

IL-6 and IL-10 gene polymorphisms in patients with aggressive periodontitis: effects on GCF, serum and clinic parameters

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Abstract: Genetic variations observed in cytokines affect periodontitis susceptibility. The aim of this study was to investigate interleukin (IL)-6(-174) and IL-10(-597) gene polymorphisms in generalized aggressive periodontitis (GAgP) patients. Also, we aimed to evaluate the effects of IL-6 and IL-10 gene polymorphisms on the clinical outcomes of non-surgical periodontal therapy and cytokine levels in gingival crevicular fluid (GCF) and serum. Fifty-three patients with GAgP and 50 periodontally healthy individuals were included in this study. Clinical parameters, GCF and blood samples were collected at baseline and at 6-week. Non-surgical periodontal therapy was performed in patients with GAgP. Gene analysis were determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). GAgP patients showed significant improvement on clinical parameters after periodontal therapy ($p < 0.05$). In the GAgP group, IL-6 GG genotype and G allele frequency were higher than in the control group. GCF IL-6 level was also significantly lower at 6-week in the GAgP group. Higher GCF IL-10 levels were observed in patients carrying the IL-6 GG genotype than in those carrying the GC+CC genotype at baseline. In conclusion, IL-6(-174) and IL-10(-597) gene polymorphisms were found to be associated with GAgP and genotype distribution did not affect the outcome of non-surgical periodontal therapy, while patients with IL-6(-174) GG genotype had higher levels of GCF IL-10 levels.

Keywords: Interleukin-6; Interleukin-10; Polymorphism, Genetic; Aggressive Periodontitis.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Aggressive periodontitis (AgP) is a rapidly progressing form of periodontal disease. Specific microbial environment and modified host response play crucial roles in the development and progression of the disease.¹ AgP patients display inadequate host response to increased expression of a variety of immunological and genetic risk factors.² The genetic risk of AgP development has been investigated in many studies in various populations.^{3,4,5}

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There is a complex network of pro- and anti-inflammatory cytokines acting in the diseased periodontal tissues.^{6,7} Also, cytokine expressions are affected by the presence of functional gene polymorphisms.⁶

Interleukin (IL)-6 is a pleiotropic cytokine released by many cell types, and its secretion levels are determined by the producing cell type and the genetic background of the source cells.⁸ Among several kinds of cytokines, IL-6 was found to be increased in gingival crevicular fluid (GCF) and gingival tissues of patients with periodontal disease.⁹ A recent study reported that chronic periodontitis (CP) and generalized aggressive periodontitis (GAgP) patients had significantly higher GCF IL-6 levels in comparison with the healthy subjects.¹⁰

Single nucleotide polymorphisms (SNPs) in the promoter region of IL-6 gene at positions -174G/C (rs 1800795), -572C/G (rs 1800796) and -597G/A (rs 1800797) have been described. First, Trevisatto et al. reported an association between IL-6(-174) gene polymorphism and CP in the Caucasian race.¹¹ After that, several studies found different results regarding the relationship between IL-6(-174) gene polymorphism and periodontal disease. Some of these studies did not find any association between IL-6(-174) gene polymorphism and CP,^{12,13,14,15} however some others found associations.^{16,17}

Studies investigating the relationship between IL-6(-174) gene polymorphism and AgP are limited.^{3,5,18,19,20} Erciyas et al.²⁰ evaluated the IL-6(-174) single nucleotide polymorphisms (SNPs) in AgP in Turkish population and found no association. However, Nibali et al.⁵ reported an association between IL-6(-174)GG genotype and AgP, and another study showed an association between IL-6(-174)GC genotype and AgP.¹⁸

IL-10, an anti-inflammatory cytokine, regulates the synthesis of pro-inflammatory cytokines such as IL-1, -2, -6 and stimulates protective antibody production.^{6,21} IL-10 is expressed in both healthy and diseased human periodontal tissues and is reported to be reduced in patients infected with *Aggregatibacter actinomycetemcomitans*.²²

