

Immunological signatures in saliva of systemic lupus erythematosus patients: influence of periodontal condition

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Abstract

Objective

The immune system has an important role in the development of systemic lupus erythematosus (SLE) and chronic periodontitis (CP). Altered cytokines levels characterise both diseases and contributes to periodontal tissue damage in CP and to macrocomplexes deposition with connective tissue destruction in SLE. This study aimed to evaluate the production of salivary cytokines in patients with SLE and its association with periodontal status.

Methods

The sample comprised 70 SLE patients and 70 paired controls. SLE activity and damage were scored using Systemic Lupus Erythematosus Disease Activity Index 2000 and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index. Subjects were classified as without or with CP. Salivary concentrations of IL-33, MMP2/TIMP2, RANK and OPG were measured by ELISA, while IL-2, IFN γ , TNF α , IL-4, IL-6, IL-10 and IL-17A were determined by Cytometric Bead Array. Linear regression models analysed association among SLE, CP and salivary cytokines.

Results

IL-6 and IL-17A concentrations were significantly higher in SLE/CP patients than controls/CP. Concentrations of IL-6, IL-17A and IL-33 were increased in SLE/CP individuals when compared to SLE without CP. Multivariate model revealed association of cumulative dose of corticoids with periodontal damage and of IL-33 salivary concentration with SLE activity.

Conclusion

Our findings suggest that long-term therapy with corticoids would contribute with periodontal destruction in SLE patients. Moreover, the increased levels of IL-6, IL-17A and IL-33 in saliva of SLE subjects with CP may signal it as possible inflammatory pathways in this process.

Key words

systemic lupus erythematosus, chronic periodontitis, cytokine, saliva

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Introduction

Chronic periodontitis (CP) is an inflammatory disease triggered by oral microorganisms of the dental biofilm that results in damage of supporting connective tissue and alveolar bone loss (1). The severity of periodontal inflammation varies among individuals, irrespective of the bacterial load indicating that the deregulation of the host inflammatory response might aggravate the periodontal destruction (2).

In this setting, chronic conditions marked by systemic inflammation such as diabetes (3), obesity, chronic kidney disease and rheumatoid arthritis (4) are associated with an increased risk of CP. Additionally, some studies indicated that CP amplifies systemic inflammation potentially affecting the development of atherosclerotic cardiovascular disease (5), adverse pregnancy outcomes (6), pneumonia, rheumatoid arthritis (4) and systemic lupus erythematosus (SLE) activity (7). SLE is a multisystem connective-tissue disorder characterised by autoimmune response to different autoantigens, and a wide range of clinical features. SLE diagnosis is based on the Systemic Lupus International Clinics Classification (SLICC) criteria (8). There are similarities in the pathogenesis of SLE and CP, but the nature of the association between these two conditions remains to be elucidated. Previous studies suggested that CP frequency in SLE individuals varies from 60 to 93.8% (9-11). Data of CP severity in SLE population are also divergent, since SLE patients exhibited similar, less severe or more severe periodontal parameters when compared to healthy controls or to non-SLE patients with CP (11-15). Interestingly, it was reported that periodontal treatment improves response to conventional therapy in SLE patients with reduction of disease activity (7).

Reports of cytokine salivary levels in periodontitis are controversial; nevertheless recent evidence suggests that IL-1 β is elevated in periodontal diseases and could discriminate between active and inactive sites (16). IL-6 is also increased in saliva of individuals with periodontitis and it is associated with periodontal tissue destruction. Some inflammatory biomarkers such as IL-17,

TNF- α , MMPs, RANK, RANKL, OPG have been studied and could be correlated with the stages of periodontal disease. However, it is unlikely that one standalone marker could be used as a diagnostic tool (16). A restrict number of studies has investigated the influence of systemic diseases such as rheumatoid arthritis, bowel disease and diabetes on salivary cytokines levels in presence of periodontitis and none has been designed specifically with this objective, consequently it was not possible to precise the local or systemic influence in salivary cytokines concentrations (16). Little information is available concerning the cytokines profile in saliva (17) and gingival crevicular fluid (GCF) (18) of SLE patients and its relation with periodontal status. For instance, the total amount of IL-1 β and IL-18 in GCF were decreased in SLE patients when compared to controls (18) and the levels of IL-6 and IL-1 β in saliva were also lower in SLE individuals with inflamed periodontal sites compared to healthy controls with similar periodontal condition (17). Considering contradictory results on the complex link between of SLE and CP, the evaluation of salivary cytokines and periodontal condition might contribute to identify markers of periodontal destruction and to define possible correlations with SLE activity or damage. Herein, this study evaluated the production of salivary cytokines in SLE patients and its relationship with periodontal parameters and SLE activity and damage.

