence study

Samples were cut at 4 μm using cryostat and then incubated in a polyvalent antibody conjugated with fluorescein isothiocyanate (Fluorescein IsoTioCyanate/FITC) (DakoCytomation) to determine the expression of IgG, IgM, IgA and C3. Preparations were examined in an Optika microscope with an ultraviolet light source at a wave emission of approximately 518 mm.

Ethical aspects

This study was developed following bioethical norms established by the Declaration of Helsinki in which the non-maleficence and autonomy of any patient was guaranteed, as well as absolute confidentiality. This work was registered and approved by The Committee of Bioethics of the Faculty of Dentistry of Universidad Central de Venezuela (CB-ESTOMATO-008-2015). All patients were informed about the aim of the research, risks and contributions, accepting and signing an informed consent approved by this committee.

Statistical analysis

The variables were analyzed through descriptive statistics. The variables were expressed in standard media ± deviation and percentages. Finally, variables were analyzed and compared through the U de Mann-Whitney non parametric test. Confidence level was established at 95% and p values < 0.05 were considered statistically significant.

Results

All patients included in the study were female (100%), aged between 17 to 77 years old. The oldest group was SCLE group (56.67 ± 22.18 years old) followed by patients with SLE without LN (47.2 ± 15.19 years old). Interestingly, LN patients were the youngest (40.14 ± 11.42 years old).

Expression of IgG, IgM and C3 in the basement membrane was observed in 9 patients (40%) of the total (N = 20), distributed in 5 NL, 2 SLE (without renal disease) and 2 SCLE. IgA was absent in SLE (without renal disease), and only 1 LN and 1 SCLE were positives. LN group demonstrated higher expression of IgG, M and C3 compared to other groups (p = 0.04).

In reference to patterns of expression observed, the most common was granular continuous immunoglobulins and C3 basement membrane deposits (Figure 1) in 4 patients with LN, 2 with SLE and 1 with SCLE. Traces of immunoglobulins and C3 were observed in 2 patients (1 patient with SCLE and 1 with LN).

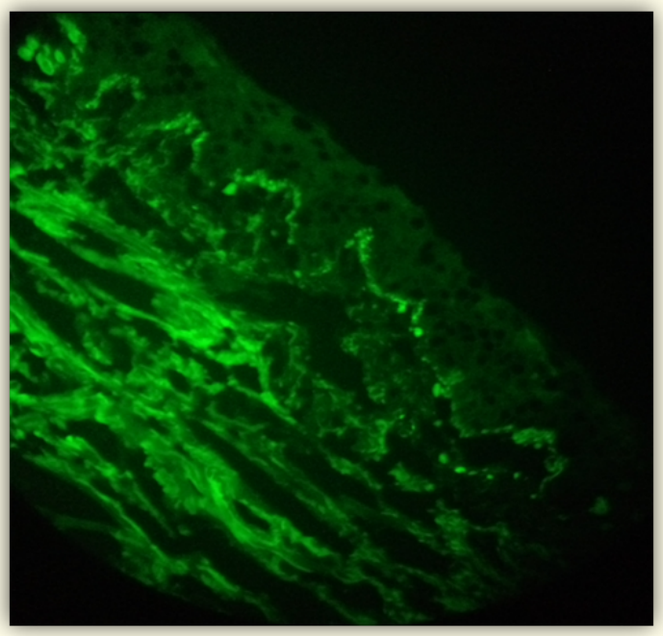

Figure 1: Microphotography of oral mucosa magnified at 40X. Observe a pattern of continuous granular IgG and C3 expression at the basement membrane in a patient with LN diagnosis.

All patients were under immunosuppressive therapy with antimalarial (hydroxychloroquine 200 mg OD or chloroquine 250 mg OD), combined with synthetic corticosteroid (prednisone between 10 mg and 40 mg OD). The average time of immunosuppressive therapy

was 16.86 ± 8.51 years in LN, followed by SLE group (without LN) (13.8 ± 10.32 years). Patients with most recent treatment corresponded to SCLE (7.67 ± 3.78 years).

Despite therapy, 40% (8 individuals) of the patients were in lupus activity, 4 of whom corresponded to the LN group, 3 to the SLE group, and 1 to the SCLE group. 65% (5 patients) of the 8 patients who were in a lupus activity showed positive profiles for deposits of IgG, IgM and C3, 4 of these patients belonging to the LN group and 1 patient to the SCLE group. However, of the 12 remaining patients who were not in activity at the moment of having the DIF test, 4 patients had positive patterns for IgG, IgM and C3, 1 belonging to the LN group, 2 to the SLE group, 1 to the SCLE group. There was no statistical association between the lupus activity and the expression of immunoglobulins and C3 (p = 0.07). Summary of results are shown in table 1.

