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Are Monocyte Chemoattractant Protein-1-2518 G and Regulated Activation Normal T Cell Expressed and Secreted -403 A Alleles Risk Factor for Periodontitis?

[MCP-1 -2518 G ve Rantes -403 A Allelleri Periodontitis İçin Bir Risk Faktörü müdür?]

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ABSTRACT

Objective: We aimed to analyze the *monocyte chemoattractant protein-1* (MCP-1) and *regulated on activation, normal T cell expressed and secreted* (RANTES) polymorphisms in relation to susceptibility to chronic periodontitis and influence of genotypes in response to non-surgical periodontal therapy.

Methods: This cross-sectional clinical study was performed during 2005-2006 in Turkey. Gene polymorphisms were investigated in 51 periodontitis patients and 48 periodontally healthy subjects. Quadrant scaling and root planning was performed to periodontitis patients. Genomic DNA was analyzed for polymorphisms by polymerase chain reaction amplification followed by restriction enzyme digestion.

Results: Genotype distributions of related genes were similar between periodontitis and healthy subjects (P>0.05). In periodontitis group, monocyte chemoattractant protein-1 -2518G allele frequencies were 25.5%, regulated on activation, normal T cell expressed and secreted-403A allele frequencies were 30.4%. Percentage of bleeding sites were significantly higher in regulated on activation, normal T cell expressed and secreted gene A negative genotypes compared to A positive (P=0.023). Significant improvements in clinical parameters were found after periodontal therapy in both genotypes (P<0.05).

Conclusion: Within the limits of present data, results may suggest that there were no association between specific alleles and periodontal treatment effect. Furthermore, these gene polymorphisms are not associated with chronic periodontitis in Turkish population.

Key words: chronic periodontitis; non-surgical periodontal therapy; monocyte chemoattractant protein-1 ((MCP-1), regulated on activation, normal T cell expressed and secreted (RANTES), gene polymorphisms

ÖZET

Amaç: Bu çalışmada, *"monocyte chemoattractant protein-1"* (MCP-1) ve *"regulated on activation, normal T cell expressed and secreted"* (RANTES) gen polimorfizmlerinin kronik periodontitise yatkınlık ile olan ilişkileri ve uygulanan cerrahisiz periodontal tedaviye yanıta genotiplerin etkisini araştırmayı amaçladık.

Yöntem: Bu kesitsel klinik çalışma 2005-2006 döneminde Türkiye'de gerçekleştirildi. Gen polimorfizmleri 51 periodontitisli ve 48 periodontal sağlıklı bireyde incelendi. Periodontitisli hastaların her yarım çenesine diş yüzeyi temizliği ve kök yüzeyi düzleştirmesi işlemleri yapıldı. Genomik DNA polimeraz zincir reaksiyonu amplifikasyonu sonrası restriksiyon enzim dijesyonu ile -403G/A ve -2518A/G polimorfizmlerinin analizleri yapıldı.

Bulgular: Periodontitisli ve sağlıklı bireylerde her iki genin de genotip dağılımları benzerdir (P>0.05). Kronik periodontitis grubunun -2518G allel frekansı 25.5%, -403A allel frekansı 30.4% dür. Sondalamada kanamalı bölgelerin oranı *„regulated on activation, normal T cell expressed and secreted"* geninde A negative genotiplerde, A pozitif genotiplere göre anlamlı olarak yüksektir (P=0.023). Her iki genin genotiplerinde cerrahisiz periodontal tedavi sonrasında klinik parametrelerde anlamlı düzelmeler bulunmuştur (P<0.05).

Sonuç: Bu verilerin sınırları içerisinde, her iki genin spesifik allelleri ile cerrahisiz periodontal tedavinin etkileri arasında bir ilişki olmadığı ileri sürülebilmektedir. Bunun yanında, türk populasyonunda *„monocyte chemoattractant protein-1"* ve *"regulated on activation, normal T cell expressed and secreted"* gen polimorfizleri ile kronik periodontitis arasında bir ilişki de bulunmamaktadır.

Anahtar Kelimeler: Kronik periodontitis; cerrahisiz periodontal tedavi; *"monocyte chemoattractant protein-1"* (MCP-1), *"regulated on activation, normal T cell expressed and secreted"* (RANTES), gen polimorfizmleri

Introduction

Periodontal diseases are infectious disorders in which environmental and genetically determined factors contribute to the individual variation (1-3). In the inflamed periodontal tissue, the regulation of leukocyte migration into the tissue is determined by the expression of adhesion molecules which are induced by pro-inflammatory cytokines as well as by a group of chemotactic cytokines, called chemokines. Chemokines are responsible for the recruitment and subsequent activation of particular leukocytes into inflamed tissues and therefore play a central role in the final outcome of the adaptive immune response (4). They are relevant in inflammatory process not only for their role in promoting directional signals for circulating leukocytes to diapedese into inflammatory sites, but also for other physiological and pathological activities, such as lymphoid trafficking, T helper (Th) 1/Th2 development and wound healing (4-7). They mainly exert their effects on target cells by binding to specific receptors and selectively attract variety of cells to the inflammatory site (8).

The chemokines are classified into four subfamilies based on number and arrangement of cysteine residues; these groups are designated C, CC, CXC and CX_3C. Monocyte chemoattractant protein-1 (MCP-1) and regulated on activation, normal T cells expressed and secreted (RANTES) are the members of CC chemokines (9,10). In addition, they are involved in the activation and recruitment of inflammatory and immune cells to the infected sites and thereby mediating a variety of pathophysiological conditions. MCP-1 shows significant chemotactic activity to cells of the monocytes/macrophage lineage, it is relatively specific for monocytes (7,9,11,12). Furthermore, RANTES has a unique spectrum of chemotactic activity and it is a potent chemoattractant for T cells, eosinophils, basophils, monocytes/macrophages and mast cells (4,7).