Materials and methods

Subjects

A total of 336 patients diagnosed with SLE and with a regular follow-up at the Rheumatology Outpatient Clinic of the Medical School Hospital of Universidade Federal de Minas Gerais were initially evaluated. Seventy SLE patients with at least 18 years and with at least eight erupted teeth were included in the present study. Exclusion criteria were: other rheumatic diseases (except from secondary Sjögren's syndrome), CP treatment in the former 6 months, presence of orthodontic appliances, use of antibiotics in the last 3 months, chronic kidney disease requiring dialysis or

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after kidney transplantation, pregnancy, lactation and neoplasia diagnosis in the last 5 years. Medical records of SLE patients were reviewed in order to collect information about the disease and medication. The SLE activity and damage were evaluated through Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (19) and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) (20), respectively. The cumulative dose of corticoids (mg/prednisone) was calculated and current use of corticoids, antimalarials and immunosuppressants were determined. Seventy individuals, with no clinical evidence of rheumatic diseases, matched for age, gender, self-referred colour, educational level and monthly income with SLE patients, comprised the control group. They were randomly selected from a population with socioeconomic and educational backgrounds similar to SLE patients, recruited among staff and escorts of patients from Faculty of Dentistry and Clinics Hospital and with no dental complaints. All participants signed a written informed consent. This study was approved by Institutional Ethics Committee for Human Studies (CAAE: 03128012.0.0000.5149/2012).

Assessment of periodontal parameters

Both groups were submitted to periodontal examination of full mouth using a periodontal probe (Hu-Friedy, PCP 15, North Carolina University, Chicago, Illinois, USA). Probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BP) were determined. Four measures were noted for each tooth (mesial, distal, lingual and buccal) (1). A site was considered a concomitant site (CS) when exhibited bleeding upon probing and PD ≥ 4 mm. Plaque index (PI) (21) was also assessed to verify oral hygiene. Two trained and calibrated examiners (JDC and SMSM) performed the periodontal examination. CP was defined as ≥ 2 interproximal sites with CAL ≥ 3 mm, and ≥ 2 interproximal sites with PD ≥ 4 mm (not on same tooth) or one site with PD ≥ 5 mm (1).

Saliva collection and processing

Saliva samples were obtained under

stimulated conditions before the periodontal examination as described elsewhere (22). The patients were instructed to wash their mouth with filtered water before collection. During 5 minutes, they were asked to chew a hyperboloid, spitting the whole produced saliva into a 50 mL sterile falcon tube. The salivary flow was determined dividing the total volume of saliva collected by five (mL/min). The samples were centrifuged at 3000 rpm for 15 minutes and 4°C. The supernatants were collected and diluted (1:1) in a phosphate-buffered saline solution (0.4 mM NaCl and 10 mM NaPO₄, pH7.4), containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM ethylenediaminetetraacetic acid, 0.01 mg/ml aprotinin A and 0.05% Tween-20) and frozen at -80°C until analysis.

Cytokines analysis

The concentrations of the cytokines IL-33, RANK, OPG and MMP2/TIMP2 were measured in stimulated saliva by sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available kits (R&D Systems, Minneapolis, MN, USA). The assay was performed according to the manufacturer's instructions. The concentrations of the cytokines IL-2, IL-4, IL-6, IL-10, IL-17A, IFN γ and TNF- α were also measured in stimulated saliva by Cytometric Bead Array (CBA) employing a BD™ CBA Human Th1/Th2/Th17 Cytokine Kit (Becton, Dickinson and Company, BD Biosciences, San Diego, CA) and were analysed on a BD FACSCalibur flow cytometer (Becton, Dickinson and Company). The concentrations of cytokines were measured using a standard curve according to the manufacturer's guidelines. The results were expressed as picogram of cytokine per mL of saliva and adjusted by stimulated salivary flux (picogram per mL^{min} of saliva).