Variables	LN	SLE	SCLE
Number of patients	7	10	3
Mean ± SD age	40.14 ± 11.42 years	47.2 ± 15.19 years	56.67 ± 22.18 years
Deposits of IgG	71.41%	20 %	66.60%
Deposits of IgM	71.41%	20 %	66.60%
Deposits of IgA	14.28%	Absent in all cases	33.30%
Deposits of C3	71.41%	20%	66.60%
Pattern of expression	Continuos granular	Continuos granular	Continuos granular
Time under treatment	16.86 ± 8.513 years	13.8 ± 10.32 years	7.67 ± 3.78 years
Lupus activity	4 cases	3 cases	1 case

Table 1: *Summary of variables studied.*

Comparison of renal and oral DIF findings

Glomerular expression of IgG and IgM was found in all patients, however, oral expression was observed in 5 cases (Table 2). On the contrary, C3 was observed in oral mucosa (4 cases) more than in renal biopsy (2 cases). Presence of IgA was scarce in both organs.

Patient	Renal biopsy Glomerular basement membrane					Oral mucosa biopsy Mucosa basement membrane				
	Antibodies				Pattern of expression	Antibodies				Pattern of expression
	IgG	IgM	IgA	C3		IgG	IgM	IgA	C3	
1	+	+	-	-	Traces	+	+	+	-	Traces
2	+	+	+	+	Granular	+	+	-	+	Continuous Granular
3	+	+	+	+	Linear	+	+	-	+	Continuous Granular
4	+	+	-	-	Linear	+	+	-	+	Continuous granular
5	+	+	-	-	Not provided	+	+	-	+	Continuous granular
6	+	+	-	-	Not provided	-	-	-	-	-
7	+	+	-	-	Not provided	-	-	-	-	-

Table 2: *Direct immunofluorescence comparison among renal and oral results.*

Discussion

SLE is a disease caused by the production of autoantibodies and immune complex deposits in different tissues of the body such as blood vessels and the connective tissue [1]. Therefore, deposits of immunoglobulins and C3 are expected in skin ad oral mucosa, named "lupus band". These deposits, along with the thickened of dermal basement membrane of SLE patients were described for the first time by Burnham., *et al.* in lesioned skin [14,15]. Later, Lai., *et al*. [16] demonstrated similar deposits on clinically normal skin. Finally, Schiödt., *et al*. [17] described the presence of immunoglobulins and complement in oral mucosa.

Lupus band is used as a diagnostic tool to assess activity disease as its sensitivity seems higher than laboratory parameters, including C3 in serum and levels of C4 of erythrocyte sedimentation, recount of lymphocytes, or the presence of antibodies anti-dsDNA [18]. Moreover, Davis and Gilliam [19] have proved that a positive skin lupus band has important predictive value identifying the group of SLE patients who may develop LN as well as significantly decreased long-term survival [19]. Deposition of IgG on non-lesional skin also correlates with antibodies anti-dsDNA and with a higher incidence of a renal disease [18], although a negative lupus band not necessarily excludes the possibility of renal affectation, as it was observed in the results of our research.

Interestingly, cutaneous positive lupus band is observed in a similar fashion to oral mucosa; however lip mucosa has shown higher expression of IgG and IgM [9]. Also, a good correlation between keratinized oral mucosa and sun-protected skin has been observed by Jonsson., *et al* [20].

There is considerable controversy in the literature about the usefulness of the lupus band test for predicting LN. Some studies have indicated that lupus band test does not correlate with the presence or severity of renal disease, nevertheless presence of deposits in vessels of normal skin correlated significantly with hypocomplementemia and with significantly higher levels of the Clq in serum [20].

Provost., *et al*. [21] demonstrated that 88% of untreated LN patients displayed positive lupus band in non-involved skin. Also, described that negative lupus band test or a positive lupus band test composed of pure lgM had a decreased occurrence of renal disease [21]. Those observations were juxtaposed by Bernstein., *et al*. [22] describing that the presence of IgM antibodies in the skin of patients with SLE was associated with a more severe and progressive form of the disease. Bernstein., *et al*. observed IgM expression in the kidney consistently accompanied by deposition of cutaneous IgM.

Concomitant expression of oral mucosa and kidney IgG and IgM were found in our results. Lack of immune complex in 2 patients was probably due to treatment or activity disease status. Contrary to our results, Jonsson., *et al*. [23] could not found connection amongst the deposition of immune complex in skin/mucosa and kidney.

Being the oral cavity an environment of easy access, the biopsy of oral mucosa might predict renal involvement. Also, it represents an advantage over skin as it is not exposed to sunlight, avoiding immunological alterations of epithelium and connective tissue. Although the renal biopsy is the "gold standard" for the diagnosis of lupus nephritis, not only because it will demonstrate whether or not there is a nephropathy, but also because it will indicate the current phase of renal insufficiency of the patient, an oral biopsy, which is far less risky, is able to predict whether the patient is at risk of having LN.

