Antibodies to periodontogenic bacteria are associated with higher disease activity in lupus patients

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Abstract

Objective

Microbial infections and mucosal dysbiosis influence morbidity in patients with systemic lupus erythematosus (SLE). In the oral cavity, periodontal bacteria and subgingival plaque dysbiosis provide persistent inflammatory stimuli at the mucosal surface. This study was undertaken to evaluate whether exposure to periodontal bacteria influences disease parameters in SLE patients.

Methods

Circulating antibodies to specific periodontal bacteria have been used as surrogate markers to determine an ongoing bacterial burden, or as indicators of past exposure to the bacteria. Banked serum samples from SLE patients in the Oklahoma Lupus Cohort were used to measure antibody titres against periodontal pathogens (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Treponema denticola) and commensals (Capnocytophaga ochracea, and Streptococcus gordonii) by ELISA. Correlations between anti-bacterial antibodies and different clinical parameters of SLE including, autoantibodies (anti-dsDNA, anti-SmRNP, anti-SSA/Ro and anti-SSB/La), complement, and disease activity (SLEDAI and BILAG) were studied.

Results

SLE patients had varying amounts of antibodies to different oral bacteria. The antibody titres against A. actinomycetemcomitans, P. gingivalis, T. denticola, and C. ochracea were higher in patients positive for anti-dsDNA antibodies, and they showed significant correlations with anti-dsDNA titres and reduced levels of complement. Among the periodontal pathogens, only antibodies to A. actinomycetemcomitans were associated with higher disease activity.

Conclusion

Our results suggest that exposure to specific pathogenic periodontal bacteria influences disease activity in SLE patients. These findings provide a rationale for assessing and improving periodontal health in SLE patients, as an adjunct to lupus therapies.

Key words

systemic lupus erythematosus, periodontitis, antibodies

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Introduction

Systemic lupus erythematosus is a complex autoimmune disorder affecting multiple organ systems such as the skin, kidneys, heart, lung, and brain (1). The presence of autoantibodies against multiple self-components is a hall mark of SLE and the autoantibody specificities are associated with distinct pathologic features of the disease. In addition to a strong genetic predisposition, environmental factors, particularly infectious agents, can play a role in SLE pathogenesis. In SLE, microbes hold the potential to initiate and exacerbate autoimmunity, and modify the course of clinical disease by affecting multiple pathways (2).

The association between antibodies to infectious agents and antibodies to self-proteins has been established in a wide spectrum of autoimmune diseases (3, 4). Over the past several years, considerable attention has been directed towards investigating how viral exposures, such as Epstein Barr virus, influence the disease (5). However, bacterial infections affecting the respiratory tract, urinary tract, and skin are often seen in SLE patients (6). In addition to these anatomic sites, the gingival mucosal surface is continuously exposed to a plethora of bacteria present in the dental plaque. The interface between the dental plaque and the gingival epithelium represents a site of constant communication between microbes and the innate immune system (7). Further, these bacteria have access to the systemic circulation through bleeding gums and through direct invasion of the gingival epithelium (8, 9). Previous work from our laboratory suggests that the dental plaque can offer a chronic source of autoantigen mimics (10). T cells reactive with a Sjögren's syndrome and lupus-associated autoantigen Ro60, can be activated by peptides derived from dental plaque bacteria. Thus, exposure of the immune system to dental plaque bacteria in SLE patients holds a significant potential to modulate the disease.

The knowledge of lupus patient responses to dental plaque bacteria is limited. The indication that lupus patients are exposed to these bacteria can be derived indirectly from reports showing higher incidence of periodontitis in SLE patients (11). In addition, a recent report on the characterisation of the subgingival microbial community showed that compared to non-lupus controls, SLE patients have significant dysbiosis in the periodontal microbiota, with a greater proportion of pathogenic bacteria (12). Whether exposure to the periodontal bacteria affects disease activity in SLE is still not clear. The goal of the present study was to determine if bacteria in the dental plaque influence autoimmune features in SLE patients. Since high titres of IgG antibodies to plaque bacteria indicate either an ongoing infection or prior history of exposure (13-16), banked sera from a well characterised SLE patient cohort were analysed for antibodies to a selected panel of oral bacteria. The associations between anti-bacterial antibodies and clinical disease were evaluated.

