Evaluation of *DEFB1* polymorphisms in individuals with chronic periodontitis and diabetes mellitus type 2 in a population of northeastern Brazil

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Abstract

Aims: The role of genetic variations in genes related to innate response, as β-defensin-1 (*DEFB1*), in the context of chronic periodontitis (CP) and diabetes mellitus type 2 (DM2), is still not clear. The present study evaluates the distribution of *DEFB1* single nucleotide polymorphisms (SNPs) 5′-untranslated (5′UTR) region and its relation with the CP in DM2 individuals in northeastern Brazilians.

Methods: Two hundred and eighty individuals participated in the study, being 116 DM2+CP, 95 CP, and 69 healthy individuals. Three known *DEFB1* functional SNPs [-52 G > A (rs1799946), -44 C > G (rs1800972), -20 G > A (rs11362)] were genotyped with allele-specific assays.

Results: Association was found for the *DEFB1* -20 G > A SNP. The G allele, the GA and GG genotypes were significantly ($P < 0.05$) more frequent in the DM2+CP (59.5%, 50%, and 34.5%, respectively) and CP (61%, 44.2%, and 38.9%, respectively) than in healthy individuals (26.8%, 36.2%, and 8.7%, respectively). The GCG and ACG combinations (-52, -44, -20) were significantly more frequent among DM2+CP and CP than in the healthy individuals.

Conclusion: The results indicate that genetic variations of *DEFB1* gene (SNP-20: G allele and GA and GG genotypes) and the *DEFB1* 5′UTR haplotypes (GCG and ACG) may be associated with a susceptibility to CP in DM2 individuals as well as CP individuals without DM2.

KEYWORDS

β-defensin, chronic periodontitis, diabetes mellitus

1 | INTRODUCTION

Diabetes mellitus type 2 (DM2) is a metabolic and multifactorial disease characterized by defects in the secretion and action of the insulin hormone leading to hyperglycemia. It is a disease with silent onset, being associated to environmental and genetic factors[1,2] and chronic periodontitis (CP) is one of the complications presented in DM2 individuals.[3]

Periodontitis develops through an imbalance between the bacterial presence and the host's immune response, generating inflammation, connective tissue destruction, and bone tissue remodeling.[4] DM2 individuals, with poor glycemic control, are more likely to develop severe and generalized periodontitis.[5] The prevalence of DM2 and CP is high and the association of these two conditions, one influencing the other, is recognized and documented.[5–8]

A severe chronic systemic inflammation in DM2 individuals may be induced or perpetuated by CP, resulting in increased insulin resistance and ineffective glycemic control. The reduction of periodontal inflammation can restore insulin sensitivity and, consequently, better metabolic control.[7,8] In this context, the host innate immunity components, such as antimicrobial peptides (defensins), are of great importance, playing a crucial role in the defense against pathogens.[9]

Human β-defensins (hBDs) belong to a family of small peptides, encoded by the gene cluster located on 8p22-23 chromosome,[10] with broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, fungi, and viruses. hBDs act on the innate immune response and also on signaling for the adaptive response.[11] At present 28 hBDs are known, however the expression have been demonstrated in only four of them,[12] of which, β-defensin-1 (hBD-1) has been reported as constitutively expressed in the oral mucosa.[13]

hBD-1 is an antimicrobial peptide responsible for maintaining a healthy state in the mucosal epithelia before infection with pathogenic bacteria.[14] It is present in the defense epithelium of various tissues[11] and saliva,[15] playing an important antimicrobial role in inflammation.[16] Despite presenting constitutive expression, it has been reported that microbial pathogens and cytokines stimulate their expression in oral epithelial cells during inflammation.[12]

Single nucleotide polymorphisms (SNPs), located at the 5′-untranslated (5′UTR) region of *DEFB1* at positions -52 G > A (rs1799946), -44 C > G (rs1800972), and -20 G > A (rs11362), are known to be related to the gene expression modulation and associated with some infectious and/or inflammatory diseases, such as periodontitis,[17] human immunodeficiency virus type 1 infection (HIV-1),[18] increased levels of *Candida albicans* in the mouth,[19] systemic lupus erythematosus,[20] and inflammatory bowel diseases.[21] Other studies did not obtain significant associations between theses SNPs and susceptibility to tuberculosis,[22] early-onset periodontitis,[23] and CP in type 2 diabetics individuals.[24]

In our study, we evaluate the distribution of *DEFB1* 5′UTR SNPs in DM2 individuals with CP, in individuals just affected by CP, as well as in healthy individuals, from Northeast Brazil aimed at disclosing a possible role of these genetic variations in the susceptibility to develop CP in DM2 individuals.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of a random and nonprobabilistic sample of individuals from the metropolitan region of Recife and small cities in the interior of the Pernambuco State, Northeast of Brazil. Two hundred and eighty individuals were enrolled in this study (116 DM2 with CP, 95 CP, and 69 healthy individuals [controls]). The subjects were recruited at the Endocrinology Clinic of the "Agamenon Magalhães Hospital (AMH)" and at the Dentistry Post-Graduation Clinic of the Federal University of Pernambuco (UFPE).