Conclusion

Oral deposits of immunoglobulin G, M, and C3 are present in most patients with LN, suggesting presence of autoantibodies in oral mucosa are potential predictors of renal disease.

Bibliography

1. American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. "The American College of Rheumatology response criteria for systemic lupus erythematosus clinical trials: measures of overall disease activity". *Arthritis and Rheumatology* 50.11 (2004): 3418-3426.

2. Hahn BH., *et al*. "American College of Rheumatology. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis". *Arthritis Care and Research* 64.6 (2012): 797-808.

3. Almaani S., *et al*. "Update on Lupus Nephritis". *Clinical Journal of the American Society of Nephrology* 12.5 (2017): 825-835.

4. Yu F., *et al*. "Redefining lupus nephritis: clinical implications of pathophysiologic subtypes". *Nature Reviews Nephrology* 13.8 (2017): 483-495.

5. Stokes MB and D'Agati VD. "Classification of Lupus Nephritis; Time for a Change?" *Advances in Chronic Kidney Disease* 26.5 (2019): 323-329.

6. Stokes MB and D'Agati VD. "Full-house glomerular deposits: beware the sheep in wolf's clothing". *Kidney International* 93.1 (2018): 18-20.

7. Pentony P., *et al*. "The initiation of autoimmunity at epithelial surfaces: a focus on rheumatoid arthritis and systemic lupus erythematosus". *Discovery Medicine* 24.133 (2017): 191-200.

8. Del Barrio-Díaz P., *et al*. "Association between oral lesions and disease activity in lupus erythematosus". *Journal of the European Academy of Dermatology and Venereology* 34.2 (2020): 349-356.

9. Burge SM., *et al*. "The lupus band test in oral mucosa, conjunctiva and skin". *British Journal of Dermatology* 121.6 (1989): 743-752.

10. Jonsson R., *et al*. "Lupus band test in uninvolved oral mucosa in systemic lupus erythematosus". *Acta Medica Scandinavica* 213.4 (1983): 269-273.

11. Schiödt M., *et al*. "Deposits of immunoglobulins and complement in oral lupus erythematosus". *Scandinavian Journal of Dental Research* 82.8 (1974): 603-607.

12. Gilliam JN., *et al*. "Immunoglobulin in clinically uninvolved skin in systemic lupus erythematosus: association with renal disease". *Journal of Clinical Investigation* 53.5 (1974):1434-1440.

13. Lopez-Labady J., *et al*. "Oral manifestations of systemic and cutaneous lupus erythematosus in a Venezuelan population". *Journal of Oral Pathology and Medicine* 3 (2007): 524-527.

14. Burnham TK., *et al*. "The application of the fluorescent antibody technic to the investigation of lupus erythematosus and various dermatoses". *Journal of Investigative Dermatology* 41 (1963): 451-456.

15. Burnham TK and Fine G. "The immunofluorescent "band" test for lupus erythematosus. I. Morphologic variations of the band of localized immunoglobulins at the dermal-epidermal junction in lupus erythematosus". *Archives of Dermatological* 99.4 (1969): 413-420.

16. Lai A Fat RF., *et al*. "An immunohistopathological study on the synthesis of immunoglobulins and complement in normal and pathological skin and the adjacent mucous membranes". *British Journal of Dermatology* 90.2 (1974): 123-136.

17. Schiödt M., *et al*. "Deposits of immunoglobulins and complement in oral lupus erythematosus". *Scandinavian Journal of Dental Research* 82.8 (1974): 603-607.

18. Reich A., *et al*. "The lupus band test in systemic lupus erythematosus patients". *Therapeutics and Clinical Risk Management* 7 (2011): 27-32.

19. Davis BM and Gilliam JN. "Prognostic significance of subepidermal immune deposits in uninvolved skin of patients with systemic lupus erythematosus: a 10-year longitudinal study". *Journal of Investigative Dermatology* 83.4 (1984): 242-247.

20. Morris RJ., *et al*. "Simultaneous immunologic studies of skin and kidney in systemic lupus erythematosus clinicopathologic correlations". *Arthritis and Rheumatology* 22.8 (1979): 864-870.

21. Provost TT., *et al*. "Lupus band test in untreated SLE patients: correlation of immunoglobulin deposition in the skin of the extensor forearm with clinical renal disease and serological abnormalities". *Journal of Investigative Dermatology* 74.6 (1980): 407-412.

22. Bernstein JE., *et al*. "Prognostic implications of cutaneous immunoglobulin deposits in systemic lupus erythematosus". *International Journal of Dermatology* 22.1 (1983): 29-34.

23. Jonsson R., *et al*. "Immune deposits in oral mucosa, skin and kidney in patients with systemic lupus erythematosus". *Clinical and Experimental Rheumatology* 4.3 (1986): 231-236.

Volume 19 Issue 10 Octosber 2020