Statistical analysis

Normal distribution of the variables was tested using Kolmogorov-Smirnov test. Chi-square test and Mann-Whitney's test were used to verify if SLE and control groups were correctly matched. Kruskal-Wallis test was applied to ana-

lyse the differences in cytokines concentrations and clinical data between SLE and control groups, subdivided according the presence (CP) or absence (non-CP) of CP. Mann Whitney test with Bonferroni correction was used to verify where was the difference when the groups were compared two by two. Mann Whitney test was applied to compare the distribution of cytokines levels between SLE patients that currently used and did not use corticoids, anti-malarial or immunosuppressant medication. Spearman's correlation analysis was used to investigate a potential relation among variables. Posteriorly, linear regression models were designed to determine the most significant associations among SLE variables (CPR, C3, C4, ESR, cumulative dose of corticoids, SLEDAI-2K, SDI and age) and parameters of periodontal destruction (PD, CAL, BP, CS and missing teeth). Linear regression models were also constructed to evaluate the interference of periodontitis and SLE chronicity and activity parameters in the levels of IL-6, IL-17A and IL-33 cytokines in saliva of SLE patients. The criterion adopted to include the variables in each model was $p < 0.2$. Significance level was determined as $p < 0.05$. All statistical evaluations were performed with SPSS software (v. 20.0 for Macintosh; IBM, Armonk, NY, USA).

Results

The demographic, laboratorial, clinical and periodontal characteristics of the studied population are presented in Table I. Among SLE patients, few subjects presented SLE activity: SLEDAI-2K > 10 (n=11, 16%); serositis (n=3, 4%); skin (n=15, 21%); nephritis (n=11, 16%), CNS (n=1, 1.4%), arthritis (n=5, 7%). Only 3 (4%) patients presented secondary Sjögren Syndrome, classified on the basis of clinical and serological parameters. CP frequency and periodontal parameters were similar when comparing SLE and controls. The plaque index was significantly higher among SLE individuals ($p=0.030$). SLE and control groups were subdivided, according to the presence or absence of CP into: control/CP, control/non-CP, SLE/CP and SLE/non-

Table I. Demographic, laboratorial, clinical and periodontal characteristics of study subjects.

	Control (n=70)	SLE (n=70)	p-value
Females	56 (80%)	63 (90%)	0.098
Age (years) mean (\pm SD)	40.9 (\pm 14.07)	37.41 (\pm 9.82)	0.200
Self-referred colour (White/non White) (%)	30 (43%)/40(57%)	19 (27%)/51(73%)	0.051
Educational level (years of study) (min-max)	11 (0-17.00)	11 (3.00-18.5)	0.150
Income (minimum wage) (min-max)	3.00 (1.00-11.00)	3.00 (0.30-20.00)	0.110
Diabetes Mellitus (%)	1 (1.4%)	4 (5.7%)	0.370
Smokers (%)	7 (10%)	7 (10%)	1.000
SLE duration (years)	-	11.29 (\pm 7.55)	-
Cumulative dose of corticoid (mg/prednisone)	-	37741.89 (\pm 27054.99)	-
Current use of corticoids	-	58 (82.9%)	-
Current use of antimalarial	-	43 (61.4%)	-
Current use of immunosuppressant	-	55 (78.6%)	-
SLEDAI-2K (min-max)	-	4.00 (0.00-18.00)	-
SDI (min-max)	-	0.00 (0.00-4.00)	-
C3 (mg/dL) (\pm SD)	-	96.60 (\pm 26.93)	-
C4 (mg/dL) (min-max)	-	18.85 (4.00-57.00)	-
ESR (mm/h)(min-max)	-	22.00 (0.00-110.00)	-
CRP (mg/dL) (min-max)	-	5.80 (0.00-87.90)	-
Missing teeth (min-max)	2.00 (0.00-20.00)	2.50 (0.00-18.00)	0.740
Stimulated sialometry (mL/min) (min-max)	2.00 (0.5-5.0)	1.65 (0.20-5.00)	0.110
Plaque Index (min-max)	0.58 (0-3.00)	0.75 (0.04-2.20)	*0.030
Periodontitis (%)	39 (56%)	46 (66%)	0.230
PD (mm) (min-max)	1.93 (1.29-4.42)	1.89 (1.3-3.17)	0.970
CAL (mm) (min-max)	2.01 (1.32-5.44)	2.09 (1.42-4.18)	0.610
BP (% sites) (min-max)	8.65 (0.00-38.00)	8.33 (0.00-47.00)	0.790
CS % sites (min-max)	0.00 (00.00-38.00)	0.00 (0.00-15.00)	0.740