Methods

Patients and controls

All experiments were performed in accordance with the Helsinki Declaration and approved by the Oklahoma Medical Research Foundation Institutional Review Board. Banked serum samples and clinical data were obtained from SLE patients seen from May 2002 to October 2014 in the Oklahoma Lupus Cohort through the Oklahoma Rheumatic Diseases Research Cores Center. Patients who met the ≥4 American College of Rheumatology (ACR) SLE criteria, and were evaluated for disease activity (n=303) were studied. The demographics of the patients in this study are shown in Table I**.** Sera from de-identified volunteers, who did not have SLE were also studied for anti-bacterial antibodies (n=75).

Clinical parameters of SLE

Anti-dsDNA antibody was measured by the *Crithidia luciliae* indirect immunofluorescence assay. Antibodies to other autoantigens including Sm protein with ribonucleoproteins (SmRNP), Sjögren's syndrome antigen A (SSA/Ro), and Sjögren's syndrome antigen B (SSB/La) were measured using a bead-based assay. C3 and C4 complement levels were determined at the

Table I. Oklahoma Lupus Cohort patient demographics.

	SLE patients
Total, n	303
Female, n (%)	279 (92.1)
Age in years, median (range)	40 (16-71)
Duration of disease in years, median (range)	2 (0-39)
Race, n (%)	
Caucasian	165 (54.5)
African American	62 (20.5)
Asian	12 (3.9)
American Indian	41 (13.5)
Mixed (2 or more)	22 (7.2)
Pacific Islander	1 (0.3)
ACR SLE criteria, median (range)	6 (4-11)
SLEDAI, median (range), n	6 (0-24), 300
BILAG total, median (range), n	11 (0-42), 303

Diagnostic Laboratory of Oklahoma. Clinical assessments of SLE were done using the modified SELENA - Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (17) and the British Isles Lupus Assessment Group (BILAG-2004) Index (18).

Bacterial strains

Bacterial strains residing in the subgingival plaque in the oral cavity were used as antigens for ELISA, and were obtained from ATCC (Manassas, VA, USA). These include: red complex organisms implicated in chronic periodontal disease: *Prophyromonas gingivalis* (ATCC 33277) and *Treponema denticola* (ATCC 35405)*;* a periodontal pathogen associated with aggressive periodontal disease: *Aggregatibacter actinomycetemcomitans serotype b* (ATCC 28522*)*; and commensal bacteria: *Streptococcus gordonii* (ATCC 51656) and *Capnocytophaga ochracea* (ATCC 33596*)*.

Detection of antibody to bacterial strains

An ELISA-based assay to measure antibacterial antibodies was used as previously described (19). Sera from the Oklahoma Lupus Cohort were tested at 1:500 dilution. Serial dilutions of a pooled serum sample were included as a calibrator for each plate. A standard curve was constructed and anti-bacterial antibody titres (units/ml) were calculated for each sample. Samples with OD readings higher than the calibration curve were re-tested at higher dilutions.