The gene encoding IL-10 was mapped to chromosome 1q31-32. The three SNPs (-1082 G/A; -819

C/T and -592 C/A) from the transcriptional start site have been associated with the altered synthesis of IL-10 in response to inflammatory stimuli.²³ In the literature, data on which of the IL-10 polymorphisms or variations contributes to periodontal disease are contradictory.^{4,21,24} The IL-10 gene polymorphism at position -597 C to A was not associated with the susceptibility to CP or AgP.⁴

Considering the key roles of pro-inflammatory and anti-inflammatory cytokines in periodontal disease, it was questioned whether genetic variations affecting transcription or function of these cytokines contribute to the development of GAgP. Also, few studies have investigated the potential association of carrying a gene variant with the clinical response to periodontal therapy. The present study was undertaken to test the hypothesis that gene polymorphisms may affect periodontal inflammation and treatment outcomes via pro- and anti-inflammatory cytokine levels in AGP patients. Thus, the aims of this study were separated in three parts: first, to determine IL-6(-174) and IL-10(-597) gene polymorphisms in GAgP patients and healthy controls; second, to evaluate the effect of these gene polymorphisms on outcomes of non-surgical periodontal therapy; and third to investigate the effect of genotype distributions on cytokine levels in GCF and serum.

Methodology

Study population

Fifty-five patients with GAgP and 50 periodontally and systemically healthy individuals were recruited from the Department of Periodontology at Cumhuriyet University from 2011 to 2013. The study protocol was approved by the Medical Research Ethics Committee of Cumhuriyet University according to the Helsinki declarations (2011/031), and signed informed consent was obtained from all individuals.

Individuals who were evaluated for the present study were excluded if they had conservative or prosthetic restorations at the anterior region, presence of systemic disease, history of hepatitis or HIV infection, immunosuppressive chemotherapy or current pregnancy or lactation, requirement

for antibiotic prophylaxis, history of antibiotic therapy in the previous 3 months, chronic usage of anti-inflammatory drugs and periodontal treatment in the previous 6 months. Also, smokers were excluded from the study.

The diagnostic criteria for GAgP was defined in accordance with the classification developed by the American Academy of Periodontology.¹ Patients who presented with a generalized severe periodontal destruction (probing depth \geq 5mm at least on three sites other than first molars and incisors, and radiographic evidence of advanced alveolar bone loss) were diagnosed with GAgP.

Control subjects were designated as healthy if they had full-mouth probing pocket depth (PD) < 3mm, gingival index scores equal to 0, and no radiographic evidence of alveolar bone loss. These individuals were included in the study as systemically and periodontally healthy volunteers. Also, all individuals came from the same geographical area.

Clinical measurements and periodontal therapy

Before crevicular fluid collection, full-mouth clinical periodontal measurements were recorded at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual), including plaque index (PI),²⁵ gingival index (GI),²⁵ PD and clinical attachment level (CAL). A periodontal probe (Williams probe, Hu-Friedy, Chicago, IL, USA) was used for periodontal measurements. In all patients, individual acrylic stents were fabricated with grooves as reference points for CAL measurement. The periodontal status of each patient was determined by a single calibrated researcher (E.P.G.), who had experience in clinical trials. In patients with GAgP, clinical periodontal measurements were performed at baseline and at 6-week. GAgP subjects received non-surgical periodontal therapy, which consists of oral hygiene instructions, scaling and root planning. The treatment procedure was performed quadrant per quadrant under local anesthesia in four visits using specific curettes (Hu-Friedy, Chicago, IL, USA). Treatment was completed within 10 days. No antibiotics were prescribed during the

treatment. All patients were treated by a single periodontist (E.P.G.).