The individuals were assessed and separated into three groups according to inclusion and exclusion criteria preestablished:

Individuals with diabetes mellitus type 2 and chronic periodontitis (DM2+CP):

Inclusion criteria: DM2 individuals (previous diagnosis in the AMH) with clinical diagnosis of CP;[25,26] with at least eight natural teeth (excluding those with an indication of extraction) and a minimum age of 35 years.

Exclusion criteria: Individuals who used antibiotics in the last 6 months and long-term anti-inflammatory drugs; who presented conditions compromising systemic immunity (ie, HIV-positive individuals, transplanted individuals, with autoimmune diseases and neoplasms); pregnant or breastfeeding; who have undergone periodontal treatment in the last 6 months; smokers and using orthodontic appliance.

Chronic periodontitis' individuals (CP):

Inclusion criteria: Individuals with CP clinical diagnosis[25,26]; with at least eight natural teeth (excluding those with an indication of extraction) and a minimum age of 35 years.

Exclusion criteria: Individuals with DM2 who used antibiotics in the last 6 months and long-term anti-inflammatory drugs; who presented conditions compromising systemic immunity; pregnant or breastfeeding; who have undergone periodontal treatment in the last 6 months; smokers and using orthodontic appliance.

Healthy individuals (healthy controls):

Inclusion criteria: Individuals without DM2 and CP, with at least eight natural teeth (excluding those with an indication of extraction) and a minimum age of 35 years.

Exclusion criteria: DM2 and/or CP individuals who used antibiotics in the last 6 months and long-term anti-inflammatory drugs; who presented conditions compromising systemic immunity; pregnant or breastfeeding; who have undergone periodontal treatment in the last 6 months; smokers and using orthodontic appliance.

For each individual, a periogram was completed to identify inflammatory changes and periodontal destruction sites, which included data on: visible plaque index,[27] bleeding index,[27] probing depth (six sites per tooth), loss of clinical attachment, mobility, and the presence of furcation involvement.

The CP diagnosis was established according to the American Periodontics Association.[25,26] Throughout the process, three calibrated examiners and annotators performed the clinical exams and the individuals record (Kappa concordance values >0.40).

All the procedures used in the study were evaluated by the Research Ethics Committees of the Health Sciences Center of UFPE (n°1310208) and the AMH (n°1368830), including the informed consent form signed by the patients.

2.2 | DNA isolation, selection of SNPs, and genotyping

Saliva samples were collected in sterile tubes of conic (Falcon type) (15 mL), requiring the individuals to spit for 3 minutes; and stored in the freezer at −20°C for subsequent DNA isolation.[15] For DNA isolation, Wizard® genomic DNA purification kit (Promega, Madison, Wisconsin, EUA) was used, following the manufacturer's protocol. The material was quantified using Nanodrop (Thermo Fischer®, Waltham, Massachusetts, EUA), and maintained at −20°C until genotyping.

The following *DEFB1* 5′UTR SNPs were chosen: -52 G > A (rs1799946), -44 C > G (rs1800972), and -20 G > A (rs11362), based on the impact of variants on gene expression and at Minimal Allelic Frequencies (MAF) equal to 10% (MAF = 10) in Utah residents with Northern and Western European ancestry (CEU) and Yoruba in Ibadan, Nigeria (YRI), being the study populations constituted by an admixture of European, African, and Amerindian genomes.[28] MAF verification was performed according to the NCBI (https://www.ncbi.nlm.nih.gov/SNP) and 1000 gemones.[29] All SNPs were genotyped by real-time polymerase chain reaction using allele specific probes (TaqMan probes) in ABI7500 platform (AppliedBiosystems®, Foster City, Califórnia, EUA), through a protocol adapted from the literature.[15]

2.3 | Statistical analysis

The allele and genotype frequencies for each SNP were calculated by direct counting using the Genotype Transposer program.[30] Genotypes distribution and their adherence to Hardy–Weinberg Equilibrium (HWE), for each group and SNP, was verified by chi-square test using the R program.[31] The linkage disequilibrium between variants and possible haplotypes was estimated using the Haploview 4.2 program.[32] The possible associations among the groups and the allelic, genotypic, and haplotype frequencies were performed using Fisher's exact test with contingency tables by the R program.