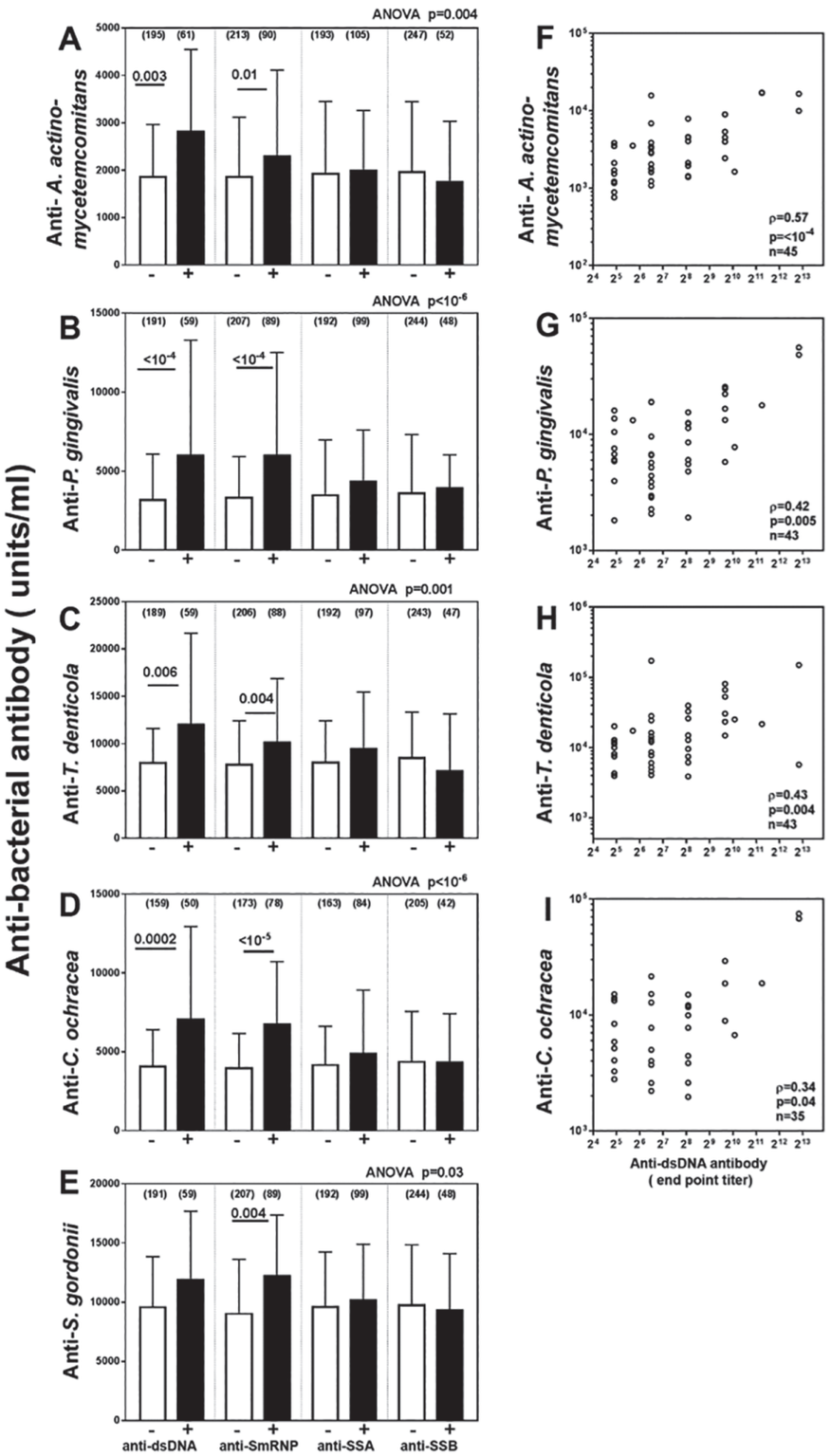

Fig. 1. Presence of anti-dsDNA antibody in SLE patients is associated with higher antibody titres against *A. actinomycetemcomitans, P. gingivalis, T.denticola and C. ochracea*.
(A-E) SLE patients were stratified into autoantibody positive or autoantibody negative groups based on reactivity to dsDNA, SmRNP, SSA/Ro, and SSB/La respectively and anti-bacterial antibody titres were compared using Kruskal Wallis test. Dunn's post-test was used to compare anti-bacterial antibodies between autoantibody positive and negative groups for each specificity. Anti-bacterial antibody titres are plotted as units/ml (median+interquartile range); (n) number of patients in each group.
(F-I) Correlation between anti-dsDNA titres and anti-bacterial antibody titres calculated by Spearman method.

Table II. Association of antibodies to periodontal bacteria and disease activity by SLEDAI.