Values are expressed as mean (\pm SD) for normal variables and median (min-max) for non-normal variables. Control: subjects without systemic lupus erythematosus or other rheumatic diseases; SLE: systemic lupus erythematosus subjects; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2K; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus; PD: probing depth; CAL: clinical attachment loss; BP: sites with bleeding upon probing; CS: concomitant sites (with bleeding upon probing and PD \geq 4mm). Chi-square test and Mann Whitney test were used to analyse the statistical differences between the groups ($p < 0.05$). *Statistical significance difference when compared controls with SLE patients.

CP groups. With regard to age, SLE/CP subjects were younger than control/CP individuals (40.09 \pm 10.09 vs. 46.67 \pm 12.36 years, $p=0.009$), but they were older than SLE/non-CP patients (40.09 \pm 10.09 vs. 32.29 \pm 6.98 years, $p=0.002$). Control/CP subjects were also older than control/non-CP individuals (46.67 \pm 12.36 vs. 33.65 \pm 12.82 years, $p < 0.0005$) (Table I).

Salivary levels of IL-6 and IL-17 were significantly higher in SLE/CP group compared to control/CP group (Fig. 1E and H). Otherwise, we observed significantly lower salivary levels of IL-33 in SLE/non-CP compared to controls/non-CP (Fig. 1G). The presence of periodontal inflammation significantly increased the levels of IL-6, IL-17A and IL-33 in saliva of SLE patients compared to SLE/non-CP (Fig. 1E, G and H). No significantly differences were seen comparing the concentra-

tions of IL-2, INF γ , TNF- α , IL-4, IL-10, MMP2/TIMP2, RANK and OPG between the groups (Fig. 1).

Table II shows the correlations between SLE variables and periodontal parameters. Positive correlations were observed between periodontal destruction (PD, CAL, CS and missing teeth) and SLE duration ($p=0.016$, $p=0.003$, $p=0.024$ and $p=0.006$ respectively). Cumulative dose of corticoids correlated with PD ($p=0.021$) and CS ($p=0.011$). The SLE index of damage (SDI) was positively correlated with two measures of periodontal damage (CAL and missing teeth, $p < 0.05$). Additionally, missing teeth positively correlated with PD ($\rho=0.359$, $p=0.002$), CAL ($\rho=0.555$, $p < 0.0005$), BP ($\rho=0.307$, $p=0.010$) and CS ($\rho=0.429$, $p < 0.0005$).

Table III presents the correlation between salivary cytokines, periodontal condition and SLE parameters of activi-

ty and damage. Salivary cytokine levels showed no correlation with periodontal parameters, SLEDAI-2K, the different subsets of SLE activity, SDI and cumulative dose of corticoids. There was no difference in salivary concentrations of all dosed cytokines between SLE patients that were or not under corticoids, antimalarial or immunosuppressive therapy at the moment of data collection ($p > 0.05$).

After multivariate analysis, the cumulative dose of corticoids was the SLE variable that remained associated with the highest number of periodontal destruction parameters such as PD ($p=0.001$), CAL ($p=0.021$) and CS ($p=0.005$). Regarding cytokines, salivary concentration of IL-33 was positively associated with SLEDAI-2K ($p=0.009$). IL-6 and IL-17A presented no correlation with periodontitis and SLE parameters.

Discussion

The main findings of this study are summarised as follows: 1) SLE patients with chronic periodontitis presented increased salivary concentration of IL-6 and IL-17A than respective controls; 2) positive correlations were seen between SLE parameters (SLE duration, cumulative dose of corticoids and SDI) and periodontal parameters (PD, CAL, CS and missing teeth); 3) the cumulative dose of corticosteroids correlated with periodontal damage and increased salivary levels of IL-33 were associated with high SLE activity in linear regression models 4) frequency and severity of CP were similar in SLE and control groups, but periodontitis seemed to affect SLE individuals earlier.

SLE is an autoimmune disease in which cytokines play a significant role priming the immune system (23). This mechanism might contribute for exacerbated periodontal inflammation triggering earlier periodontitis observed in SLE individuals. Moreover, chronic periodontal inflammation might induce systemic effects (4-7) that in turn can perpetuate inflammatory response increasing SLE damage.

