

## Association of *TGF- $\beta$ 1* Codon 10 Gene Polymorphism with Chronic Periodontal Disease in Type 2 Diabetics Patients from Bahia-Brazil

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### Abstract

**Background:** Differences in the host response may determine susceptibility or resistance to periodontal disease. Studies have linked the increased propensity of diabetic subjects to develop periodontal disease to genetic predisposition. There is growing evidence that genetic factors play a role in the onset and severity of periodontal disease, since the expression cytokines may be modulated by genetic polymorphisms.

**Methods:** Ninety-one subjects were divided into three groups: the DP group was formed of 31 diabetic subjects with chronic periodontal disease, DSP consisted of 30 diabetic subjects without periodontitis and NDP was formed by 30 metabolically healthy patients with severe chronic periodontal disease. DNA was extracted from samples using a modified salting out technique. The concentration was determined by optical density readings. The polymorphisms studied were *TNF- $\alpha$*  (-308G/A), *TGF- $\beta$ 1* (codon 10C/T, codon 25C/G), *IL-10* (-1082A/G; -819T/C; -592A/C), *IL-6* (-174G/C) and *IFN- $\gamma$*  (+874T/A). Genotyping for cytokine polymorphism was performed by standard polymerase chain reaction using sequence-specific primers with a cytokine genotyping kit.

**Results:** A statistically significant difference was observed in the genotype frequency of the polymorphisms of the *TGF- $\beta$ 1* gene on codon 10 between DP and DSP patients. The proportion of subjects that exhibited the TT genotype was significantly higher in the DP group than in the DSP group. No statistically significant differences were found for allele, genotypic and phenotypic frequencies for the genes that code for *IL-6*, *IL-10*, *TNF- $\alpha$* , *IFN- $\gamma$*  and *TGF- $\beta$ 1* codon 25 between the groups.

**Conclusions:** These results demonstrate that at *TGF- $\beta$ 1* polymorphism gene at codon 10C/T is associated with chronic periodontitis in diabetic patients in Bahia, Brazil. Further studies are warranted to determine if these polymorphisms are a risk factor for susceptibility to periodontal disease.

**Keywords:** Chronic Periodontitis; Cytokines; Genetic Polymorphism; Transforming Growth Factor-B1

## Introduction

Periodontitis is a nosological entity of an infectious nature, characterized by the triggering of a potent inflammatory response responsible for the destruction of the supporting tissues of the dental units. A study by Papapanou [1] stated that microorganisms present in the biofilm are the etiological agents responsible for this disease. According to Fox [2], each individual's response to bacterial aggression is unique, with marked differences even occurring between sites in the same individual.

Susceptibility to periodontitis involves a triad formed by bacteria, host and environmental factors [3]. Smoking and diabetes are risk factors for the development of periodontitis, since they increase the likelihood of involvement of this infectious disease. However, neither smoking or diabetes are determinant for the development of periodontitis [4].

Differences in the immunological response to microbial challenge are partially determined by genetic risk factors [5]. Evidence for a genetic influence on the pathogenesis of periodontitis has been published over the past decade [6-8]. Van Der Velden, *et al.* concluded that hereditary factors are responsible for the family aggregation observed in periodontitis [9].

Periodontitis has between 10 and 20 disease modifying genes [10]. These contribute to the susceptibility and severity of periodontitis, but their presence does not determine its development, and it is necessary that other factors, such as a pathogenic microbiota, are present to trigger it [11].

Genetic polymorphisms of cytokines and HLA are hereditary risk factors [5]. Such factors may influence the natural history of periodontitis, exacerbating the inflammatory process, induced by an altered pattern in the production and secretion of proinflammatory cytokines and/or regulatory cytokines. This exerts a direct influence on the beginning, regulation and perpetuation of the infectious periodontal inflammatory process [7,11-13]. Cytokines play a central role in the periodontal inflammatory process, alveolar bone loss and regulation of the adaptive immune response with effect on immunoregulation and determining the Th1/Th2 profile of the immune response [14].

