


DNMT3B (rs2424913) polymorphism is associated with systemic lupus erythematosus alone and with co-existing periodontitis in a Brazilian population

Abstract


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The association between Periodontitis and Systemic Lupus Erythematosus (SLE) has been primarily based on their similar pathophysiology and both are associated with genetic polymorphisms. Objectives: To investigate an association between the methylation-related gene polymorphisms *DNMT3B* (rs2424913) and *MTHFR* (rs1801133) to Systemic Lupus Erythematosus (SLE) and Periodontitis. Methodology: In total, 196 individuals of all genders aged 24 to 60 years old were allocated into four groups based on their systemic and periodontal status, namely: Healthy control (n=60), periodontitis (n=51), SLE (n=47), and SLE + periodontitis (n=38). Individuals with SLE were stratified according to disease activity (SLEDAI) in inactive or active. We performed polymorphism analysis using PCR-RFLP with genomic DNA from mouthwash. We analyzed data using Fisher's Exact, Chi-square test, and regression models. Results: Periodontal status were similar in subjects with periodontitis alone and combined with SLE. SLE patients with periodontitis had a longer SLE diagnosis than SLE only (p=0.001). For *DNMT3B* polymorphism, the periodontitis, SLE, and Inactive SLE + periodontitis groups showed a higher frequency of T allele and TT genotypes compared to healthy controls (p<0.05). Regression analyses showed that the TT genotype is a strong risk factor for periodontitis (OR=4.53; CI95%=1.13–18.05) and also for SLE without periodontitis (OR=11.57; CI95%=3.12–42.84) and SLE with periodontitis (OR=5.27; CI95%=1.25–22.11) when compared to control. Conclusion: SLE patients with periodontitis had a longer length of SLE diagnosis. The *DNMT3B* (rs2424913) polymorphism was associated with periodontitis and SLE alone or combined with periodontitis. Our study contributes to understanding the genetic mechanisms involved in periodontitis and SLE susceptibility.

Keywords: Periodontitis. Systemic lupus erythematosus. Polymorphism. *MTHFR*. *DNMT*. Inflammation.

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Introduction

The association between Periodontitis and Systemic Lupus Erythematosus (SLE) has been primarily based on their similar pathophysiology.¹ Periodontitis is a chronic inflammatory disease, in which attachment loss and bone destruction around teeth result from an imbalance in the host immune response against a dysbiotic dental biofilm in susceptible individuals.² SLE is a systemic autoimmune disease characterized by connective tissue destruction in multiple organs under abnormal T cell activity and high autoantibody levels.³

Increasing epidemiologic evidence has shown a high prevalence and an increased risk of periodontitis in SLE patients.^{4,5} Conversely, the literature shows controversial data concerning periodontal status in SLE patients, and some studies reported a milder,⁶ a similar,⁷⁻⁹ and a more severe form of periodontitis in SLE patients.¹⁰ Moreover, interventional studies have demonstrated the beneficial effect of periodontal therapy in reducing SLE disease activity¹¹ and ameliorating the response to treatment. Indeed, few mechanistic studies¹² elucidate the relationship between periodontitis and SLE; however, most of the underlying mechanisms remain unknown.¹²

Studies have reported genetic polymorphisms in both periodontitis¹³ and SLE¹⁴ in genes bonded to inflammatory pathways. Despite the evidence of epigenetic mechanisms involved in both diseases, epigenetic pathways related genes are, so far, neglected. Researchers have reported DNA hypermethylation and hypomethylation in a variety of genes in individuals with periodontitis and SLE.^{15,16} These epigenetic changes, in turn, can be caused by an increase or decrease of enzymes involved in epigenetic mechanisms such as methylenetetrahydrofolate (MTHFR) and DNA methyltransferase (DNMT), for example.