Bacteria	Antibody titres (n)	SLEDAI SCORES			
		0-2	3-9	>10	*p*-value*
A.actino-mycetemcomitans	**median**	**1618**	**2021**$^{\#}$	**2318**$^{\#\#}$	**0.011**
	range	17-14520	275-21855	456-60160	
	(n)	(63)	(184)	(53)	
P. gingivalis	**median**	**2823**	**3616**	**6062**$^{\#\#\#}$	**0.0095**
	range	525-13550000	268-48470	225-88350	
	(n)	(62)	(179)	(52)	
T. denticola	**median**	**7524**	**8505**	**9903**	0.15
	range	894-94190	845-149900	1046-172500	
	(n)	(61)	(179)	(51)	
C.ochracea	**median**	**3929**	**4668**	**4383**	0.07
	range	943-24030	677-122500	714-107800	
	(n)	(54)	(160)	(37)	
S.gordonii	**median**	**9999**	**9848**	**9562**	0.86
	range	1393-30840	1630-45610	1826-32980	
	(n)	(62)	(179)	(52)	

*by Kruskal Wallis test; $^{\#}p$=0.04, $^{\#\#}p$=0.007, $^{\#\#\#}p$=0.0047 by Dunn's post-test.

Table III. Association of antibodies to periodontal bacteria and disease activity by BILAG.

Bacteria	Antibody titres (n)	TOTAL BILAG		
		Mild (C/D)	Mod. Severe to severe (≥3B or ≥1A)	*p*-value
A. actinomycetemcomitans	**median**	**1794**	**2534**	**0.043**
	range	17-16960	350-21855	
	(n)	(90)	(43)	
P. gingivalis	**median**	**3408**	**5026**	0.056
	range	572-307000	307-88350	
	(n)	(90)	(43)	
T. denticola	**median**	**8066**	**10565**	0.23
	range	893-149900	1922-80900	
	(n)	(90)	(42)	
C. ochracea	**median**	**4197**	**5369**	0.08
	range	943-122500	761-95120	
	(n)	(76)	(35)	
S. gordonii	**median**	**10012**	**9907**	0.46
	range	1393-45610	3528-25180	
	(n)	(90)	(43)	

Statistical analyses

Graph Pad Prism 7.0 and Systat software were used to perform statistical analyses. Normality tests were performed on each dataset and non-parametric tests were used for non-Gaussian distributions. Mann-Whitney test was used to compare two populations; Kruskal-Wallis test followed by Dunn's post-test for multiple comparisons. Correlations were determined by Spearman's method. A *p*-value of less than 0.05 at a 95% confidence interval was considered significant.

Results

Higher anti-bacterial antibody titres against specific bacteria are associated with the presence of anti-dsDNA

Patients were stratified into groups based on the presence or absence of antibodies to the major lupus-associated autoantigens: dsDNA, Sm/RNP, SSA/Ro, and SSB/La. The anti-bacterial antibody titres in the antibody positive and negative group for each autoantigen were compared. As shown in Figure 1A-E, patients with anti-dsDNA have higher antibodies to *A. actinomycetemcomitans, P. gingivalis, T. denticola,* and *C. ochracea*, but not to *S. gordonii*, when compared to patients lacking anti-dsDNA. The presence of anti-SmRNP antibodies was associated with higher anti-bacterial antibody titres against all the bacteria, while anti-SSA and anti-SSB failed to associate with antibodies to any of the periodontal bacteria.

Among the anti-dsDNA positive patients, anti-*A. actinomycetemcomitans* antibodies showed the strongest correlation (Spearman rho=0.57; $p<10^{-4}$) with anti-dsDNA antibody titres (Figure 1F). Anti-*P. gingivalis (*rho=0.42; *p*=0.005), anti-*T. denticola* (rho=0.43; *p*=0.004) and anti-*C. ochracea* (rho=0.34; *p*=0.04) also showed statistically significant, albeit more modest correlations (Fig. 1G-I).

Antibodies to specific periodontal pathogens correlate with indicators of higher disease activity in SLE patients

SLE patients were categorised based on disease activity into mild (SLEDAI ≤2), moderately severe (SLEDAI 3-9) and severe (SLEDAI ≥10) groups. As shown in Table II, a comparison of anti-bacterial antibody titres between these 3 groups showed a statistically significant association between increased disease activities with higher anti-*A. actinomycetemcomitans* antibody (*p*=0.011) and higher *anti-P. gingivalis* antibody titres (*p*=0.0095).

The anti-*A. actinomycetemcomitans* antibody titres in patients with the lowest disease activity scores were significantly lower than the patients with moderate (*p*=0.04) or highest activity (*p*=0.007). Anti-*P. gingivalis* antibodies were significantly lower in the patients with lowest disease activity compared to the highest activity scores (*p*=0.0047).