The likelihood ratio independence test was applied to verify associations with the genotype when there was no Pearson's chi-square test by the IBM SPSS Statistics 20.0 trial version program (IBM, Armonk, NY).

In all analyses, the 95% confidence interval (CI) and *P*-value <0.05, were considered as significant. Additionally, the study power for each SNP was verified using G*power software 3.1.9.2.[33]

3 | RESULTS

Two hundred and eighty individuals were recruited for the study, of which 116 (41.4%) were DM2+CP, with a mean age of 58.5 ± 9.0 years (ranging from 38 to 80 years); 95 (33.9%) were CP, mean age 53 ± 9.6 years (ranging from 43 to 62 years); and 69 (24.6%) were healthy individuals, with a mean age of 49.6 ± 10.7 (ranging from 35 to 77 years). In all groups, female gender was predominant (74.1% of DM2+CP, 80% of CP, and 91.3% of healthy individuals). Table 1 shows the distribution of allelic and genotypic frequencies for the three *DEFB1* 5′UTR SNPs in our population. All SNPs studied were in HWE equilibrium in both groups, except the SNP rs1800972 (-44) in healthy individuals.

Significant differences were observed for the rs11362 (-20 G > A) SNP. The G allele was significantly more frequent in DM2+CP (59.5%) and CP individuals (61%) than in healthy individuals (26.8%) (odds ratio [OR] = 3.99, 95% CI = 2.48–6.53, *P*-value = $1.12e^{-09}$ and OR = 4.26, 95% CI = 2.59–7.10, *P*-value = $9.507e^{-10}$, respectively). Similarly, the GA and GG genotypes were significantly more frequent in DM2+CP (50.0% and 34.5%, respectively) and CP individuals (44.2% and 38.9%, respectively) than in healthy individuals (36.2% and 8.7%, respectively) (Table 1), suggesting an association with CP susceptibility in individuals with and without DM2. The remaining SNPs did not present significant differences between the analyzed subjects (Table 1).

SNPs at the *DEFB1* 5′UTR were found to be in moderate linkage disequilibrium (D' > 0.7), forming eight haplotypes (Table 2). The GCG and ACG haplotypes were significantly more frequent in DM2+CP (28.0% and 23.3%, respectively) and CP individuals (31.1% and 17.4%, respectively), compared to healthy controls (15.2% and 6.5%, respectively), suggesting a possible association with CP susceptibility in individuals with and without DM2. The other haplotypes did not present significant differences (Table 2) within the groups enrolled.

Additionally, the genotypic distribution for rs11362 (-20 G > A) SNP among DM2+CP individuals was associated with severity (*P*-value = 0.021) and severity/extent of CP (*P*-value = 0.046) (Table 3). For the other SNPs no

TABLE 1 Allelic and genotypic frequencies distribution of *DEFB1* 5′UTR SNPs in diabetic mellitus type 2 patients with chronic periodontitis, in subjects with chronic periodontitis, and healthy individuals from Northeast Brazil