The enzyme encoded by *MTHFR* originates during folate metabolism the S-adenosyl methionine (SAM) radical (a methyl donor), whereas *DNMT* uses SAM as a substrate in the DNA methylation process.¹⁷ In particular, C677T *MTHFR* (rs1801133) polymorphism is related to a decreased enzyme activity of methylenetetrahydrofolate reductase, and -149C→T *DNMT3B* (rs2424913) polymorphism is related to an increased expression of *DNMT3B*. Changes in the activity or concentration of these enzymes can influence the DNA methylation profile,

which can, in turn, lead to changes in gene expression.^{18,19}

Studies have shown the involvement of *MTHFR* or *DNMT3B* polymorphisms in inflammatory diseases.^{20,21} Recently, our group revealed the association of the 149C→T *DNMT3B* (rs2424913) polymorphism with periodontitis,²² but not for C677T *MTHFR* (rs1801133). Our study aimed to study the association of these polymorphisms in individuals with SLE, as well as in individuals with SLE and periodontitis.

Methodology

Study design

This is a cross-sectional study comprising 196 individuals of all genders, aged 24 to 60 years old.

Research ethics

This study followed the Helsinki Declaration and was approved by the Institutional Research and Ethics Committee at the Federal University of Paraíba (UFPB) (CAAE: 03948118.0.0000.5183). All subjects signed an informed consent form.

Study local

The study was conducted at the dental (Department of Clinical and Social Dentistry) and medical (Center of Rheumatology at the Lauro Wanderley University Hospital) clinics of the Federal University of Paraíba, João Pessoa-PB, Brazil. The latter is a reference center for diagnostic and treatment of patients with SLE in the State of Paraíba. Individuals were selected to compose the study from new attendances and diagnosis realized during 2019.

Sample size

Adopting a medium effect size of proportion (0.50) for interest outcome of our study (difference in genotypic profile between SLE with and without periodontitis), a minimal necessary sample of 47 individuals with the outcome of interest (SLE) was estimated, considering a study power of 80% and a confidence interval of 95%. The *a posteriori* sample size was evaluated based on effect sizes for comparisons between the different subject groups and resulted in a study with an inference power of 80.24%. These estimates were performed using the G*Power software (version 3.1.9.2, Faul F., Universität

Kiel, Germany).

SLE and periodontal assessment

SLE status – All patients with SLE fulfilled at least four of the 11 criteria for the diagnostic classification of SLE established by the American College of Rheumatology.²³ This index comprises both clinical and laboratory characteristics to determine the activity of the disease. A rheumatologist classified in the first visit SLE activity into inactive (Systemic Lupus Erythematosus Disease Activity Index: SLEDAI <5) or active (SLEDAI ≥5) stages according to the guidelines of the American Academy of Rheumatology.²³ SLEDAI aims to assess disease activity in the last 10 days, by clinical and laboratory criteria.²⁴ The scale ranges from 0 to 105, with a grade being attributed to each clinical and laboratory domain evaluation. Moreover, medical history, including time since SLE diagnosis and medications protocol, were analyzed from the patient's medical record.

Periodontal status– Periodontal diagnosis was assessed by a single examiner previously calibrated ($\kappa=0.90$) and included plaque index (PI), probing depth (PD), bleeding on probing (BOP), clinical attachment level (CAL) at six sites per tooth as well as the number of missing teeth (at least 15 remaining natural teeth). Periodontal assessment was based on the current Classification of Periodontal and Peri-Implant Diseases and Conditions.²⁵ The control group (periodontal health) consisted of CAL=0 and PD<3 mm and BOP in <10% of sites. The primary criteria considered for the diagnosis of periodontitis was interproximal clinical attachment loss at least of 3 mm detected in two or more nonadjacent interproximal sites and secondarily PD≥5mm (which corresponds to stages II, III, and IV).²⁵ As that current periodontal inflammatory status does not influence the polymorphism analysis, SLE patients with stable periodontitis in a reduced periodontium according to the current Classification of Periodontitis²⁵ were included in the SLE + periodontitis group.

According to systemic condition (systemic health or with SLE diagnosis) and periodontal status (absence or presence of periodontitis), a convenience sample was categorized into four groups as follows: healthy control (n=60), periodontitis (n=51), SLE (n=47) and SLE + periodontitis (individuals with SLE also diagnosed with periodontitis) (n=38). Demographic and medical history of participants were assessed

using a questionnaire or the medical chart patient analysis. The exclusion criteria considered individuals under 18 years old, history of HIV or hepatitis, current diabetes, pregnancy, orthodontic treatment, cognitive disorders, smoking habits, and other autoimmune and chronic diseases (except Periodontitis and Systemic Lupus Erythematosus)