The British Isles Lupus Assessment Group (BILAG) index uses different clinical measures for evaluation of disease activity based on individual organ involvement (18). The disease activity in each patient was classified as mild (only C and D organ scores; n=90), moderate (1- or 2- B scores; n=168), and moderately severe to severe (≥3- B or ≥1- A score; n=43). As shown in Table III, the anti-*A. actinomycetemcomitans* antibody titres were significantly higher in patients with moderately severe to severe activity compared to the patients with mild disease (*p*=0.043). A similar trend was seen in anti-*P. gingivalis* antibody titres, although it failed to reach statistical significance (*p*=0.056). Associations were not seen between disease activity and antibody titres to *T. denticola, C. ochracea* or *S. gordonii*.

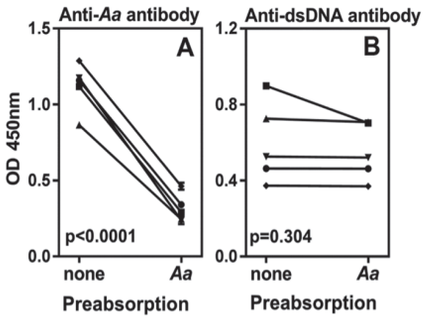

Fig. 2. Limited cross reactivity between anti-*A. actinomycetemcomitans* and anti-dsDNA antibodies in SLE patients. IgG purified from five SLE patients were pre-absorbed with *A. actinomycetemcomitans*, and the residual antibodies to *A. actinomycetemcomitans* **(A)** and dsDNA **(B)** were measured by ELISA. Sera not pre-absorbed with *A. actinomycetemcomitans* were used as controls. Each line connects control and *A. actinomycetemcomitans* absorbed serum for the same patient. *p*-values were calculated by paired *t*-test.

Discussion

By using banked serum samples from a well characterised cohort of SLE patients, this study demonstrates that high titre of IgG antibodies against the periodontal pathogen *A. actinomycetemcomitans* was significantly associated with higher disease activity in SLE patients. Considered together with previous reports investigating the association between periodontal disease and lupus (20-23), our study supports the notion that bacterial infections in the subgingival mucosa modulate SLE.

In this study we used the presence of circulating anti-bacterial antibodies as evidence for exposure to the bacteria. This strategy is well established and antibody titres against bacteria from the subgingival plaque have been used as indicators for exposure to and burden of specific bacteria (13-16). A recent study showed a strong correlation between the detection of antibodies to *A. actinomycetemcomitans* and its leukotoxin with the presence of *A. actinomycetemcomitans* in the sub-gingival isolates from individual patients (24). Further, anti-*A. actinomycetemcomitans* and anti-*P. gingivalis* antibody titres reflecting exposure to those bacteria are known to persist for 15 years (25). A similar clinical observation was also made in another study where elevated titres of anti-*P. gingivalis* antibodies persisted when monitored for 24 months even when the disease was inactive (26). Thus, detecting high titre of antibodies against organisms associated with periodontitis in our cohort of SLE patients suggests that the patients immune system was exposed to these oral pathogens.

The presence of anti-dsDNA antibody is a hallmark of SLE, and it showed strongest correlation with anti-*A. actinomycetemcomitans*. Although the precise specificity of anti-*A. actinomycetemcomitans* antibodies is not known, they do not appear to cross-react with DNA. While absorption of anti-*A. actinomycetemcomitans* IgG with *A. actinomycetemcomitans* significantly reduced anti-bacterial reactivity, it failed to reduce dsDNA reactivity (Fig. 2). These data suggest that the *A. actinomycetemcomitans* mediated periodontal disease process might contribute towards amplifying anti-dsDNA in SLE patients. This is highly plausible considering the study by Konig et al which implicates *A. actinomycetemcomitans* as a trigger for rheumatoid arthritis (24).

