ORIGINAL RESEARCH

Association between TNF- α , TGF- β 1, IL-10, IL-6 and IFN- γ gene polymorphisms and generalized aggressive periodontitis

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Abstract

Objective: The aim of this study was to investigate links among cytokine genetic variants and generalized aggressive periodontitis (GAgP).

Methods: Thirty-five patients with generalized aggressive periodontitis and 85 healthy controls without periodontitis were included in the study. Probing depth (PD), clinical attachment loss (CAL), plaque index (PI), and gingival index (GI) were recorded as clinical parameters. Polymorphisms of IL-6, IL-10, IFN- γ , TGF- β 1 and TNF- α gene were analysed using the polymerase chain reaction sequence-specific primer method (PCR-SSP).

Results: No significant differences were observed for IL-6, IL-10, IFN- γ , and TGF- β 1 cytokine polymorphisms, from the genotype distribution and allele frequency, between GAgP and healthy control groups. In contrast, significant differences were observed in the TNF- α gene polymorphism between GAgP and healthy control groups ($P = 0.002$).

Conclusion: Our data suggest that TNF- α (-308) may be associated with the development of generalized aggressive periodontitis. These results should be replicated in a larger and more diverse population of patients diagnosed with

generalized aggressive periodontitis to determine if these findings are generalizable.

Generalized aggressive periodontitis (GAgP) is characterized by severe and rapid periodontal attachment loss and bone destruction in young and otherwise healthy individuals.¹ To explain the severity of periodontal tissue destruction, various studies have concentrated on the specific bacterial etiology associated with GAgP^{2,3} or on a modified host defense mechanisms in response to specific bacterial plaque.^{4,5} In addition, previous studies have investigated factors that may increase host susceptibility to tissue destruction in GAgP; including genetic factors^{5,6} and high levels of inflammatory mediators and cytokines.^{4,7,8}

Cytokines play a major role in a number of biological activities including proliferation, development, homeostasis, regeneration, repair and inflammation.⁷ Synthesis profiles of cytokines can be considered as

either T helper cell type 1 (Th1) responses, promoting cell-mediated immunity and interleukin-2 (IL-2) and interferon gamma (IFN- γ), or Th-2 responses, promoting humoral immunity (IL-4, IL-5, IL-6, IL-10) and Th-3 subset characterized by the transforming growth factor beta (TGF- β).⁹ Polymorphisms likely to occur in regulatory regions of cytokine genes may not only increase susceptibility to some infectious diseases, but also influence the course and prognosis of the disease.⁹ Many studies have evaluated the putative relevance of cytokine gene polymorphism in the pathogenesis of periodontal disease^{4,8,10}; however, the effect of genetic factors in the pathogenesis of periodontal disease remains unclear.

The purpose of our study was to compare the relative frequencies of the cytokine genotypes (IL-6, IL-10, TNF- α , TGF- β , IFN- γ) in subjects with and without GAgP.

Methods

Study population

This study was performed at the Department of Periodontology, Faculty of Dentistry, Gaziantep University, Turkey. The study was approved by the Ethical Committee of Gaziantep University School of Medicine. The study group included 35 patients with GAgP and 85 healthy volunteers. The patients were informed about the purpose and the method of the study, and their consents to participate were obtained. All of the subjects were free of any systemic or oral disease other than periodontitis and were nonsmokers. Exclusion criteria included the use of drugs affecting the oral flora, immune system or inflammatory response in the six months prior to assessment. A detailed medical, oral, and family medical history was taken, followed by a complete periodontal examination. For periodontal patients, measurements of probing depth (PD) and clinical attachment loss (CAL) were made at six sites (mesiobuccal, buccal, distobuccal, mesiolingual/palatal, palatal/lingual, and distolingual/

palatal) for each tooth, using a manual periodontal probe (Williams' periodontal probe designed with 1, 2, 3, 5, 7, 9, and 10 mm calibrations). Gingival index (GI) and plaque index (PI) were recorded at four sites (mesiobuccal, buccal, distobuccal, and palatal/lingual).^{11,12} Periodontal examination was carried out by a single trained periodontist. GAgP was characterized as follows: patients were generally healthy; age of onset of disease < 35 years; attachment loss of 4 mm or in at least 30% of the teeth; at least three of the affected teeth were not first molars and incisors; and the severity of attachment loss was inconsistent with the amount of mineralized plaque. The absence of periodontal disease was determined as the absence of both CAL and sites with probing depth > 3 mm.