Sample collection

Genomic DNA samples were obtained from buccal mucosa cells by a mouthwash. Briefly, the cells were harvested by a one-minute mouthwash with 3% dextrose solution (6 mL) followed by TNE addition (3 mL). The homogenates were centrifuged at 3000 rpm for 10 minutes, and pelleted cell samples were stored in lysis solution at -20°C until DNA extraction.²⁶

DNA extraction and genetic polymorphism analysis

Genomic DNA was purified of oral epithelial cells using 8 M ammonium acetate as previously described.²⁶ The quantity and purity of DNA were determined on a Nanodrop spectrophotometer. The analysis of the single nucleotide polymorphisms (SNPs) C677T *MTHFR* (rs1801133) and -149C→T *DNMT3B* (rs2424913) was performed using the PCR-RFLP technique (polymerase chain reaction–restriction fragment length polymorphism), which consisted of DNA amplification by PCR and digestion of DNA fragments by a restriction enzyme. Restriction enzyme activity determined the presence or absence of the SNP. Briefly, PCR was performed using 100 ng of total DNA in a 15 µL final volume reaction containing 7.5 µL of GoTaq® G2 Hot Start Green Master Mix (Promega), 4.5 µL of DNA, and nuclease-free water in the presence of 1 µL of each primer (10 µM). Primer sequences, as well as both PCR and enzymatic digestion conditions, were used as previously described. PCR products were resolved by electrophoresis on 6% (w/v) polyacrylamide gels containing GelRed® (Biotium) or silver nitrate. The 677 CC/CT/TT and -149 CC/CT/TT genotypes were identified by their band pattern.²² SNPs were selected using the dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and were chosen based on functional significance and its relationship to inflammatory diseases. Minor allele frequency according to 1000Genomes database is 0.24 for rs1801133 and 0.31 for rs2424913.

Statistical analysis

The collected data were inserted in a Microsoft Excel platform and analyzed by descriptive and inferential statistic using the R Software (version 3.6.1). Demographic data were analyzed descriptively and the study groups ((1) control; (2) periodontitis; (3) SLE; (4) SLE+Periodontitis) were compared by One Way ANOVA with Tukey *post-hoc* test (for age, number of missing teeth, and CAL comparisons), by Chi-Square test with Yates correction (for gender comparisons), Student's *t*-test (for SLE duration comparisons), by Chi-Square test (for SLE activity, Medication use, Periodontal status, Sites with periodontal pockets, and Probing depth comparisons), by Kruskal-Wallis test (for Bleeding sites comparisons) and Mann-Whitney U test (for Sites with periodontal pockets and Probing depth comparisons). Hardy-Weinberg equilibrium (HWE) was estimated for each polymorphism using

the Chi-Square test. The Fisher's Exact test and Chi-Square test with and without Yates' correction were used to analyze possible associations among allele and genotypic frequencies, SLE and periodontitis. Binary logistic regression models were used to evaluate the variables associated to outcomes of interest. Five models were adjusted for regression analysis using the backward method: (Model 1): Periodontitis (1) × Control (0); (Model 2): SLE (1) × Control (0); (Model 3): SLE × Periodontitis (1) × Periodontitis (0); (Model 4): SLE with periodontitis (1) × Control; (Model 5): SLE with periodontitis (1) × periodontitis (0); (Model 6): SLE (1) × SLE with Periodontitis (0). In adjust of regression models, an univariate analysis was performed and, sequentially, the variables of interest that showed a significance level $\leq 0,20$ were included in multiple models for adjustment by backward method, remaining in adjusted models for

Table 1- Demographic data and clinical and periodontal status of the study population including the mean age, the mean time of SLE diagnosis (in years), disease activity (inactive or active), gender distribution, current use of drugs as well as periodontal status and the number of missing teeth are described as the mean \pm SD or total number (n) and percentage (%); periodontal parameters (bleeding sites, sites with periodontal pockets, probing depth and clinical attachment level are described as median (p25,p75)