Several studies have reported on the existence of an association between the frequency of certain IL-1 $\beta$  gene alleles (IL-1 $\beta$  allele 2 + 3953 and the single nucleotide polymorphism (T  $\rightarrow$  G) at position - 330) and the severity of periodontitis [11,16]. A meta-analysis performed to evaluate the IL-1 $\beta$  C (3953/4) T polymorphism and chronic periodontal disease found a strong association between them. Also, the genetic polymorphism of IL-1 $\alpha$  -899 (+4845) C  $\rightarrow$  T has been associated with an increased risk of chronic periodontitis [17]. Additionally, concentrations of IL-1 $\beta$ , TNF- $\alpha$ , IL-2 and IFN- $\gamma$  cytokines are, on average, higher in serum and gingival tissue samples from individuals with periodontitis than in periodontally healthy individuals [17]; IL-6 and IFN- $\gamma$  mRNA have been shown to be significantly higher among diseased tissues when compared to levels in healthy tissues [18-20]. Also, specific haplotypes and the single nucleotide polymorphism of the IL-10 gene are associated with greater susceptibility to chronic periodontitis in the population of southeastern Brazil [21]. In contrast to these studies, Gonzales, *et al.* [22] concluded that there was no association between genetic polymorphisms (A-597C) and (T-824C) and chronic and aggressive periodontal diseases. In addition, allele frequencies at position 5 in the TGF- $\beta$ 1 gene have been evaluated, but there was no association between these polymorphism and the severity of periodontitis [23].

The immune system is responsible for the defense of the body. Paradoxically, plays a direct role in the destruction of periodontal tissues by activating osteoclasts [24-29]. Elevated levels of prostaglandin E2, IL-1 $\beta$  and TNF- $\alpha$  in diabetic individuals with periodontitis results in an exacerbated reaction in these individuals to microbial challenge [25].

The objective of this study was to describe and compare the allelic, genotypic and predicted phenotypes of TGF- $\beta$ 1 codon 10 and codon 25, IL-6 -174 G/C, IL-10 -1082 G/A, -819 C/T and -592 C/A, TNF- $\alpha$  -308 G/A and IFN- $\gamma$  +874 A/T between groups of mestizos composed of diabetic individuals with severe generalized chronic periodontitis, diabetics without periodontitis, and non-diabetic individuals with periodontitis.

## Material and Methods

### Population

The sample of convenience was composed of 61 individuals, unrelated mestizos according to the criteria of Azevedo [30], aged between 40 to 70 years, recruited from patients treated at the clinic of Cooperfeira in Feira de Santana, Bahia. Subjects were distributed into three groups: DP – 31 type 2 diabetic individuals with severe chronic periodontal disease; DWP – 30 type 2 diabetic individuals which at the periodontal clinical exam did not show periodontitis. The third group was formed of 30 metabolic healthy individuals with severe chronic periodontal disease, screened by the Bahiana Foundation for Development of Science (NDP), resulting in a total sample of 91 individuals. All subject provided written informed consent. The severity criteria were described by Gomes-Filho., *et al* [31].

Individuals who had already undergone previous periodontal treatment, individuals who had an oral infection with drainage indication or who were smokers were excluded. Other exclusion criteria were the use of antibiotics, anti-inflammatory drugs, corticosteroids, antidepressants, hormones other than insulin or anticonvulsants in the four months preceding the start of the study.

### Collection of periodontal clinical data

After screening, the selected individuals from the three groups were submitted to a periodontal clinical evaluation when they were registered to provide dental summary data and clinical periodontal parameters using the plaque index of O'Leary described by Badersten., *et al.* [32]; the percentage of the bleeding faces after probing [33]; a measure of the probing pocket depth of the sulcus/pocket [34], classified according to the criteria of Badersten., *et al.* [35]; and a measurement of the clinical attachment level [36], according to the classification proposed by [37]. The evaluation and frequency distribution of the IL-6, IL-10, TGF- $\beta$ , IFN- $\gamma$  and TNF- $\alpha$  gene alleles was performed using genomic DNA, obtained from a peripheral blood sample.