Increased salivary levels of IL-17A were observed in SLE patients with periodontitis. TH17-cells play a central role in the pathogenesis of auto-

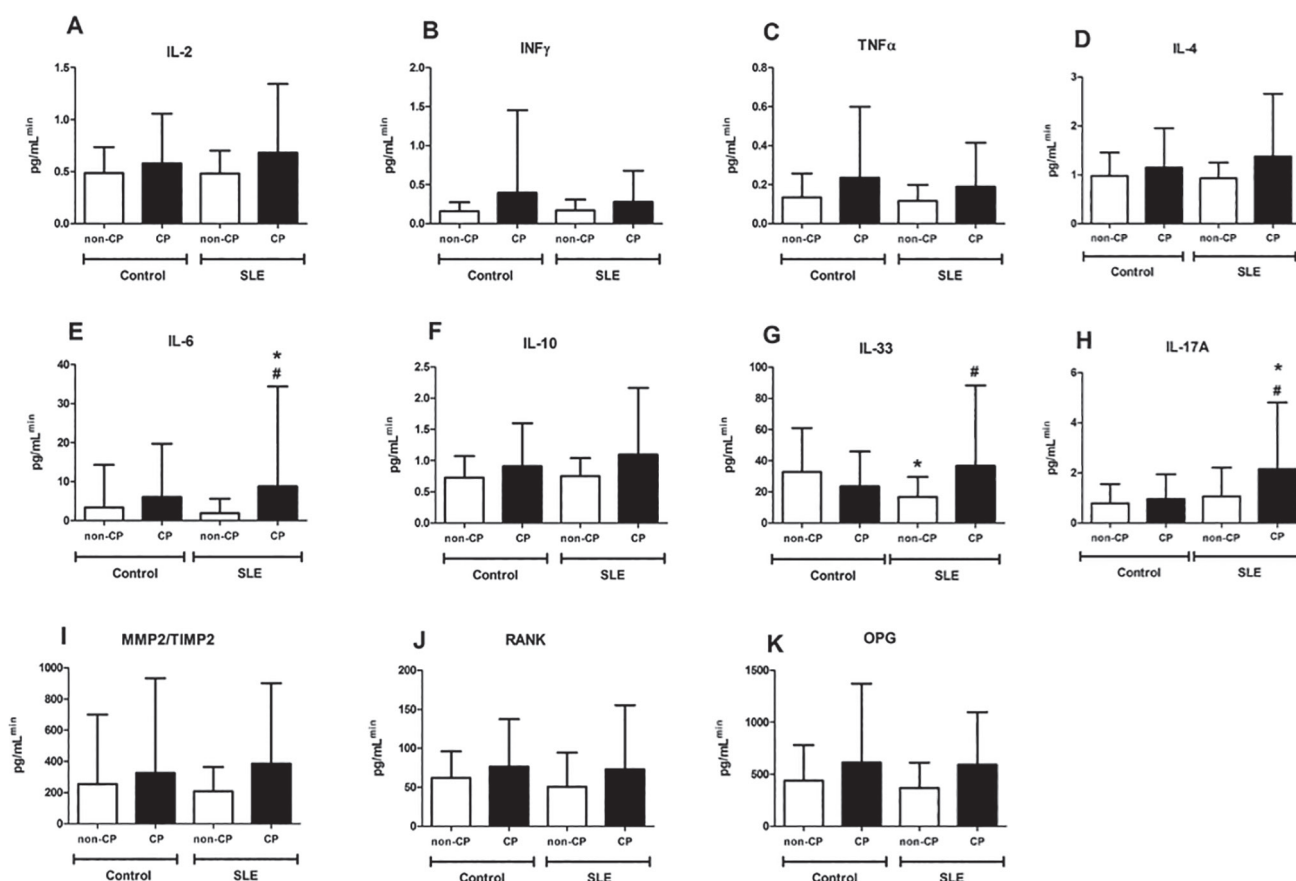


Fig. 1. Concentration of IL-2 (A), INF γ (B), TNF- α (C), IL-4 (D), IL-6 (E), IL-10 (F), IL-33 (G), IL-17A (H), MMP2/TIMP2 (I), RANK (J) and OPG (K) in saliva of study subjects. Control: subjects without systemic rheumatic diseases; SLE: systemic lupus erythematosus subjects; Non-CP: subjects without chronic periodontitis (CP); CP: subjects with CP. #statistically different compared to non-CP subjects within the same group. *Statistically different compared to controls with the same periodontal condition. $p < 0.05$, Kruskal-Wallis and Mann-Whitney test.