Sample Collection and DNA Extraction

Peripheral blood samples were collected in EDTA sterile tubes and stored at -20 °C until analysis. DNA was extracted by a salting-out method as described previously.¹³

Cytokine Gene Polymorphism

Cytokine genotyping was performed by the polymerase chain reaction sequence-specific primer method (PCR-SSP), using the Cytokine Genotyping Tray kit according to the manufacturer's instructions. Single nucleotide polymorphisms (SNP) for five cytokines (IL-6, IL-10, IFN- γ , TGF- β 1, TNF- α) were analyzed.¹⁴

Statistical analysis

All data were analyzed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The data were analyzed for appropriateness between the observed and expected genotypes as well as for Hardy-Weinberg Equilibrium (HWE) as described elsewhere.^{15,16} All analyses and differences were interpreted as statistically significant when $P < 0.05$.

TABLE 1. Demographic and clinical data of patients with generalized aggressive periodontitis and healthy controls

Variable	GAgP (n=35)	Healthy Controls (n=85)
Sex (Female/Male)	21/14	52/33
Mean age	17-33 (27.3)	18-39 (32.6)
Parental consanguinity	15	13
PD (mm)	5.6 $\pm$ 1.4	1.9 $\pm$ 0.4
CAL (mm)	5.9 $\pm$ 1.5	2.1 $\pm$ 0.2
PI	1.7 $\pm$ 0.31	0.6 $\pm$ 0.09
GI	2.03 $\pm$ 0.39	0.4 $\pm$ 0.1

PD: probing depth, CAL: clinical attachment loss, PI: plaque index, GI: gingival index, GAgP: generalized aggressive periodontitis

Results

Of 35 patients with GAgP, 14 were males and 21 were females, from 17 to 35 years of age (mean age 27.3 years). Of the healthy control volunteers, 52 were females and 23 were males, from 18 to 39 years of age (mean age 32.6 years). The patients and controls were from the same geographical areas. Demographic and clinical data are shown in Table 1.

Genotype frequencies of IL-6 (-174), INF- γ (+874), IL-10 (-1082, -819, -592), and TGF- β 1 (codons 10 and 25) polymorphisms were not significant different between patients and controls, whereas genotype frequencies of TNF- α (-308) were significantly

higher in patients in comparison with controls ($P = 0.002$). Genotype frequency and expression and the results of HWE ($P > 0.05$) in patients with GAgP and controls are summarized in Tables 2 and 3.

Discussion

Periodontal diseases affect millions of people around the world. Individual factors play an important role in progression of periodontitis, and host defense in particular is thought to be under genetic control. Studies on periodontal disease have revealed a genetic basis; however, the specific nature of the genetic risk remains unclear.¹⁷ Cytokines play a key role in the regulation of the immune response. The maximal capacity of cytokine production varies among individuals and correlates with polymorphisms in the cytokine gene promoters.¹⁸ Polymorphic gene sequences of certain cytokines could be the potential markers of susceptibility and clinical outcome of different infectious diseases in humans.¹⁹ The aim of this study was to determine potential associations between cytokine gene polymorphisms and GAgP. We found no significant differences between allele frequency and genotype distribution of IL-6 (-174), INF- γ (+874), IL-10 (-1082, -819, -592) and TGF- β 1 (codon 10 and 25) polymorphisms between patients and controls, indicating no detectable correlation between genetic poly-

TABLE 2. Comparison of frequencies of TNF- α , IL-6 and IFN- γ gene polymorphisms between patients with generalized aggressive periodontitis and healthy controls