The clinical periodontal parameters were assessed with the aid of a calibrated and standardized Williams XP23/UNC15 periodontal probe (Hu-Friedy). Six sites per dental unit were investigated: midvestibular, mesiovestibular, distovestibular, metalingual, distolingual and mesiolingual, as described by Pihlstrom., *et al* [34].

All periodontal evaluation parameters were measured by a single specialist researcher, which provided an intra-examiner agreement of 92.68% for this longitudinal study. For this, 10 patients were examined at two different times, with a 21-day interval. A total of 1,104 faces from 184 dental units were examined.

### Genotyping

Genomic DNA was purified from peripheral blood leukocytes using the GFX™ Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech). The concentration and purity of the DNA was determined by reading the optical density at 260 and 280 nm. The cytokine genotyping kit from One Lambda Incorporation (Cytokine Genotyping Tray) was used, comprised of 15 PCR tubes, each containing two pairs of primers with a specific sequence that selectively amplifies polymorphic regulatory regions within the genes encoding *TNF- $\alpha$*  (-308G/A), *TGF- $\beta$ 1* (10C/T, 25C/G), *IL-10* (-1082A/G, -819T/C, -592A/C), *IL-6* (-174C/G) and *IFN- $\gamma$*  (+874T/A).

Sequentially at amplification, the amplified DNA fragments were separated by agarose gel electrophoresis and visualized by ethidium bromide staining and exposure to ultraviolet light. The electrophoresis cell used was developed by One Lambda, in order to allow the concomitant analysis of 96 PCR products in a short migration time. Thus, in each gel, the results of the amplification of six different DNA samples (16 reactions for each sample) were analyzed. The electrophoretic run was then performed at 120-130V for 4 to 5 minutes, or until the dye (phenol red) migrated about two-thirds the distance between two lines of wells in the gel.

Interpretation of the PCR-SSP results was based on the presence or absence of a specific amplified DNA fragment. Positive reactions to an allele or group of cytokine alleles were visualized on the gel as a DNA fragment amplified between the internal control product band and the unincorporated primer pool.

After electrophoresis on the gel, the amplified products were visualized using ultraviolet light and photographed using a transilluminator. The results were interpreted using maps of the cytokine genotyping plates provided with the PCR plates.

**Methodology of statistical analysis**

The genotypic frequencies were determined by direct counting of the data obtained with the polymerase chain reaction. The allele frequency was calculated by dividing the number of each of the alleles found by the total number of possible alleles in each group studied. The frequency of predicted phenotypes was obtained by adding the absolute values of different genotypes that show the same predicted phenotype for cytokine production. The comparisons of allele frequencies, genotypes and predicted phenotypes among the different groups were performed using Fisher’s exact test, with a significance level of  $p < 0.05$ .

**Results**

The sample of diabetic patients was formed of 61 mestizo individuals, classified according to the pathogenesis of the disease, and treated at the Diabetes Center at the City Hall of Feira de Santana, Bahia. The subjects were divided into three groups. The DP group was composed of 31 type 2 diabetic subjects who were metabolically uncontrolled, with chronic, generalized and severe periodontitis. The DSP group was composed of 30 type 2 diabetics, metabolically uncontrolled, but without periodontitis. The NDP group consisted of 30 non-diabetic individuals with chronic, generalized, severe periodontitis, screened at the Bahia Foundation for the Development of Sciences.

The distribution of individuals according to age was similar in the DP and DSP groups and significantly different in the NDP group when compared to both groups ( $p = 0.0489$  and  $p = 0.0452$ ), respectively. The duration of diabetes was significantly higher in the DSP group than in the DP group ( $p = 0.002$ ). The gender distribution did not present a statistically significant difference between the groups ( $p = 0.2411$ ) (Table 1).