immune diseases and IL-17, specifically, amplifies the immune response in SLE by increasing the production of autoantibodies by B cells contributing to injury of target organs (24). Consistently, plasma levels of IL-17A were positively correlated with SLEDAI and poor prognosis in SLE nephritis (23). Therefore, this cytokine is emerging as potential therapeutic target for SLE treatment (23, 24). The elevated concentration of IL-17A in saliva of SLE patients with periodontitis might suggest periodontium as SLE target structure. In line with these results, IL-17A has been detected at SLE target organs including central nervous system, skin and kidney (23, 25, 26) IL-17A also plays an important role in the pathogenesis of periodontitis, mainly affecting osteoclastogenesis and neutrophil recruitment to periodontal sites (27). We also observed an increase of IL-6 in saliva of SLE patients with periodonti-

Table II. Correlation between SLE variables and periodontal parameters (rho values).

	PD mean (mm)	CAL mean (mm)	BP (% sites)	CS (% sites)	Missing teeth (number)
Age (years) mean (\pm SD)	0.194	^a 0.475	0.076	^a 0.277	^a 0.621
SLE duration (years)	^a 0.288	^a 0.350	0.076	^a 0.269	^a 0.326
Cumulative dose of corticoids (mg/prednisone)	^a 0.276	0.220	0.172	^a 0.301	0.121
SLEDAI-2K	0.136	0.168	-0.079	-0.008	-0.039
SDI	0.186	^a 0.257	0.073	0.115	^a 0.371
C3 (mg/dL) (\pm SD)	-0.054	-0.073	0.091	-0.003	0.011
C4 (mg/dL) (min-max)	0.037	0.018	0.031	0.053	0.027
CRP (mg/dL) (min-max)	0.079	0.078	-0.217	-0.077	-0.015

PD: probing depth; CAL: clinical attachment loss; BP: sites with bleeding upon probing; CS: concomitant sites (with bleeding upon probing and PD \geq 4mm). SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2K; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus. ^aStatistically significant correlation ($p < 0.05$).

tis. IL-6 participates in the differentiation process of naïve T cells into TH-17 cells and promotes activation and/or differentiation of macrophages, neutrophils, T cells and B cells that are involved in SLE systemic auto-immunity (28). High levels of IL-6 were identified

in serum of SLE patients and correlated with SLEDAI (29). Besides its systemic effects, IL-6 also mediates local inflammation in lupus nephritis, cardiopulmonary complications, neuropsychiatric problems and joint damage (30, 31). Accordingly, IL-6 might be a link between

Table III. Correlation between salivary cytokines levels and periodontal, laboratorial and clinical parameters in SLE subjects (rho values).

	IL-2	IFN γ	TNF- α	IL-4	IL-6	IL-10	IL-33	IL-17A	RANK	OPG	MMP2 TIMP2
PD mean (mm)	0.009	-0.009	-0.010	0.058	0.209	0.037	0.166	0.055	0.038	0.130	0.028
CAL mean (mm)	0.011	-0.015	0.053	0.054	0.195	0.033	0.171	0.107	-0.005	0.126	0.042
BP (% sites)	0.128	0.011	0.081	0.146	0.146	0.149	-0.116	0.166	-0.047	0.185	0.073
CS (% sites)	0.041	-0.023	0.066	0.118	0.118	0.088	0.035	0.118	-0.122	0.094	0.032
C3 (mg/dL)	-0.126	-0.141	-0.151	-0.092	-0.124	-0.087	-0.214	-0.106	-0.140	*-0.261	-0.052
C4 (mg/dL)	-0.028	0.004	-0.008	0.004	-0.005	0.030	0.006	-0.084	-0.101	-0.107	-0.030
SLEDAI-2K	0.115	0.066	0.171	0.098	0.107	0.098	0.199	0.135	0.109	0.131	-0.022
SDI	-0.014	-0.014	0.051	0.022	0.171	0.006	-0.044	-0.026	0.046	0.134	0.148
Cumulative dose of corticoids (mg/prednisone)	-0.161	-0.177	-0.064	-0.143	0.069	-0.124	-0.158	0.017	-0.182	0.118	0.018

Spearman's correlation coefficient (rho values). *Statistically significant correlation ($p < 0.05$).

systemic disease and local inflammation and a potential marker of periodontitis activity. Indeed, IL-6 is a key molecule for alveolar bone resorption and connective tissue destruction (32) and subjects with periodontitis present higher levels of IL-6 in serum, saliva and GCF than healthy controls (33).