We had previously reported that bacterial proteins from oral commensal bacteria, specifically *C. ochracea* were capable of activating T cells reactive with the Sjögren and lupus associated autoantigen, SSA/Ro60. Therefore, the lack of association between antibodies to periodontal bacteria and SSA and SSB was a surprising result. Since SSA and/or SSB antibody positivity was significantly associated with the history of sicca or dry mouth in our lupus patients (p=0.015, n=299), anti-bacterial antibodies were compared between patients with or without sicca. As shown in Supplementary Fig. 1, anti-bacteria antibody titres between patients with or without sicca were not significant. These results support previous literature showing lack of association between clinical and microbiologic parameters of periodontal disease and Sjögren's syndrome, a disease characterised by reduced saliva production and progressive loss of salivary gland function (27-29). In contrast to periodontal disease, lack of saliva has been found to be strongly associated with dental caries and supra-gingival bacteria, which are distinct from the periodontal bacterial populations (30).

Our clinical analyses showed a significant association between higher anti-*A. actinomycetemcomitans* antibody titres and increased disease activity with both SLEDAI and BILAG indices. Higher anti-*P. gingivalis* antibody titres were also strongly associated with higher SLEDAI scores. Anti-dsDNA antibodies are a scoring criterion for SLEDAI, and multivariate analyses showed that the SLEDAI association with anti-*P. gingivalis* antibodies was significantly driven by anti-dsDNA positivity. However, this fails to explain the trend between higher *P. gingivalis* antibodies and higher BILAG indices. Further, despite the comparable association of anti-*T. denticola* antibodies with higher anti-dsDNA as well as, C3 and C4 complement depletion (Supplementary Fig. 2), there was a lack of association between anti-*T. denticola* antibodies and either measure of disease activity. An important factor to consider is that the BILAG and SLEDAI are very different kinds of measurement. While SLEDAI scores are more affected by global increases in disease activity, the BILAG index is more sensitive and fluctuates with changes even in an individual manifestation of the disease. Taken together, these results suggest the need for additional studies to investigate the contribution of exposure to *P. gingivalis* in SLE. The present study suggests that the relationship between SLE and periodontal disease is defined by the specific pathogen, *A. actinomycetemcomitans* driving the periodontal disease.

A unique characteristic of *A. actinomycetemcomitans* is their ability to induce citrullination of host proteins. A recent report showed that Leukotoxin A, an exotoxin produced by *A. actinomycetemcomitans,* induces permiabilisation of the cell plasma membranes leading to an unregulated influx of calcium in toxin susceptible cells (24, 31). This activates the endogenous protein arginine deiminase (PAD) enzymes in the cytosol (PAD2) and the nucleus (PAD4) and causes hypercitrullination of self-proteins, which in neutrophils, leads to cell death. Thus, exposure to *A. actinomycetemcomitans* and not the other periodontal pathogens in lupus patients holds the potential to amplify a pathogenic autoimmune response through the release of modified self-antigens.

This is a cross sectional study, and therefore it does not allow us to postulate causal associations between periodontal bacterial exposure and SLE. Further, in a study of this size, the heterogeneous medications given to the patients, and the lack of this information prevents the ability to make proper adjustments of data for treatment. It is possible that immune modulators may influence periodontal bacteria, and future studies with medication controlled protocols will be helpful. The sera were obtained from samples banked over an extended period of time and therefore, retroactive dental information and periodontal health in the patient and control populations were not available. This lack of data precluded the ability to set cut-offs for pathogenic antibacterial antibody positivity since it is possible that the non-SLE controls were also exposed to some of these bacteria (Supplementary Fig. 3). Despite these limitations, our data suggest an association between exposure to specific periodontal pathogens and lupus disease activity in SLE patients. Previous studies show a higher incidence of periodontal disease in SLE patients (20, 21). Taken together, all these studies confirm the interaction between periodontal disease and SLE, and specifically, suggest a role for gingival bacterial infections as a factor for increasing morbidity in SLE.

An interesting clinical study in SLE patients (n=49) showed that aggressive treatment of periodontitis resulted in a significant improvement in the responses to lupus therapy (23). This report complements our finding of higher lupus activity in patients with potential exposure to specific bacteria. Prevention of periodontal disease and management of periodontal health have been advocated as simple tools for reducing morbidity in debilitating systemic disease states (32). Our study provides support for conducting systematic clinical investigations into periodontal disease in SLE and a rationale for aggressive management of periodontal health in SLE patients.

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