Group	N	Age		Gender (%)		Diagnostic time	
		<i>x</i>	DP	M	F	<i>x</i>	DP
DSP	30	54.83	± 1.65	34	66	13.13	± 1.13
DP	31	56.54	± 1.59	55	45	7.87	± 1.18
NDP	30	47.00	± 1.38	42	58		

**Table 1:** Descriptive statistics of demographic variables.

*DP: Diabetic patients with severe chronic periodontitis; DSP: Diabetics without severe chronic periodontitis; NDP: Non-diabetic patients with severe chronic periodontitis; X: average; DP: standard deviation of the mean; M: male gender; F: Female gender.*

Regarding the plaque index and bleeding index, statistically significant differences were observed when comparing the indices of the DP and NDP groups with the indices of the DSP group. On the other hand, comparing both the bleeding index and the plaque index between the DP and NDP groups, no significant differences were observed. In relation to the percentage of framed faces in the three subgroups in relation to the depth of probing, considering the depth of probing as shallow (0 to 3 mm), moderate (4 to 6 mm) or deep

(greater or equal to 7 mm), when comparing the between the DP and NDP groups, no statistically significant differences were observed. On the other hand, when comparing the three subgroups of both the DP group and the NDP group to the three subgroups of the DSP group, a statistically significant difference was observed in all the comparisons (Table 2).

The percentage of framed faces according to the criteria described by Armitage in relation to clinical attachment loss can be seen in table 2. This score was divided into mild attachment loss (L, 1 to 2 mm), moderate (M, 3 to 4 mm) and severe (S, greater than or equal to 5 mm). A fourth subgroup was also described, composed of the faces that were not submitted to loss of attachment (SP). Comparing the three subgroups between the DP and NDP groups, no statistically significant differences were observed. On the other hand, when comparing the four subgroups of both the DP group and the NDP group to the four subgroups of the DSP group, a statistically significant difference was observed in all the comparisons (Table 2).

Group	IP		ISS		PS			PI			
	X	DP	X	DP	R x	M X	P x	SP X	L x	M X	S x
DP	85,1	5,28	77,6	7,74	75,6	22	2,4	30	13,9	21	35,1
DSP	57,1	10,39	47,9	6,47	100	0	0	85	15	0,0	0,0
NDP	8,3	6,11	81,9	6,68	77,2	21,3	1,5	35,2	16,5	12,3	36,0

**Table 2:** Distribution of the plaque index, percentage of tooth faces with bleeding on probing, the percentage of tooth faces according to the three probing depths and the percentage of tooth faces according to the loss of attachment levels between the three study groups (DP and DSP).

DP: Diabetic patients with severe chronic periodontitis; DSP: Diabetics without severe chronic periodontitis; NDP: Non-diabetic patients with severe chronic periodontitis. IP: Plaque index, ISS: Probe bleed index, PS: Probe depth, PS R: Shallow probe depth, M: moderate, P: deep, PI: Insertion loss, SP: no insertion loss, PI L: Light insertion loss, PI M: Moderate insertion loss and PI S: Severe insertion loss.

Comparison of the genotype frequency of *TGF-β1* (TT, TC and CC) at codon 10 between the DP and DSP groups resulted in a statistically significant difference (p = 0.0127). It was also observed that the PD group presented a trend (p = 0.0674) for the higher frequency of TT genotype when compared to NDP. Comparisons of the allelic (G/C) and genotype frequencies of codon 25 (GG, GC and CC) between the groups did not result in a statistically significant difference. The comparison of predicted phenotype frequencies among the DP, DSP and NDP groups did not show statistical significance (Table 3).

	DP n	n = 31 (%)	DSP n	n = 30 (%)	NDP n	n = 30 (%)	DPxDSP	DPxNDP
<b><i>TGF-β1</i> codon 10</b>								
Allele T	40	(64.52)	35	(58.33)	32	(53.33)	0.6062	0.2695
Allele C	22	(35.48)	25	(41.67)	28	(46.67)		
Genotype TT	16	(51.61)	8	(26.67)	8	(26.67)	0.0127	0.0674
Genotype TC	8	(25.81)	19	(63.33)	16	(53.33)		
Genotype CC	7	(22.58)	3	(10.00)	19	(20.00)		
<b><i>TGF-β1</i> codon 25</b>								
Allele G	58	(93.55)	52	(86.67)	55	(91.67)	0.3311	0.9592
Allele C	4	(6.45)	8	(13.33)	5	(8.33)		
Genotype GG	28	(90.32)	22	(73.33)	26	(83.33)	0.0705	0.2953
Genotype GC	2	(6.45)	8	(26.67)	5	(16.67)		
Genotype CC	1	(3.22)	0	(0.00)	0	(0.00)		
<b>Phenotype</b>								
High Producer	23	(74.19)	21	(70.00)	21	(70.00)	0.9271	0.9271
Intermediate Producer	6	(19.35)	7	(23.33)	7	(23.33)		
Low producer	2	(6.46)	2	(6.67)	2	(6.67)		

**Table 3:** Distribution of the allelic frequencies, genotypes and predicted phenotypes of the *TGF-β1* gene codons 10 T/C and 25 G/C.