Cytokine gene	Genotype	GAgP n^a (%)	Healthy Control n^b (%)	HWE	p
TNF α (-308)	GG ^{&}	20 (57.1)	71 (83.5)	0.168 ^b	<0.001
	AG [#]	15 (42.9)	12 (14.1)	0.041 ^a	0.002
	AA [#]	0 (0)	2 (2.4)		0.360
IL-6 (-174)	GG [#]	22 (62.9)	49 (57.6)	0.973 ^b	0.727
	GC [#]	12 (34.3)	31 (36.5)	0.662 ^a	0.597
	CC ^{&}	1 (2.8)	5 (5.9)		0.489
IFN γ (+874)	TT [#]	7 (20.0)	8 (9.4)	0.159 ^b	0.078
	AT [#]	14 (40.0)	45 (53)	0.324 ^a	0.110
	AA ^{&}	14 (40.0)	32 (37.6)		0.809

^an=35, ^bn=85, [#]high expression, [&]low expression, ^βintermediate expression, GAgP: generalized aggressive periodontitis, HWE: Hardy-Weinberg Equilibrium

TABLE 3. Comparison of frequencies of TGF- β and IL-10 haplotypes between patients with generalized aggressive periodontitis and healthy controls.

Cytokine gene	Haplotype	GAgP	Healthy Control	HWE	p
		n^a (%)	n^b (%)		
IL-10	GCC GCC [#]	4 (11.4)	17 (20.0)	0.177 ^b	0.243
	GCCACC, GCC ATA ^{β}	17 (48.6)	35 (41.2)		
	ACC ACC, ACC ATA, ATA ATA &	14 (40.0)	33 (38.8)	0.732 ^a	0.261 0.904
TGF β	TTGG, TCGG [#]	23 (65.7)	55 (64.7)	0.684 ^b	0.817
	TCGC, CCGG, TTGC ^{β}	12 (34.3)	26 (30.6)	0.220 ^a	0.916
	CCGC, CCCC, TTCC, TCCC &	0 (0)	4 (4.7)		0.191

^a $n=35$, ^b $n=85$, [#]high expression, &low expression, ^{β} intermediate expression, HWE: Hardy-Weinberg Equilibrium

morphisms and susceptibility to GAgP in this population.

IFN- γ , a product mainly of natural killer and activated T cells, plays an important role in host defense: it modulates the activation of macrophages, increases class II molecule expression and antigen presentation, and increases differentiation of lymphocyte populations.^{20,21} The IFN- γ (+874) polymorphism has been found to be associated with infectious diseases, such as brucellosis and tuberculosis.^{14,22} Reichert *et al.* reported no association between IFN- γ (+874) polymorphism and aggressive and chronic disease.²³ Our data show no correlation between IFN- γ polymorphism (+874) and GAgP.

IL-10 is known as a cytokine synthesis inhibitory factor because of its ability to inhibit cytokines, such as IL-2, and IFN- γ . On the other hand, IL-10 also fosters B-cell antibody production and has a direct mitogenic effect on T cells and B cells.²⁴ Fernandes *et al.* showed in the murine model that IL-10 may down regulate the immune response to *B. abortus* by affecting both macrophage effector function and the production of IFN- γ .²⁵ A limited number of studies have investigated IL-10 promoter polymorphisms at three positions (-1082, -819, -592) in patients with periodontitis in comparison to healthy controls.²⁶⁻³¹ The results were inconsistent. For example, two studies failed to demonstrate associations between periodontitis and polymorphisms in the IL-10 gene.^{27,28} In contrast, studies with Swedish and Turkish populations re-

vealed that allelic variants of the IL-10 (-1087 and -592) polymorphisms are associated with severe chronic periodontitis.^{29,30} Similarly, Brazilian Caucasian populations show an association between IL-10 (-819 and -592) polymorphisms and chronic periodontitis.³¹ Our data suggest that IL-10 (-1082, -819, -592) gene polymorphisms may not affect susceptibility to GAgP.