Variable	Control (n = 60)	Periodontitis (n = 51)	SLE (n = 47)	SLE + Periodontitis (n = 38)	p value
Age (mean SD)	38.48 (\pm 8.16)	48.96 (\pm 11.30)	33.17 (\pm 8.95)	38.87 (\pm 10.03)	0.110 ^I
Gender n (%)					
Female	47 (78.30%)	29 (56.90%)	43 (91.50%)	36 (94.70%)	<0.001 ^{II}
Male	13 (21.70%)	22 (43.10%)	4 (8.50%)	2 (5.30%)	
SLE duration (years) (mean SD)	-	-	5.21 (5.17)	10.34 (7.81)	0.001 ^{III}
SLE activity					
Inactive SLE n (%)	-	-	20 (42.60%)	24 (63.20%)	<0.001 ^{IV}
Active SLE n (%)	-	-	27 (57.40%)	14 (36.80%)	
Medication use					
Use of corticosteroid n (%)	-	-	18 (38.30%)	16 (42.10%)	0.086 ^{IV}
Use of immunosuppressive n (%)	-	-	30 (63.80%)	17 (44.70%)	0.825 ^{IV}
Use of hydroxychloroquine n (%)	-	-	42 (89.40%)	34 (89.50%)	0.987 ^{IV}
Periodontal status					
Stage II n (%)	-	25 (49.00%)	-	18 (47.40%)	0.877 ^{IV}
Stage III or IV n (%)	-	26 (51.00%)	-	20 (52.60%)	
Number of missing teeth (mean SD)	-	7.84 (5.84)	8.04 (5.15)	9.16 (4.54)	0.332 ^I
Bleeding sites (median (p25; p75))	-	41.00 a (24.00; 56.00)	3.00 b (1.00; 8.00)	19.50 c (7.00; 40.00)	<0.001 ^V
Sites with periodontal pockets (median (p25; p75))	-	25.00 (16.00; 46.00)	-	12.00 (6.00; 23.75)	0.002 ^{VI}
Probing depth (median (p25; p75))	-	7.00 (6.00; 8.00)	-	5.50 (5.00; 7.00)	0.032 ^{VI}
CAL (mean SD)	-	4.77 (+1.57)	-	5.14 (+1.42)	0.253 ^{III}

I One-way ANOVA (Tukey post-hoc test); II Chi-Square test with Yates Correction; III Student T-test; IV Chi-Square test; V Kruskal-Wallis test; VI Mann-Whitney U test. Significance level=5%.

each outcome only variables with significance level of 5%. For all the analysis the significance level adopted was 5%.

Results

Demographic and periodontal conditions

Table 1 shows the demographic data and the clinical and periodontal status of the study population. The subjects in the control, Periodontitis, SLE, and SLE + Periodontitis groups had a similar mean age with no significant differences: Control (38.48±8.16 years old), Periodontitis (48.96±11.30 years old), SLE (33.17±8.95 years old), SLE + Periodontitis (38.87±10.03 years old); (p=0.110).

According to the medical records, patients with SLE without periodontitis showed a shorter time of SLE diagnosis (5.21±5.17 years), whereas patients from the SLE + Periodontitis group had a longer length of SLE diagnosis (10.34±7.81 years); (p=.001). The number of inactive cases (n=20; 42.6%) was lower than of active cases (n=27; 57.40%) in the SLE group

(p<0.001). For the SLE + periodontitis group, 63.20% (n=24) of subjects had inactive SLE, while 36.80% (n=14) of individuals had active SLE (p<0.001). No difference occurred in the profile of medication used by SLE subjects and patients with SLE and periodontitis (p>0.05).

Periodontal status was similar in both the periodontitis and SLE + periodontitis groups, showing no significant differences (p=0.877). We diagnosed subjects from the periodontitis group as Stage II (n=25; 49.0 %) and Stage III/IV (n=26; 51.0%); individuals with SLE + periodontitis were classified as Stage II (n=18; 47.4%) and Stage III/IV (n=20; 52.6%). The number of missing teeth was similar in all groups: periodontitis (7.84±5.84), SLE (8.04±5.15) and SLE + periodontitis (9.16±5.54); (p=0.332).