GCF and serum sampling

GCF sampling was carried out during the same session as the clinical measurements. Only the upper anterior teeth were included in the study to facilitate the access and to reduce the risk of salivary contamination during the procedures. GCF samples were collected from a proximal site of a single-rooted tooth with moderate pocket size in GAgP patients and one site in control group. The GCF samples were collected using an absorbent paper strip (Periopaper; ProFlow Inc., Amityville, NY, USA), which was placed into the sulcus/pocket until mild resistance was felt and then held there for 30s.²⁶ The procedure was performed carefully to avoid mechanical injury. Strips with contamination of blood were excluded. All strips with GCF were immediately and individually placed into sterile Eppendorf tubes and kept at -80°C until required for further analysis.

Blood samples (5 ml) were taken from the antecubital vein of all individuals and collected in tubes. Venous blood samples were allowed to stand for 30min before being centrifuged at 6000rpm for 10min. All samples were kept in -80°C conditions until the day of analysis.

Cytokine assay

On the day of the assay, 100 μl phosphate buffer solution was added to GCF samples in Eppendorf tubes. GCF samples were mixed with a vortex for 1min to mix the solution. After removal of the strips, the supernatants were divided into two aliquots for the determination of each cytokine. The amounts of IL-6 and IL-10 in the serum and GCF were determined by using ELISA kits (Invitrogen, Carlsbad, USA) according to manufacturer's instructions. After color development was stopped, the absorbance was measured using a microtiter plate-computerized reader set to a wavelength of 450nm. GCF cytokine levels were calculated from the standard curves and defined as picogram/site for total amount of cytokine levels. The sensitivities for IL-6 and IL-10 ELISAs were 2 and 1 pg/mL, respectively. Sites with cytokine levels below the limits of the assay's detectability were scored as 0.

Genotype analyses

Two mL of blood was taken from each individual at baseline. The blood samples were collected in sodium EDTA vacutainers and stored at -80°C until genetic analysis were performed. The extraction of DNA was performed with a commercial kit (Invitrogen, Cambrillo, USA) following the manufacturer's protocol.

Each sample of DNA was analyzed for polymorphisms in the IL-6 gene at position -174, and in the IL-10 gene at position -597 with PCR-RFLP. Primer sequences are shown for each primer pair in Table 1.

DNA samples were amplified by PCR (PTC-200 thermal cycler, MJ Research, Watertown, MA, USA) with specific primers for each examined polymorphism. PCR reaction mixtures (50 μl) contained 50 μl PCR buffer, 0.2 mM each dNTP, 10 pmol of each primer (forward and reverse, control and allele specific), 1.5 mM MgCl_2 , 200 ng of genomic DNA, 0.5U Taq DNA polymerase (Fermantes, Maryland, USA) and each internal control primer.

For IL-6(-174) G/C, the following PCR protocol was used: 94°C for 3 min (initial denaturation), 94°C for 30s (denaturation), 62.6°C for 40s (annealing), and 72°C for 40s (extension). The total number of cycles was 35 followed by a final extension of 72°C for 5min. We used NlaIII and a cleavage protocol following the manufacturer's instructions. NlaIII cleaves a restriction site at position -174 when G is present, leading to fragments of 29, 176, 202, and 233 base pairs (bp) in length or 29, 111, 122, 176 and 202 bp when C is present. In heterozygote individuals (A/C), fragments have 42, 66, 232, 240 and 306 bp in length.

For IL-10 (-597)A/C, the following PCR protocol was used: 94°C for 3min (initial denaturation), 94°C for 30s (denaturation), 48°C for 40s (annealing), and 72°C for 40s (extension). The total number of cycles was 35 followed by a final extension of 72°C for 5 min.

We used RsaI and a cleavage protocol following the manufacturer's instructions. RsaI cleaves a restriction site at position -597 when A is present, leading to fragments of 42, 66, 232, and 240 bp in length or 42, 232, and 306 bp when C is present. In heterozygote individuals fragments have (A/C), 42, 66, 232, 240 and 306 bp in length.