The function of IL-6 in GAgP is not completely known. Yoshie *et al.* reviewed the role of IL-6 polymorphisms in periodontitis²² and reported that the GC SNP at the (-174) position correlated with chronic periodontitis susceptibility in Brazilian Caucasian, but not in Czech Caucasian, populations.³² Several studies indicated the importance of considering the cooperative influence of the IL-6 promoter variants (-174, -190, and -597) in determining the response to disease.^{33,34} In our study, no difference was found in the distribution of the IL-6 (-174) polymorphism between the patients and controls.

TGF- β 1 is a multifunctional cytokine that regulates several events in growth and differentiation of many cell types.³⁵ TGF- β 1 suppresses the cellular immune response at multiple levels, including proliferation, and inhibits lymphocyte proliferation and function.³⁵ As TGF- β 1 mRNA appears to be expressed in the regulatory T cells (T_{regs}) present in the gingival tissue, the possible engagement of TGF- β 1 in receptor activator of nuclear factor kappa B ligand (RANKL) mediated periodontal disease is yet to be clarified.³⁶ In

particular, TGF- β 1 has been reported to increase osteoprotegerin expression by bone marrow stromal cells. Therefore, it is possible that T_{regs} plays a suppressive regulatory role in the bone destruction lesion of periodontal disease.³⁶ Nonetheless, further *in vitro* and *in vivo* studies are required to identify the role of T_{regs} in periodontal bone destruction.³⁶ Babael *et al.* reported the TGF- β 1 (codon 25) GG genotype more frequently in control subjects than in chronic periodontitis patients.³⁷ In contrast, our results did not show an association between TGF- β 1 (codon 10 and 25 polymorphisms) and GAgP.

TNF- α is a potent immunologic mediator of acute and chronic inflammatory responses and mediates bone resorption.¹¹ Several case-control studies have been conducted to evaluate the role of TNF- α variants in periodontitis^{32,38-44}; however, the results are inconsistent. While two studies suggest that TNF- α polymorphisms are factors affecting disease,^{38,39} other researchers could not confirm any links between genetic variants and periodontitis.⁴⁰⁻⁴⁴ These inconsistent data may be partly explained by the different clinical, methodological and statistical settings, as well as by variances in TNF genotype distributions within distinct races and /or populations.^{45,46}

In this study, we detected a statistically significant correlation between the genetic variant of TNF- α (-308G) and the occurrence of GAgP (Table 2). The frequency of the AG genotype of TNF- α polymorphisms is higher in GAgP patients in comparison with healthy controls. Accordingly, the AG genotype may be considered a genetic factor leading to susceptibility to GAgP. Moreover, the GG genotype at the TNF- α gene was more prevalent in controls than in patients, suggesting that the GG genotype may serve as a protective factor against GAgP.

Conclusion

In conclusion, our data suggest that TNF- α gene (-308) may be associated with an increased risk of GAgP. Nevertheless, the small group of patients in our

study limited the investigation of cytokine gene polymorphisms in relation to the development of GAgP. The findings of the present study highlight the need for prospective longitudinal studies to elucidate the relative contributions of various factors in diseases with a multifactorial etiology where there is interplay among genetic susceptibility and exogenous factors.

Abbreviations

GAgP	generalized aggressive periodontitis;
PD	probing depth;
GI	gingival index;
PI	plaque index;
CAL	clinical attachment level;
IL	interleukin;
Th1	T helper cell type 1;
IFN- γ	interferon gamma;
TGF- β	transforming growth factor beta;
Tregs	regulatory T cells;
RANKL	receptor activator of nuclear factor kappa B ligand;
PCR-SSP	polymerase chain reaction sequence-specific primer method;
TNF- α	tumor necrosis factor alpha;
EDTA	ethylenediamine tetraacetate;
SNP	single nucleotide polymorphism;
HWE	Hardy-Weinberg equilibrium

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