DP: Diabetic patients with severe chronic periodontitis; DSP: Diabetics without severe chronic periodontitis; NDP: Non-diabetic patients with severe chronic periodontitis. The production of cytokines related to the predicted phenotype is in agreement with Hoffman, et al. (2001).

Statistically significant differences were not observed for the genetic polymorphism of IFN-γ, IL-6, TNF-α and IL-10 when comparing genotype allelic frequencies and predicted phenotypes between groups (Table 4).

The observed differences in allele frequencies, genotype and predicted phenotypes between the DP and DSP and DP and NDP groups were not statistically significant for the IFN-γ gene polymorphism (+874 T/A) (Table 4).

The possible combinations of the IL-10 polymorphisms at positions -1082, -819 and -592 form six possible genotypes: GCC/GCC, GCC/ACC, GCC/ATA, ACC/ACC, ACC/ATA and ATA/ATA. The former is the predicted phenotype of a high producer, while the next two genotypes initiated by the GCC sequence are the predicted phenotypes of intermediate producers. The other genotypes are probably responsible for low production of this cytokine. The results of the comparisons between allele frequencies, genotype and predicted phenotypes of this cytokine, among the evaluated groups, did not result in statistically significant differences (Table 4).

Genotype (Phenotype)	DP n	N=31 (%)	DSP n	n =30 (%)	NDP n	n =30 (%)	DPxDSP	DPxNDP
<b><i>TNF-α</i><sup>-308 G/A</sup></b>								
G/G (Low producer)	20	(64.52)	20	(66.67)	20	(66.67)	0.8597	0.8597
GA+AA (High producer)	11	(35.48)	10	(33.33)	10	(33.33)		
<b><i>IL-6</i><sup>-174G/C</sup></b>								
GG+GC (High producer)	29	(93.55)	28	(93.3)	30	(100)	1.0000	0.4918
CC (Low producer)	2	(6.45)	2	(6.7)	0	(0)		
<b><i>IFN-γ</i><sup>+874T/A</sup></b>								
TT (High producer)	1	(3.22)	4	(13.33)	2	(6.67)	0.1232	0.7108
TA (Intermediate producer)	15	(48.39)	8	(26.67)	12	(40.0)		
AA (Low producer)	15	(48.39)	18	(60.0)	16	(53.33)		
<b><i>IL-10</i><sup>-1082G/A,-819C/T,-592C/A</sup></b>								
GCC/GCC (High producer)	3	(9.68)	3	(10.00)	2	(6.67)	0.9910	0.9123
GCC/ACC; GCC/ATA (Intermediate producer)	15	(48.39)	14	(46.47)	15	(50.00)		
ACC/ACC; ACC/ATA; ATA/ATA (Low producer)	13	(41.93)	13	(43.33)	13	(43.33)		

**Table 4:** Distribution of genotype frequencies and predicted phenotypes of IFN-γ + 874T/A, IL-6-174G/C, TNF-α-308G/A and IL-10-1082G/A, -819C/T genes, -592C/A

DP: Diabetic patients with severe chronic periodontitis; DSP: Diabetics without severe chronic periodontitis; NDP: Non-diabetic patients with severe chronic periodontitis; DP X DSP: Statistical result of the comparison between groups; DP X NDP: Statistical result of the comparison between groups; The production of cytokines related to the predicted phenotype is in agreement with Hoffman., et al. (2003), Pravica., et al. (1997), Turner., et al. (2000) and Wilson., et al. (1993).