The alleles were separated by 3% agarose gel electrophoresis and stained with ethidium bromide. Following electrophoresis, the PCR products were visualized under ultraviolet light.

Statistical analysis

The chi-square test was used to test for deviation of genotype frequencies from Hardy-Weinberg equilibrium and to compare the genotype distributions among individuals with GAgP and healthy controls. Odds ratios (ORs) and 95% confidence intervals were calculated to determine the strength of the association. A p-value of < 0.05 was considered significant.

Statistical analyses were performed using the program SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Demographic data and clinical parameters were reported as mean \pm standard deviation. Genotype frequencies were tabulated by direct counting and allele frequencies were calculated from the observed number of genotypes. Kolmogorov-Smirnov test was used to assess the distribution of data. Comparisons among groups were performed using the Mann-Whitney U test. In the GAgP group, differences between baseline and 6-week values were analyzed by Wilcoxon rank test. The relationships among GAgP group and gene polymorphisms were analyzed by logistic regression analysis. The power of the analysis was defined by utilizing data from a previous publication.²¹ The sample size was calculated under a 5% error giving a required sample size of 55 in each group, with a statistical power of 80%.

Table 1. Primer sequences and reaction properties for genes.

Marker	Primer Sequences	tA ($^{\circ}\text{C}$)	Bp
IL-6(-174)	5'-GGGCTGCGATGGAGTCAGAG-3'	62,6	639
	5'-TCCCTCACACAGGGCTCGAC-3'		
IL-10(-597)	5'-ATCCAAGACAACACTACTAA-3'	48	412
	5'-TAAATATCCTCAAAGTTC -3'		

tA: Annealing temperature; bp: base pairs of amplicon size.

Results

Clinical findings

Two of the GAgP patients did not complete the periodontal therapy and were excluded from the study. The mean age and sex distribution showed no difference between the groups ($p > 0.05$) (Table 2). In the GAgP group, all clinical parameters at 6-week were significantly lower than in baseline ($p < 0.05$) (Table 3). Also, there was no significant difference between the GAgP and healthy control groups regarding PI, GI and PD values at 6-week.

Distribution of genotypes and allele frequencies

The frequency of IL-10 genotypes in the GAgP and healthy control was found in accordance with Hardy-Weinberg equilibrium ($p > 0.05$), whereas IL-6 genotypes showed disequilibrium ($p < 0.05$).

IL-6 (-174) G/C and IL-10 (-597) A/C genotype distributions were significantly different between groups ($p = 0.007$, $p = 0.006$, respectively) (Table 4). In the GAgP group, IL-6 GG genotype was associated with 2.1 times higher risk of GAgP compared to IL-6 GC/CC genotype, however, this risk was not statistically significant (OR:2.1, 95%CI: 0.96–4.64, $p > 0.05$). Conversely, IL-10 AA genotype was significantly associated with 7 times higher risk of GAgP compared to IL-10 AC/CC genotype (OR:7, 95%CI: 1.48–33.22, $p < 0.05$).

Considering allele frequencies in both groups, IL-10 A allele frequency was similar in the GAgP and healthy control ($p > 0.05$). However there was a significant difference between the study groups in IL-6 G allele frequency. The frequency of G allele was

Table 2. Demographic characteristic of study groups.

Variable	GAgP (n = 53)	Healthy controls (n = 50)
Age (years)		
Mean ± SD	29.7 ± 6.2	31.8 ± 5.2
Range	15–44	23–43
Gender		
Male n(%)	24(45.3)	17(34)
Female n(%)	29(54.7)	33(66)

GAgP: generalized aggressive periodontitis; SD: standard deviation.

higher in the GAgP group compared to the healthy control group ($p < 0.05$).

Cytokine levels in GCF and serum

In GAgP group, GCF IL-6 levels were significantly decreased after periodontal treatment ($p < 0.05$, Table 5). Also in GAgP group, GCF IL-6 cytokine levels at baseline and 6-week were significantly higher compared to the control group ($p < 0.05$). After periodontal treatment, serum IL-6 levels were decreased, but not significantly.