SNPs/ alleles/ genotypes	DM2+CP n = 116 (%)	CP n = 95 (%)	Healthy individuals n = 69 (%)	Fisher's exact test OR (95% CI), *P*-value	
				DM2+CP vs healthy individuals	CP vs healthy individuals
rs1799946 (-52 G > A)					
G	117 (50.4)	111 (58.4)	72 (52.2)	Reference	Reference
A	115 (49.6)	79 (41.6)	66 (47.8)	1.07 (0.69–1.67), 0.748	0.78 (0.49–1.24), 0.311
GG	32 (27.6)	31 (32.6)	22 (31.9)	Reference	Reference
GA	53 (45.7)	49 (51.6)	28 (40.6)	1.30 (0.60–2.80), 0.474	1.24 (0.57–2.70), 0.586
AA	31 (26.7)	15 (15.8)	19 (27.5)	1.12 (0.47–2.66), 0.842	0.56 (0.21–1.46), 0.271
HWE	*X^2 = 0.86; P = 0.353*	*X^2 = 0.36; P = 0.547*	*X^2 = 2.41; P = 0.121*		
rs1800972 (-44 C > G)					
C	197 (84.9)	155 (81.6)	115 (83.3)	Reference	Reference
G	35 (15.1)	35 (18.4)	23 (16.7)	0.89 (0.48–1.66), 0.768	1.13 (0.61–2.12), 0.770
CC	86 (74.1)	65 (68.4)	51 (73.9)	Reference	Reference
CG	25 (21.5)	25 (26.3)	13 (18.8)	1.14 (0.51–2.65), 0.850	1.50 (0.66–3.54), 0.345
GG	5 (4.3)	5 (5.3)	5 (7.2)	0.59 (0.13–2.72), 0.506	0.79 (0.17–3.62), 0.750
HWE	*X^2 = 2.93; P = 0.087*	*X^2 = 1.47; P = 0.225*	*X^2 = 7.14; P = 0.007*		
rs11362 (-20 G > A)					
A	94 (40.5)	74 (39.0)	101 (73.2)	Reference	Reference
G	138 (59.5)	116 (61.0)	37 (26.8)	3.99 (2.48–6.53), 1.12e^{-09}*	4.26 (2.59–7.10), 9.507e^{-10}*
AA	18 (15.5)	16 (16.8)	38 (55.1)	Reference	Reference
GA	58 (50.0)	42 (44.2)	25 (36.2)	4.83 (2.22–10.89), 1.35e^{-05}*	3.94 (1.74–9.26), 0.0004*
GG	40 (34.5)	37 (38.9)	6 (8.7)	13.64 (4.66–46.80), 2.35e^{-08}*	14.15 (4.74–49.48), 1.79e^{-08}*
HWE	*X^2 = 0.16; P = 0.688*	*X^2 = 0.47; P = 0.493*	*X^2 = 0.41; P = 0.523*		

DM2+CP, diabetes mellitus type 2 with chronic periodontitis; CP, chronic periodontitis; HWE, Hardy–Weinberg equilibrium; X^2, chi-square test; OR, odds ratio; 95% CI, 95% confidence interval.
[a]$P < 0.05$.

TABLE 2 Haplotype frequencies of *DEFB1* 5′UTR SNPs in diabetic mellitus type 2 patients with chronic periodontitis, in subjects with chronic periodontitis, and healthy individuals from Northeast Brazil

Haplotypes						Fisher's exact test OR (95% CI), *P*-value	
-52 G > A	-44 C > G	-20 G > A	DM2+CP 2n (%)	CP 2n (%)	Healthy individuals 2n (%)	DM2+CP vs healthy individuals	CP vs healthy individuals
A	**C**	**A**	47 (20.3)	30 (15.8)	49 (35.5)	**Reference**	**Reference**
G	**C**	**A**	31 (13.4)	33 (17.4)	36 (26.1)	0.90 (0.46–1.76), 0.752	1.49 (0.74–3.04), 0.247
G	**C**	**G**	65 (28.0)	59 (31.1)	21 (15.2)	3.20 (1.64–6.43), 0.0002[a]	4.54 (2.22–9.54), 6.67e^{-06}[a]
A	**C**	**G**	54 (23.3)	33 (17.4)	9 (6.5)	6.18 (2.65–15.88), 1.79e^{-06}[a]	5.89 (2.36–16.05), 2.19e^{-05}[a]
G	**G**	**A**	12 (5.2)	10 (5.3)	8 (5.8)	1.56 (0.53–4.82), 0.463	2.03 (0.64–6.64), 0.193
A	**G**	**A**	4 (1.7)	1 (0.5)	8 (5.8)	0.52 (0.11–2.12), 0.369	0.21 (0.004–1.67), 0.151
G	**G**	**G**	9 (3.9)	9 (4.7)	7 (5.1)	1.34 (0.40–4.60), 0.788	2.08 (0.62–7.34), 0.265
A	**G**	**G**	10 (4.3)	15 (7.9)	0 (0.0)	nc	nc

DM2+CP, diabetes mellitus type 2 with chronic periodontitis; CP, chronic periodontitis; OR, odds ratio; 95% CI, 95% confidence interval.
[a]$P < 0.05$.

TABLE 3 Genotype distribution of the rs11362 (-20 G > A) SNP according to the severity and severity/extent of chronic periodontitis in DM2 patients

Chronic periodontitis (CP)	rs11362 (-20 G > A) GG n(%)	GA n(%)	AA n(%)	*P*-value*
Severity				
Light	12 (30.0)	8 (13.8)	2 (11.1)	**0.021***
Moderate	19 (47.5)	19 (32.8)	9 (50.0)	
Severe	9 (22.5)	31 (53.4)	7 (38.9)	
Classification of CP				
Light localized	8 (20)	6 (10.3)	1 (5.6)	**0.046***
Light generalized	4 (10)	2 (3.4)	1 (5.6)	
Moderate localized	1 (2.5)	4 (6.9)	2 (11.1)	
Moderate generalized	18 (45)	15 (25.9)	7 (38.9)	
Severe localized	0 (0.0)	0 (0.0)	1 (5.6)	
Severe generalized	9 (22.5)	31 (53.4)	6 (33.3)	

[a]Probability Ratio Test.
*$P < 0.05$.

significant associations were observed in CP individuals (data not shown). In relation to the other CP clinical variables (probing depth, clinical attachment level, bleeding on probing, and visible plaque index), no association with genotypic distribution was observed for the SNPs studied.