A correlation analysis of the genotype with the probable predicted phenotype of each of the cytokines was not performed in this study, since this correlation has already been described in the literature [26-28].

## Discussion

In the present study, the frequencies of cytokine polymorphisms for IFN-γ (directly involved in the Th1 response), IL-6 and TNF-α (proinflammatory cytokines) and TGF-β1 and IL-10 (regulatory cytokines associated with a Th2 profile) were evaluated.

A statistically significant difference was observed in the TGF- $\beta$ 1 codon 10 genotype frequencies between the DP and DSP groups. In the DSP group, the most prevalent genotype was CT (63.3%) and in the PD group, the TT genotype was the most frequent (51.6%). This may be considered a marker for predisposition to the development of severe chronic periodontitis in the studied population. The statistically significant difference in relation to the aforementioned genotypes did not result in a significant difference in relation to the predicted phenotypes. In all groups, the expected high producer phenotype was the most prevalent, followed by intermediate producers and low producers.

De Souza, *et al.* [38], when evaluating the C-509T polymorphism of TGF- $\beta$ 1, observed that the T allele and the TT genotype are the most frequent in patients with chronic periodontitis and concluded that this polymorphism may play a role in modulation of the inflammatory process. In the present study, it was also observed that the TT genotype at codon 10 may be associated with a more intense inflammatory process, since it was more frequent in the DP group, where the inflammatory process was more severe. However, in a study by Holla, *et al.* [23], evaluation of the -988 (C/A), -800 (G/A) and -509 (C/T) polymorphisms in the 5' region and the L10P polymorphism in codon 10 and R25P at codon 25 did not result in an association between these polymorphisms and the severity of periodontitis. These divergent findings may be the result of the different ethnic origins evaluated. The two articles cited above evaluated Caucasian individuals while the present study evaluated a population characterized by an interrelationship between distinct peoples, resulting in a genetically hybrid ethnicity of tri-racial origin, i.e. whites, blacks and aboriginals, leading to a wide variety of mestizo phenotypes [30].

The present study did not find a statistically significant difference when comparing the groups in relation to allele frequency, genotype and predicted IFN- $\gamma$  phenotype. In all groups, the genotype AA homozygote at position +874 was the most prevalent; this is associated with a predicted low producer phenotype, followed by the heterozygote TA genotype associated with intermediate production of this cytokine and the homozygous TT genotype associated with high secretion of this cytokine. The homogeneity between the groups in relation to the prevalent AA genotype was not expected in this study, since Gemmel and Seymour [39] concluded that stable lesions are associated with a Th1 response and higher secretion of IFN- $\gamma$ , while advanced lesions demonstrate a Th2 profile, characterized by intense secretion of IL-4, IL-5 and IL-10 cytokines and by a reduction in Th1 cytokine secretion.

The allelic, genotypic and predicted phenotypes for the -174 G/C polymorphism of the IL-6 gene between the groups did not result in a statistically significant difference. In all groups, the G allele and GG homozygous genotype were the most frequent. A high prevalence of the expected phenotype was observed in all groups, despite the significant difference observed when periodontal inflammatory conditions were compared between the DP and DSP groups. Trevilatto, *et al.* [40], studying the IL-6 -174 polymorphism in Caucasians from southeastern Brazil, also observed a higher frequency of the G allele and GG genotype among individuals with moderate and severe chronic periodontitis. The authors concluded that the IL-6 -174G/C polymorphism was associated with susceptibility to chronic periodontitis in the studied population. It is important to note that, although the current study and the work by Trevilatto, *et al.* [40] were both carried out in Brazil, the studied populations differ considerably; in our study, the population was mestizo, with a predominance of the black race, while Trevilatto, *et al.* assessed Caucasians influenced by Japanese and European immigration. The evaluation of the genetic polymorphism of IL-6 at position -174 in Caucasians indicated that the G allele is associated with type 2 diabetes mellitus and that this polymorphism is associated with serum levels of IL-6 [41]. Sharma, *et al.* [42] observed that, in the Malaysian population, the GA/AA genotype and IL-6 (-597) allele appear to be a protective factor for chronic periodontitis in type 2 diabetics [42]. Kubaszak, *et al.* [43] reported that the CC genotype of IL-6 at position -174 poses a two-fold increased risk of developing type 2 diabetes mellitus from a state of glucose intolerance. In the present study, both type 2 diabetic and non-diabetic subjects presented the GG genotype most frequently.