GCF and serum IL-10 levels at 6-week were higher than those of baseline, but these differences were not statistically significant in the GAgP group.

In the GAgP group, individuals were categorized according to the IL-6 and IL-10 genotype distribution, and then changes in clinical and biochemical parameters were evaluated (Table 6). Significant improvements in clinical parameters were found after non-surgical periodontal therapy in individuals with both IL-6 and IL-10 genotypes ($p < 0.05$).

In bivariate comparisons, higher PD values were found in patients carrying IL-6 GG genotype than the IL-6 GC+CC genotype at baseline ($p < 0.05$) and higher CAL values were observed in patients carrying the IL-6 GG genotype at 6-week ($p < 0.05$).

In patients carrying IL-6 GC+CC genotype, there was a statistically significant decrease in GCF IL-6 levels at 6-week. However, in comparisons between IL-6 genotypes, serum and GCF IL-6 levels were not significantly different at 6-week. At baseline, higher

Table 3. Clinical parameters at baseline and 6-week for study groups (mean ± SD).

Parameter (mean ± SD)	GAgP	Healthy controls
PI	Baseline	1.5 ± 0.7 ^b
	6-week	0.3 ± 0.5 ^a
GI	Baseline	2 ± 0.5 ^b
	6-week	0.6 ± 0.5 ^a
PD	Baseline	5.2 ± 0.6 ^b
	6-week	3.1 ± 1 ^a
CAL	Baseline	8.7 ± 2.5
	6-week	6.9 ± 2.6 ^a

^a $p < 0.05$ different from baseline; ^b $p < 0.05$ different from healthy controls; GAgP: generalized aggressive periodontitis; PI: plaque index; GI: gingival index; PD: pocket depth; CAL: clinical attachment level; NA, not applicable.

Table 4. Distribution of the IL-6 (-174) and IL-10 (-597) genotypes and allele frequencies for study groups. n(%).

Variable	GAgP n = 53	Healthy controls n = 50	p-value
Genotypes			
IL-6(-174)			
CC	4(7.5)	16(32)	-
GC	18(34)	14(28)	0.007*
GG	31(58.5)	20(40)	-
IL-10(-597)			
CC	7(13.2)	3(6)	-
AC	34(64.2)	45(90)	0.006*
AA	12(22.6)	2(4)	-
Allele frequencies			
IL-6(-174)			
C	26(24.5)	46(46)	0.001*
G	80(75.5)	54(54)	-
IL-10(-597)			
C	48(45.28)	51(51)	0.274
A	58(54.8)	49(49)	-

*p < 0.05; GAgP: generalized aggressive periodontitis.

GCF IL-10 levels were found in patients carrying the IL-6 GG genotype than in those carrying the IL-6 GC+CC genotype (p < 0.05).

GCF IL-6 level was a significantly decreased in patients carrying the IL-10 AC+CC genotype after periodontal treatment. Also, there was no significant differences in GCF and serum IL-10 levels after non-surgical periodontal therapy in individuals with both IL-10 genotypes (p > 0.05).

Finally, we evaluated whether IL-6 and IL-10 genotype distributions affect the response to non-surgical periodontal therapy. Differences in clinical parameters were analyzed according to genotype distribution and no significant difference was found (Table 7).

In GAgP patients, logistic regression analysis was used to evaluate the association of the IL-6 GG and IL-10AA genotypes with periodontal disease susceptibility, while adjusting for confounders such as age and gender. It was found that neither age nor gender had effects on genotype diversity.

Discussion

Polymorphisms in the regulatory regions of the cytokine genes may change cytokine expression.

Table 5. Gingival crevicular fluid(GCF) and serum IL-6 and IL-10 levels at baseline and 6-week (pg/30s, mean±SD).