Finally, we estimated the power of the study for each SNP. We verified that the SNPs -52 (rs1799946) and -44 (rs1800972) presented a low power (0.18 and 0.22, respectively), while -20 (rs11362), presented a high power value (1.0).

4 | DISCUSSION

DEFB1 5′UTR polymorphisms have been related to protein levels alteration[15,34] and associated with some oral diseases, such as caries[35,36] and periodontitis.[17,37] The -20A (rs11362) and -52 A (rs179946) alleles have been associated, respectively, with the increased (high DMFT—Decayed, Missing teeth due to caries, Filled Teeth; and DMFT—Decayed, Missing teeth due to caries, Filled Surface of a tooth) and decreased (low DMFT) risk of caries in US population.[35] Similarly, the same *DEFB1* SNPs (-20G/G and -52T/T genotypes) were associated with DMFT index in an Italian population, also suggesting a potential risk to develop caries.[36] In the context of periodontal disease, the -44G/C *DEFB1* SNP (rs1800972) was associated with a risk to develop periodontitis in individuals of Ural region (Caucasian).[37] In Japanese individuals, the -44C/C genotype has been associated with susceptibility to periodontitis, particularly with severe CP and with combined severe and moderate CP.[17]

In the present study, besides the variants associated with susceptibility to CP, we also found that genotypic distribution of -20 SNP was related with the severity and severity/extent of CP in DM2 individuals, probably because this systemic condition directly influences the control of periodontal disease. These results are in disagreement with those found by Wohlfahrt et al[38] in North American population. They did not find association for severe CP. The differences may be related to ethnic background of population. In addition to the different ethnicities of the study groups investigated in these two studies, smoking may be another reason for the different results obtained in these studies.

In our population, we did not observe association for -52 and -44 *DEFB-1* variants with CP in DM2 individuals, but this relation cannot be ruled out, because the study power analysis for theses SNPs revealed that sample number used was quite small. The low sample number was directly related to rigorous inclusion/exclusion criteria of individuals in the study, which despite reducing possible bias, made difficult the formation of a larger sample number, principally, in relation to healthy individuals groups. This fact may also relate with the lack of adherence to HWE among healthy individuals.

In healthy individuals, the relation among *DEFB-1* 5′UTR SNPs and hBD-1 concentration in the saliva was evaluated by Polesello et al.[15] The authors described significant associations among high protein levels and *DEFB-1* variants (-52 G/G and -44 C/G genotypes; and ACxxx/ACxxx haplotypes). The genotype distribution of -20 *DEFB-1* SNP was not significantly associated with hBD-1 levels, but individuals with -20 A/A genotype produced a major protein level in saliva than -20 G/G individuals.

All this considered, we hypothesize that DM2 individuals with -20G/G genotype for *DEFB-1* possibly produce a lower amount of hBD-1 in the saliva.[15] The lower hBD-1 production results in an antimicrobial activity reduction, with consequent increase in the amount of pathogenic bacteria in the oral environment, thus deregulating the local immune response and increasing the risk of developing periodontitis.

5 | CONCLUSION

For the population studied, the results indicate that genetic variations of *DEFB1* (SNP-20: G allele and GA and GG genotypes) may be associated with a susceptibility to CP in DM2 individuals as well as CP individuals without DM2. Additionally, the *DEFB1* 5′UTR haplotypes (GCG and ACG) may also be involved in increasing this susceptibility. All these results indicate the importance of innate immunity in the multifactorial context of CP, suggesting that polymorphisms in *DEFB1* can be considered as potential risk markers for CP. Other studies are needed for further conclusions, evaluating other SNPs and with a greater number of individuals.

SHORT INFORMATIVE

The results indicate that genetic variations of *DEFB1* gene (SNP-20: G allele and GA and GG genotypes) and the *DEFB1* 5′UTR haplotypes (GCG and ACG) may be associated with a susceptibility to chronic periodontitis in DM2 individuals as well as CP individuals without diabetes mellitus type 2.

CONFLICT OF INTEREST

None of the authors has any potential financial conflict of interest related to this manuscript.

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