Genetic polymorphism

Table 2 and 3 shows the allelic and genotypic distributions of the study population. Genotypic frequencies are in accordance with HWE for both studied polymorphisms: *MTHFR* (rs1801133): Control (p=0.57), Periodontitis (p=0.13), SLE (p=0.25) and SLE + periodontitis (p=0.27), and

Table 2- Comparative analysis of allele frequencies of the studied polymorphisms (MTHFR and DNMT3B) among the control, periodontitis, SLE and SLE + periodontitis groups

Gene SNP	<i>MTHFR</i> C677T(rs1801133)		<i>DNMT3B</i> C46359T (rs2424913)	
Allelic frequency	C	T	C	T
Control¹ n=60	85 (70.80%)	35 (29.20%)	82 (68.30%)	38 (31.70%)
Perio² n=51	69 (67.60%)	33 (32.40%)	48 (47.06%)	54 (52.94%)
p value^{1,2}	0.181		0.011	
SLE³ n=47	70 (74.50%)	24 (25.50%)	37 (39.36%)	57 (60.64%)
p value^{1,3}	0.565		<0.001	
Inactive SLE⁴ n=20	28 (70.00%)	12 (30.00%)	13 (32.50%)	27 (67.50%)
p value^{1,4}	0.897		0.002	
Active SLE⁵ n=27	42 (77.78%)	12 (22.22%)	24 (44.44%)	30 (55.56%)
p value^{1,5}	0.360		0.002	
SLE + Perio⁶ n=38	51 (67.10%)	25 (32.90%)	41 (53.95%)	35 (46.05%)
p value^{1,6}	0.529		0.138	
Inactive SLE + Perio⁷ n = 24	32 (66.67%)	16 (33.33%)	24 (50.00%)	24 (50.00%)
p value^{1,7}	0.810		0.020	
Active SLE + Perio⁸ n= 14	19 (67.86%)	09 (32.14%)	17 (60.71%)	11 (39.29%)
p value^{1,8}	0.773		0.370	

Chi-Square test and Chi-Square test with Yates's correction. Significance level = 5%.

DNMT3B (rs2424913): Control (p=0.54), Periodontitis (p=0.89), SLE (p=0.72) and SLE + periodontitis (p=0.80).

For MTHFR, the C allele and genotypes CC and CT were the most common distributions in all groups, and we observed no significant differences (p>0.05).

For the DNMT3B polymorphism, the frequency of the T allele and the TT genotype respectively was higher in the periodontitis (52.9%; 27.5%) and in SLE (60.5%; 38.3%) groups compared to the control group (31.7%; 8.3%), even when the groups with inactive (67.5%; 40%) and active (55.5%; 37%) form of SLE were compared separately with the control group (p<0.05). When the comparison was made between the control (31.7%; 8.3%) and the SLE + periodontitis (46%; 21.1%) group, the difference was no longer observed (p>0.05). However, when stratifying the group according to the activity of SLE we detected a higher frequency of the T allele and the TT genotype

in the SLE group with inactive form (50%; 29.2%) in comparison to the control group (p<0.05).

Binary logistic regression analysis

Table 4 shows Binary Logistic regression (adjusted and non-adjusted) for different outcomes. We can evaluate that the TT allele it's a strong risk factor for periodontitis compared to control (OR=4.53; CI95%= .13 - 18.05) and also for SLE without periodontitis compared to control (OR=11.57; CI95%= .12 - 42.84) and SLE with periodontitis (OR=5.27; CI95% = 1.25 - 22.11) compared to control. As expected, the chance of observing bleeding sites is 1.05 times smaller in people with SLE without periodontitis compared to people with SLE with periodontitis (OR: 0.95 [0.92- 0.98]).

Table 3- Comparative analysis of genotype frequencies of the studied polymorphisms (MTHFR and DNMT3B) among the control, periodontitis, SLE and SLE + periodontitis groups