There was no statistically significant difference between the groups in the distribution of the allelic frequencies, genotypes and predicted phenotypes of the -1082 G/A, -819 C/T and -592 C/A polymorphisms of IL-10. In contrast to the results obtained in this study, in which the A allele at position -1082 was the most prevalent, the G allele was the most prevalent in a sample of Swiss Caucasian subjects

[14]. In this same population, the GG genotype was significantly more frequent among patients with severe periodontitis when compared to controls [14]. However, a Japanese study did not reveal the presence of the G allele at position -1082; rather it was found that the GCC haplotype was present in 68% of the Japanese population, supporting the conclusion that the frequency of haplotypes in the Japanese population differs greatly from that observed among Caucasians [44]. In this study, the GCC haplotype was present in 56% of the study population.

In the present study, the IL-10 -819C and -592C alleles were the most prevalent in all groups. In all three groups, the most frequent genotypes were GCC/ATA and ACC/ATA and, in relation to predicted phenotypes, the most prevalent was associated with intermediate production, despite the different degrees of severity observed among the three groups. A similar evaluation in Caucasians with severe chronic periodontitis and in periodontally healthy individuals indicated that polymorphisms at positions -597 and -874 are not associated with periodontitis, it was also observed that the A and T alleles were the most frequent at positions -597 and -874, respectively [14]. Conversely, in the mestizo population studied here, the most frequent allele was C at both positions. In a study on Caucasians from southeastern Brazil, a statistically significant difference was observed in the distribution of the genotype frequency, at positions -819 and -592, among patients with chronic periodontitis and periodontally healthy individuals. In this case, the C allele was the most frequent in both groups and at these positions [21].

The distribution of frequencies for the -308 G/A polymorphism of the TNF- $\alpha$  gene did not present statistically significant differences between the groups. The G allele and the GG genotype were the most frequent in all groups, despite the significant differences observed when the periodontal inflammatory conditions were compared between the DP and DSP groups. Craandijk, *et al.* [45] also concluded that the genetic polymorphism TNF- $\alpha$  -308 (G/A) may not be associated with susceptibility to or severity of periodontitis, independently of smoking. On the other hand, a study on this polymorphism in the Czech population found an association with chronic periodontitis when associated with the lymphotoxin- $\alpha$  +252 (A/G) polymorphism [46]. Kubaszek, *et al.* [43] concluded that the TNF- $\alpha$  gene polymorphism G-308A is a predictor for the conversion of glucose intolerance status to type 2 diabetes mellitus and, similar to the data obtained in the present study, the GG genotype was the most frequent (74%), followed by the GA (25%) and AA (1%) genotypes. The latter was absent among the mestizos evaluated in this study. In the Malaysian population, the A allele at TNF- $\alpha$  -308 appears to be a risk factor for chronic periodontitis [43].

A careful evaluation of the periodontal indices revealed that the DP and NDP groups, with periodontitis, showed a higher index of plaque and bleeding faces after probing. Also, they presented a higher percentage of faces framed in the subgroups associated with a greater probing pocket depth and greater loss of attachment, demonstrating more severe periodontal destruction in these two groups. On the other hand, in the DSP group, plaque and bleeding indices were much smaller and no faces were observed in the subgroups associated with a probing pocket depth above 3 mm and moderate to severe loss of attachment, demonstrating periodontal health.

Thus, this study showed an association between severe chronic periodontitis in type 2 diabetic individuals and a higher frequency of the TT genotype for TGF- $\beta$ 1 gene codon 10. This genotype can be considered a possible genetic marker for periodontitis. It was not possible to associate the polymorphisms of IFN- $\gamma$  +874T/A, IL-6 -174G/C, TNF- $\alpha$  -308G/A, TGF- $\beta$ 1 codon 25 and IL-10 -1082G/A, -819C/T, -592C/A to severe conical periodontitis.

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