Variable		GAgP	Healthy controls
GCF			
IL-6	Baseline	126.1 ± 214.9 ^b	-
	6-week	52.9 ± 92.9 ^{a,b}	21.3 ± 52.3
IL-10	Baseline	5.5 ± 10.7	-
	6-week	10.5 ± 23.2	6.9 ± 13.3
Serum			
IL-6	Baseline	14.2 ± 30.8	-
	6-week	8.8 ± 22.8	3.1 ± 11.4
IL-10	Baseline	4.4 ± 19.4	-
	6-week	6.4 ± 24.4	0.8 ± 2.6

^ap < 0.05, different from baseline; ^bp < 0.05, different from healthy controls; GAgP: generalized aggressive periodontitis.

Therefore, the polymorphisms of cytokine genes are potentially important as genetic predictors of the disease susceptibility and clinical outcomes.²⁷ In our study, periodontal therapy improved clinical results and decreased GCF IL-6 level, consistently with the literature.²⁸ Also, in patients with IL-6 GC+CC and IL-10 AC+CC genotypes, GCF IL-6 level was decreased at 6-week. We showed that reduced clinical inflammation was related to lower GCF IL-6 cytokine level. Contrary to our study, Oliveira et al.²⁹ reported that GCF IL-6 level was increased in GAgP patients after treatment, but not significantly. The authors explained this situation by affirming that IL-6 acts both as pro- and anti-inflammatory cytokine at the same time. Furthermore, similar to our results, Becerik et al.¹⁰ demonstrated that in GAgP group, GCF IL-6 cytokine level was higher than in the control group and plasma levels of IL-1beta and IL-6 were similar among GAgP, CP and gingivitis patients, and controls. Also, in this study, serum IL-6 level decreased after non-surgical periodontal therapy, but the difference was not significant. Contrary to our findings, Shimada et al. and D'Aiuto et al. reported that serum IL-6 level was decreased after periodontal therapy.^{28,30} GCF and serum IL-10 levels increased after periodontal therapy, but no significant difference was detected. Lappin et al.³¹ suggested that the numbers of inflammatory leucocytes that express the IL-10 were increased in aggressive periodontitis compared to chronic periodontitis, and the numbers of inflammatory leucocytes that express

IL-10 are more widely distributed than the IL-6 and TNF- α . It was demonstrated that periodontal therapy improved GCF cytokine profiles by lowering IL-1 β and increasing IL-10 levels but the mean change in IL-10 did not differ between AgP and control groups.²⁹ Similarly, these findings were observed in our previous study.⁷

In the literature, there are few studies investigating the relationship between AgP and IL-6 gene

polymorphisms.^{6,20,32,33} Supporting our results, Nibali et al. reported that IL-6 GG genotype was associated with susceptibility to AgP.⁵ Similarly, ameta-analysis indicated that the IL-6(-174) G allele did not modify the risk of CP, but increased the risk of AgP.³⁴ However, similar to Erciyas et al.,²⁰ Brett et al.¹⁸ evaluated IL-6(-174) gene polymorphisms in CP, AgP and control groups in Caucasians, and they found that association between GG genotype

Table 6. Clinical Parameters, GCF and serum cytokine levels in generalized aggressive periodontitis group distributed by IL-6 and IL-10 genotypes.