Gene SNP Genotype frequencies	MTHFR C677T (rs1801133)			DNMT3B C46359T (rs2424913)		
	CC	CT	TT	CC	CT	TT
Control¹	31	23	6	27	28	5
n=60	(51.70%)	(38.30%)	(10.00%)	(45.00%)	(46.70%)	(8.30%)
Perio²	21	27	3	11	26	14
n=51	(41.20%)	(52.90%)	(5.90%)	(21.60%)	(51.00%)	(27.50%)
p-value^{1,2}		0.275			0.005	
SLE³	27	16	4	8	21	18
n=47	(57.40%)	(34.00%)	(8.50%)	(17.00%)	(44.70%)	(38.30%)
p-value^{1,3}		0.842			<0.001	
Inactive SLE⁴	10	8	2	1	11	8
n=20	(50.00%)	(40.00%)	(10.00%)	(5.00%)	(55.00%)	(40.00%)
p-value^{1,4}		0.991			<0.001	
Active SLE⁵	17	8	2	7	10	10
n=27	(63.00%)	(29.60%)	(7.40%)	(26.00%)	(37.00%)	(37.00%)
p-value^{1,5}		0.618			0.003	
SLE + Perio⁶	19	13	6	11	19	8
n=38	(50.00%)	(34.20%)	(15.80%)	(28.90%)	(50.00%)	(21.10%)
p-value^{1,6}		0.735			0.105	
Inactive SLE+ Perio⁷	11	10	3	7	10	7
n=24	(45.80%)	(41.70%)	(12.50%)	(29.20%)	(41.70%)	(29.20%)
p-value^{1,7}		0.876			0.042	
Active SLE+ Perio⁸	8	3	3	4	9	1
n=14	(57.20%)	(21.40%)	(21.40%)	(28.60%)	(64.30%)	(7.10%)
p-value^{1,8}		0.378			0.620	

Chi-Square test and Chi-Square test with Yates's correction. Significance level = 5%.

Table 4- Results for binary logistic regression (non-adjusted and adjusted) for different outcomes, considering gender, age, DNMT3B, bleeding sites, and sites with periodontal pocket

Outcomes	Variable	Categories	Non-adjusted			Adjusted		
			p value	OR	IC 95%	p value	OR	IC 95%
Periodontitis x Control	Gender	Female	–	1.00	–	–	–	–
		Male	0.017	2.74	1.20–6.27	–	–	–
	Age	<0.001	1.11	1.06–1.16	<0.001	1.11	1.05–1.16	
	DNMT3B	CC	–	1.00	–	–	1.00	–
		CT	0.067	2.27	0.94–5.50	0.363	1.60	0.58–4.43
	TT	0.002	6.87	1.20–23.71	0.032	4.53	1.13–18.05	
SLE x Control	Gender	Female	–	1.00	–	–	–	–
		Male	0.074	0.33	0.10–1.11	–	–	–
	Age	0.003	0.92	0.88–0.97	0.008	0.92	0.87–0.98	
	DNMT3B	CC	–	1.00	–	–	1.00	–
		CT	0.061	2.53	0.95–6.68	0.094	2.37	0.86–6.53
	TT	<0.001	12.15	3.42–43.11	<0.001	11.57	3.12–42.84	
SLE x Periodontitis	Gender	Female	–	1.00	–	–	1.00	–
		Male	<0.001	0.12	0.03–0.39	0.023	0.11	0.01–0.73
	Age	<0.001	0.85	0.79–0.91	0.003	0.82	0.72–0.93	
	Bleeding sites	<0.001	0.92	0.89–0.95	<0.001	0.91	0.87–0.95	
SLE with periodontitis x Control	Gender	Female	–	1.00	–	–	–	–
		Male	0.042	0.20	0.04–0.94	0.026	0.15	0.03–0.79
	Age	0.834	1.00	0.96–1.05	–	–	–	
	DNMT3B	CC	–	1.00	–	–	1.00	–
		CT	0.273	1.66	0.66–4.14	0.240	1.75	0.68–4.45
	TT	0.042	3.92	1.05–14.68	0.023	5.27	1.25–22.11	
SLE with Periodontitis x Periodontitis	Gender	Female	–	1.00	–	–	–	–
		Male	0.001	0.07	0.01–0.33	–	–	–
	Age	<0.001	0.91	0.87–0.96	0.004	0.90	0.83–0.96	
	Sites with periodontal pocket	0.047	0.96	0.93–0.98	0.024	0.95	0.92–0.99	
SLE x SLE with Periodontitis	Bleeding sites	0.002	0.95	0.92–0.98	0.002	0.95	0.92–0.98	

* Some variables were not evaluated for all groups and, for this reason, only those that were evaluated in the compared groups are shown for each outcome.

Discussion

Considering the marked role of genetic, epigenetic, and environmental factors on the onset of both SLE and periodontitis, our study investigated for the first time the contribution of methylation-related gene polymorphisms to the clinical association between SLE and periodontitis. Here we identified that the polymorphism -149C→T *DNMT3B* (rs2424913) was bonded with both diseases. Indeed, demographic and clinical findings suggest that periodontitis develops similarly in SLE patients and that its establishment may be associated with the length of SLE diagnosis.