In our study, periodontitis increased the salivary concentration of IL-33 in SLE patients. Although experimental studies indicated that IL-33 plays a role in periodontal disease (34, 35), clinical studies failed to prove that IL-33 concentration was increased in GCF, saliva and serum of patients with periodontitis (36, 37). Furthermore, the precise function of IL-33 in SLE pathogenesis remains unclear (38). A study reported that SLE patients and healthy volunteers showed similar concentration of IL-33 in serum (39). On the other hand, another study found that IL-33 serum concentration was significantly increased in SLE patients compared to healthy controls (40). Additionally, the serum levels of IL-33 correlated with inflammatory markers (ESR and CRP) in SLE patients, but no association of serum levels of IL-33 and SLEDAI was found, suggesting that IL-33 may have a role in acute phase of SLE, but could not reflect the entire progression of this disease expressed through SLEDAI (40). In the present study, all SLE patients were under treatment for approximately 11.29 (± 7.55) years and most of them did not present high SLE activity what could explain reduced levels of IL-33 in saliva of SLE/non-CP patients and no difference between SLE/CP and control/CP group. However, after mul-

tivariate analysis, salivary IL-33 levels correlated exclusively with SLEDAI-2K suggesting that lupus activity interfered with IL-33 concentration in oral cavity and that IL-33 might be a link between two diseases.

The other cytokines analysed (IL-2, IFN- γ , TNF- α , IL-4, IL-10, MMP2/TIMP2, RANK and OPG) did not differ between SLE and controls. Contradictory results were reported when levels of IFN- γ , IL-10, IL-17, IL-1 β and IL-4 were measured in saliva of SLE patients with and without CP. This study failed to identify a significant difference in IL-17 salivary concentrations, but it also found no differences in salivary concentrations of IL-4, IL-10 and IFN- γ between these groups (17). In the present study, the frequency and severity of CP were similar in SLE and control groups. Similar to our finding several studies found no difference in some periodontal parameters of CP severity between SLE and control groups (12, 13, 15, 18, 41). However, others studies showed that some periodontal parameters were less severe (12, 13, 17, 18) or more severe (12, 13, 42, 43) in SLE group. The truly CP frequency in SLE is debatable in the literature with a wide range of variability, probability due to methodological issues, such as application of different criteria for CP diagnosis and sample recruitment (1, 11). We verified that CP occurred earlier in SLE patients in accordance with previous results (15). SLE patients have high risk to infections due to frequent use of immunosuppressive medications and reduced host resistance (44). It is possible that the long-term therapy

with corticoids has contributed to anticipate the onset of CP and increase of periodontal destruction since corticoids reduce the immune response and consequently the control of periodontal pathogens. Thus, increased bacterial load at periodontal sites resulted in high damage of periodontal structures at younger ages (45). Indeed, in our study, we found that cumulated dose of corticoids was associated with periodontal damage in a multivariate model.

Possible limitations of the present study are its cross-sectional design and the SLE population evaluated, with long-lasting disease, low SLEDAI-2K and frequent cumulative damage, as evaluated by SDI score greater than 1 in half of patients. Furthermore, the heterogeneity of the individuals enrolled, *e.g.* organ involvement, therapies and comorbidities, and the limited sample size might have an impact on the results. A longitudinal study in which individuals were followed just after SLE diagnosis, for monitoring periodontal condition and cytokines levels in saliva and serum would produce substantial information about diseases progression and onset biomarkers.

To our knowledge this is the first study that analysed the association between salivary cytokines, periodontal status and SLE parameters. In conclusion, despite the similar frequency and severity of CP in SLE and control subjects, CP occurs earlier in SLE subjects. Our findings suggest that the long-term therapy with corticoids would contribute with periodontal destruction in SLE patients. Moreover, the increased levels of IL-6, IL-17A and IL-33 in saliva

of SLE subjects with CP may point it as possible inflammatory pathways in this process. Future clinical studies using larger and homogenous SLE population and concomitant analysis of serum and saliva are important to confirm our findings.

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