Clinical parameters	IL-6 (mean \pm SD)		IL-10 (mean \pm SD)	
	GG (n = 31)	GC + CC (n = 22)	AA (n = 12)	AC+CC (n = 41)
PI				
Baseline	1.6 \pm 0.7	1.4 \pm 0.7	1.3 \pm 0.7	1.6 \pm 0.7
6-week	0.4 \pm 0.5**	0.1 \pm 0.5	0.2 \pm 0.4	0.3 \pm 0.5
p-value	p = 0.001*	p = 0.001*	p = 0.006*	p = 0.001*
GI				
Baseline	2 \pm 0.5	2.1 \pm 0.4	1.8 \pm 0.7	2.1 \pm 0.4
6-week	0.7 \pm 0.5	0.5 \pm 0.5	0.5 \pm 0.5	0.6 \pm 0.6
p-value	p = 0.001*	p = 0.001*	p = 0.005*	p = 0.001*
PD(mm)				
Baseline	5.4 \pm 0.6**	4.9 \pm 0.7	4.9 \pm 0.6	5.3 \pm 0.6
6-week	3.3 \pm 1	3 \pm 0.9	3 \pm 0.7	3.2 \pm 1
p-value	p = 0.001*	p = 0.001*	p = 0.004*	p = 0.001*
CAL(mm)				
Baseline	9.1 \pm 2.8	8.1 \pm 2.1	8.1 \pm 2.7	8.9 \pm 2.5
6-week	7.5 \pm 2.7**	6.1 \pm 2.2	6.1 \pm 2.5	7.2 \pm 2.6
p-value	p = 0.001*	p = 0.001*	p = 0.003*	p = 0.001*
GCF IL-6				
Baseline	124.3 \pm 209.1	128.6 \pm 227.8	209.7 \pm 335.6	101.6 \pm 162.4
6-week	39.6 \pm 55.3	71.6 \pm 128.0	65.9 \pm 121.4	49.1 \pm 84.3
p-value	p = 0.136	p = 0.017*	p = 0.131	P = 0.013*
GCF IL-10				
Baseline	8 \pm 13**	2.1 \pm 4.7	6.6 \pm 9.4	5.2 \pm 11.1
6-week	9 \pm 19.5	12.5 \pm 28.1	16.1 \pm 26.4	8.8 \pm 22.3
p-value	p = 0.291	p = 0.171	p = 0.594	p = 0.746
SERUM IL-10				
Baseline	4.5 \pm 22.5	9.2 \pm 27.2	7.1 \pm 24.5	9.7 \pm 41.6
6-week	0.5 \pm 3	10 \pm 29.4	8.3 \pm 29	6.9 \pm 39
p-value	p = 0.655	p = 0.500	p = 0.317	p = 0.866
SERUM IL-6				
Baseline	27.7 \pm 91.5	38.3 \pm 116.5	34.1 \pm 43.8	8.4 \pm 23.5
6-week	7.2 \pm 16.9	36 \pm 116.4	17.5 \pm 32.8	6.2 \pm 18.7
p-value	p = 0.051	p = 0.893	p = 0.068	p = 0.575

*p < 0.05 different from baseline; **p < 0.05 different from IL-6 GC+CC; PI: plaque index; GI: gingival index; PD: pocket depth; CAL: clinical attachment level; GCF: gingival crevicular fluid.

Table 7. Differences of clinical parameters in generalized aggressive periodontitis patients (at baseline and 6-week) distributed by IL-6 and IL-10 genotypes.

Parameter	IL-6 (mean ± SD)			IL-10 (mean ± SD)		
	GG	GC+CC	p-value	AA	AC+CC	p-value
PI	1.19 ± 0.74	1.31 ± 0.64	0.58	1.08 ± 0.79	1.29 ± 0.67	0.40
GI	1.35 ± 0.55	1.63 ± 0.78	0.10	1.41 ± 0.66	1.48 ± 0.67	0.49
PD (mm)	2.09 ± 0.94	2.0 ± 1.06	0.63	1.91 ± 1.08	2.09 ± 0.96	0.80
CAL (mm)	1.64 ± 1.01	1.95 ± 1.13	0.32	2.0 ± 1.27	1.70 ± 1.0	0.57

PI: plaque index; GI: gingival index; PD: pocket depth; CAL: clinical attachment level.