Indeed, we observed a lower prevalence of periodontitis in SLE patients (25.9%) in comparison with findings from other studies (42.59% to 88.53%).^{10,27,28} We can also attributed it to variations and a lack of clarity of periodontal case definitions used to diagnose periodontitis.^{4,8} Tooth loss is an important parameter that has been incorporated into the current classification of periodontal disease used in our study.^{25,29} In our data, the mean tooth loss was similar among systemically healthy and SLE subjects with periodontitis. To date, there is an emerging discussion regarding the inclusion of patients with extensive tooth loss and stable remaining teeth (with no current

active periodontal disease) in periodontal medicine studies.²⁹ Notably, we observed SLE patients with this clinical profile: tooth loss and signs of previous history of periodontitis, which were included in the SLE + periodontitis group. However, a longitudinal follow-up is important to accurately distinguish the causes of high tooth loss due to periodontal or other dental problems, including caries, in SLE patients.³⁰

Regarding periodontal diagnosis, we observed a similar periodontal condition between the Periodontitis and SLE + periodontitis groups, as verified in other investigations,^{8,9} contrasting to a worse periodontal condition in SLE patients found by others.¹⁰ Moreover, SLE patients without periodontitis showed a protective factor in the number of bleeding sites in comparison to SLE patients with periodontitis, in agreement with a previous study.³¹ Intriguingly, patients with SLE + periodontitis had a smaller chance of observing sites with periodontal pockets when compared to patients with periodontitis only. These findings may be related to the chronic use of medications by SLE patients, which may affect the infectious and inflammatory processes. For instance, increased doses of immunosuppressive agents in nonresponding patients may possibly contribute to the control of periodontal inflammation.^{32,33} In contrast, prolonged use of corticosteroids at high doses has been associated with periodontal destruction in patients with SLE.⁸ Importantly, periodontitis was most frequent in active SLE patients with a longer length of SLE diagnosis ($p=0.001$) and, consequentially, under prolonged treatment. This finding highlights the importance of an early periodontal diagnosis to prevent the onset and progression of periodontal disease in patients with SLE.

The study of genetic polymorphisms is extremely important in gene mapping and the search for genes involved in common disorders, with important applicability for personalized medicine.³⁴ In particular, *MTHFR* or *DNMT3B* polymorphisms have been associated with inflammatory diseases.^{20,21} The *MTHFR* and *DNMT* genes participate in the DNA methylation process, which in turn is an epigenetic marker that regulates gene expression. The methylenetetrahydrofolate reductase enzyme encoded by the *MTHFR* gene generates the methyl radical donor (SAM), and the DNA methyltransferase 3B, encoded by the *DNMT3B* gene, transfers the methyl radical from SAM to the DNA.¹⁷ In particular, the rs1801133 polymorphism is associated with decreased methylenetetrahydrofolate

activity and consequent decrease in SAM levels, which may lead to DNA hypomethylation,^{35,36} while the rs2424913 polymorphism is associated with increased DNMT3B expression, which may lead to DNA hypermethylation.³⁷ As previously mentioned, DNA methylation is an epigenetic marker that regulates gene expression, and generally, hypomethylation is associated with increased transcription and hypermethylation is associated with decreased transcription.¹⁷

In our study, we observed that individuals with periodontitis, SLE and inactive SLE + periodontitis showed a higher frequency of the T allele and the TT genotype for *DNMT3B*. The data on periodontitis confirm our previously results²² and for the first time we show an association between rs2424913 polymorphism and SLE and SLE concomitant with periodontitis. The TT genotype (no CT genotype) has been associated with an upregulation of DNMT3B and increased hypermethylation status.³⁷ Hypermethylation has been reported in periodontitis in genes involved in prostaglandin synthesis, gene encoding receptors that recognize molecular patterns associated with pathogens, gene encoding pro-inflammatory cytokines and genes that regulate the translation process.³⁸ In SLE, hypermethylation occurs in genes involved in: leukocyte activation, regulation of cell proliferation and death, control of granulocyte differentiation and positive regulation of metabolic processes.³⁹ However, the relationship between hypermethylation at specific sites and the presence of the rs2424913 polymorphism in these populations remains to be clarified.

Intriguingly, the frequency of the T allele and TT genotype observed in Inactive SLE + periodontitis group was not observed in the Active SLE + periodontitis group. We may hypothesize that this can be explained by the smaller sample size in the Active SLE + periodontitis group or possibly by a different biological mechanism with the influence of other polymorphisms in patients with concomitant active form of SLE and periodontitis. Recently, a review emphasized that SLE is the most heterogeneous disease treated by physicians, making diagnosis and treatment a challenge.^{40,41} Other authors have argued that SLE is not just one disease, but many, based on different clinical presentations and their degrees of severity.^{40,42} Thus, based on the data from our study, we can speculate that the heterogeneity of the disease may also be related to different

metabolic pathways, which in turn are determined by different polymorphisms. In fact, the authors state that genetics is not only involved in susceptibility to SLE but is also involved in the clinical course of the disease. Moreover, the literature shows that SLE may have a multifactorial etiology (environmental factors associated with genetic polymorphisms) or monogenic etiology (mutations).⁴²⁻⁴⁴

While data on polymorphisms related to SLE or periodontitis are relatively vast,^{44,45} to the best of our knowledge, few studies have evaluated genetic polymorphisms in a population with SLE + periodontitis. Kobayashi, et al.¹⁰ (2003) showed an association of the *FcgammaR* polymorphism with SLE + periodontitis in a Japanese population. In a Mendelian randomization study, the *IGF2R* polymorphism was associated with periodontitis and SLE in a European population.¹² Both studies disregarded the degree of SLE severity, as in our study.

In contrast to the findings for *DNMT3B* (rs2424913), the C677T *MTHFR* polymorphism was not associated with periodontitis and/or SLE, as the C allele and the CC and CT genotypes were the most frequent genotypes in the entire population. Individuals who are homozygous for the wild-type C allele have normal enzyme activity, whereas heterozygotes retain 65% of wild-type *MTHFR* enzymatic activity.³⁵ The data in relation to periodontitis confirm our results previously found²² and in relation to SLE are consistent with Salimi, et al.⁴⁴ (2017) that found no association too. However, TT genotype that have only 30% of the *MTHFR* enzymatic activity³⁵ was already found in non-syndromic cleft lip, lichen planus, cardiovascular risk, and squamous cell carcinoma.⁴⁶⁻⁴⁸

Our study focused on methylation-related gene polymorphisms associated with inflammatory diseases, an investigation which studies had never been focused on before in diagnosed SLE patients with periodontitis. However, several target genes still need to be analyzed. The limitation of our study relates to its cross-sectional design, small sample size due to the well-defined inclusion criteria as well as a narrow sample collection period. Therefore, other studies should further explore this data in larger populations with different ethnic backgrounds which could provide definitive conclusions.

Conclusion

This study demonstrates the association of the polymorphism -149C→T *DNMT3B* (rs2424913) with the occurrence of SLE and concomitant SLE + periodontitis in individuals with the inactive form of SLE. Moreover, SLE patients with periodontitis had a longer length of SLE. Genetic studies provide a biological database that can be used as tools for precision medicine, enabling a more accurate line of diagnosis, treatment and prophylaxis.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Authors' contributions

Dias, Larissa NS: Data curation (Equal); Formal analysis (Lead); Investigation (Lead); Methodology (Equal); Writing – original draft (Equal). **Aquino, Sabrina Garcia:** Conceptualization (Equal); Data curation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Lead); Writing – original draft (Equal); Writing – review & editing (Equal). **Oliveira, Naila FP:** Conceptualization (Equal); Data curation (Equal); Methodology (Equal); Project administration (Equal); Writing – original draft (Equal); Writing – review & editing (Equal). **Coêlho, Marina Castro:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Writing – review & editing (Equal). **Persuhn, Darlene Camati:** Data curation (Equal); Methodology (Lead); Writing – review & editing (Equal). **Freire, Eutílica AM:** Data curation (Equal); Investigation (Equal); Methodology (Equal); Writing – review & editing (Equal). **Ribeiro, Isabella Lima Arrais:** Data curation (Supporting)

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