prevalence was higher in CP/AgP patients compared to control, but no association between IL-6 GG genotype and AgP was detected. IL-6(-174)G/C and (-572)G/C polymorphisms are important regulators of transcription and have been shown to affect IL-6 production. Patients with juvenile rheumatoid arthritis carrying the GG genotype have been observed to have twice as high IL-6 serum levels as patients with the CC genotype.³⁵ Moreover, the IL-6(-174) polymorphism was associated with increased odds of the concomitant detection of *Aggregatibacter actinomycetemcomitans* and *P. gingivalis*.³ In contrast, D’Aiuto et al.²⁸ observed that in patients with severe periodontal disease, higher serum IL-6 levels were associated with the carriage of allele C for the IL-6(-174) polymorphism. Terry et al.³⁶ reported that genetic polymorphism in the IL-6 promoter region influence IL-6 gene transcription by rather complex interactions determined by the haplotype. Also, in a recent report, it was suggested that detecting IL-6 haplotype-positive periodontitis patients might become helpful in identifying subjects who are prone to excessive inflammatory response. However, in this study, one SNP of IL-6 gene was assessed and we could not find any differences in GCF and serum IL-6 levels between GG and GC+CC genotypes, but GCF IL-6 levels were decreased in patients with the IL-6 GC+CC and IL-10 AC+CC genotypes compared with the IL-6 GG patients after periodontal treatment. These findings partially support that GG genotypes affect IL-6 production in GCF but not its serum level.

Although there are limited studies about AgP in the literature, different results for IL-10(-592) gene polymorphism were demonstrated. Racial and ethnic characteristics play a role on these results. Gonzales et al.⁴

searched gene polymorphism in IL-10(-592) loci in AgP and CP Caucasian patients. Contrary to our results, they found no association between IL-10 AA genotype and AgP; similar results were found by Hu et al.²³ in Chinese population. Likewise, A allele in position -592 is more frequent in the Chinese population than in a Caucasian subgroup.²³ In a Jordanian population, the frequencies of the IL-10(-1087)A and (-592)A alleles were significantly more common in CP patients but there was no difference for genotype distribution between localized AgP group and controls.³⁷ A recent large-scale replication study also suggested that only IL-10SNP(rs 61815643) was associated with Dutch and German/Austrian AgP samples.³⁸ Claudino et al.³⁹ studied the effect of IL-10(-592) gene polymorphism on cytokine expression in 116 CP patients and 173 control subjects. In CP, a significantly lower IL-10 mRNA expression was verified in both CA and AA genotypes when compared to CC genotype carriers. Also, in a study that investigated the possible association between IL-10(-592) and (-1082) SNPs and periodontal disease progression in a five year period was found that patients with IL-10 haplotypes experienced around 20% fewer PD ≥ 4mm compared to subjects with other genotypes.⁴⁰ Based on the present findings, IL-10(-592)AA genotypes did not seem to play a role in the periodontal therapy outcomes. Conversely, GCF IL-10 levels were higher in patients carrying IL-6 GG genotype compared to those of the IL-6 GC+CC genotype. This may be explained as a result of the production of anti-inflammatory cytokine IL-10 in order to reduce inflammation and reestablish homeostasis.

Our study has some plausible limitations. The studied population was small, and therefore our findings need to be corroborated in larger samples

and should be tested in groups of different ethnic origins. Also, other SNP of these genes and the effect of haplotype combinations have not been estimated. A precise statement about a possible association of a genetic locus with a disease can be only made if the full haplotype information has been assessed.³⁸

Conclusion

To the best of our knowledge, this is the first study evaluating outcomes of periodontal therapy on these gene polymorphisms in AgP patients in the Turkish population. IL-6(-174) and IL-10(-597)

gene polymorphisms might be associated with AgP, but genotype distribution for the two cytokines did not influence periodontal treatment outcomes. However, GCF IL-10 levels were higher in the IL-6 GG genotype carriers at baseline and GCF IL-6 levels only decreased in patients with the IL-6 GC+CC and IL-10 AC+CC genotypes after periodontal treatment.

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