Garlet et al. examined the prevalence of mRNA expression for chemokines and chemokine receptors in gingival tissue and indicated strong positive and differential message expression in the periodontitis patients (8). Emingil et al. have shown that MCP-1 and RANTES have been found in the periodontal tissues and the levels of these chemokines in the tissue from aggressive periodontitis patients were higher than the healthy tissue (13).

In human cell lines, the -28G and -403A polymorphisms were shown to increase promoter activity of RANTES comparing with the more frequent -28C and -403G, respectively, suggesting that these polymorphisms increase RANTES expression in the human body (14). Recently a biallelic A/G polymorphism has been found in the MCP-1 distal gene regulatory region at position -2518 that affects the level of MCP-1 expression in response to an inflammatory stimulus (15).

The effect of the G allele at -2518 appears to be dose dependent as cells from individuals homozygous for G at -2518 produced more MCP-1 than cells from G/A heterozygotes(16).

Periodontitis is a chronic multifactorial disease with a strong genetic component.[2] Gene polymorphisms that modulate host immune responses to the microbial challenge have been associated with different clinical forms of periodontitis (3,17). Several studies have shown that allelic variations in genes encoding molecules of the host defense system such as cytokines could affect the susceptibility and progression of periodontal disease (18).

Although MCP-1 and RANTES polymorphisms were widely investigated in several diseases with chronic inflammation such as atopic dermatitis, atherosclerosis, systemic lupus erythematosus and inflammatory renal disease (16,19,20,21,22), there is no report about the role of MCP-1 and RANTES polymorphisms in periodontitis and influence of genotypes on response to the non-surgical periodontal therapy

Therefore, the purposes of the present study were; 1) to comparatively analyze the frequency of the MCP-1 and RANTES alleles and genotype distributions in chronic periodontitis and healthy groups; 2) to investigate the potential impact of genotypes in response to the non-surgical periodontal therapy; 3) to study the association of the MCP-1 and RANTES genes polymorphism with clinical periodontal parameters in a sample of Turkish population.

Materials and Methods

Study population

A total of 99 unrelated Caucasians of Turkish descent were included in the present study. Fifty one chronic periodontitis (CP) patients and 48 subjects with healthy periodontal conditions recruited from the Department of Periodontology, School of Dentistry, Ege University between February 2005 and December 2006. All of the patients and healthy controls were from the western region of Turkey and were of low to moderate socio-economical level. The study protocol was approved by the ethics committee of the Ege University Faculty of Medicine (15/03/2005, 05-1/7). All participants gave written informed consent in accordance with the Helsinki Declaration. Medical and dental histories were taken. All of the CP patients had at least 20 teeth in the mouth. None of the subjects had a history or current manifestation of serious systemic disease, which could impair the immune response. Patients with medical disorders (such as diabetes mellitus, immunological disorders, hepatitis and HIV infections) and pregnant females were excluded from the study (23). Smokers in both CP and healthy groups were smoking >10 cigarettes/day for more than 5 years. CP patients were diagnosed according to the clinical criteria for CP agreed by

consensus at the World Workshop in Periodontitis in 1999 as follows (24).

CP group: The CP group consisted of 18 females and 33 males between the ages of 32 and 65 with a mean age of 45.94 ± 8.08 years (Table 1). They had ≥20 teeth, severe CP and exhibited at least four sites with clinical attachment loss (CAL) ≥5 mm and ≥50% alveolar bone loss in at least two quadrants. Assessment of the extent and severity of alveolar bone loss was done radiographically. Bitewing radiographs were evaluated for interproximal bone loss from the cemento-enamel junction (CEJ) of the tooth to the bone crest. These subjects also had bleeding on probing (BOP) at >80% of the proximal sites. A diagnosis of CP was made if the CAL was commensurate with the amount of supragingival plaque.

Healthy group: The healthy group consisted of 24 females and 24 males who exhibited (mean age 46 ± 8.58 years; range 37-51; Table 1). They had ≥20 teeth, ≥90% of the measured sited exhibited probing pocket depth (PPD) <3 mm and CAL ≤1 mm, and no bleeding on probing (BOP) at examination and no alveolar bone loss on radiographs (i.e., a distance of <3 mm between the CEJ and bone crest at >95% of the proximal tooth sites). This group included healthy volunteers from the staff and other patients from the Dental School (Table 1).

Determination of periodontal status

The clinical periodontal parameters were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) for the whole mouth, excluding third molars, and included PPD, CAL, BOP, and supragingival plaque accumulation. CAL was assessed from the CEJ to the bottom of the probable pocket. BOP (deemed positive if it occurred within 15 seconds after periodontal probing) and supragingival plaque accumulation were recorded dichotomously by visual examination (25). All measurements were performed by a single calibrated examiner using a manual Williams periodontal probe (Hu-Friedy, IKK UNITY, USA).

Treatment procedures

In conjunction with the screening examination the patients were given careful instructions in self-performed plaque control measures: twice-daily tooth-brushing using the Modified Bass brushing technique and once-daily inter-dental cleaning using dental floss and/or inter-dental brushes. In the baseline treatment protocol, quadrant scaling and root planning was performed over four consecutive sessions at 1-weekly intervals on each patient by an experienced periodontist (Ö.Ö.).

Genomic DNA preparation and quantification

Two milliliter of whole blood samples was collected into EDTA-anticoagulated tubes by a standard venipuncture method. Genomic DNA was extracted from whole blood samples using a commercially available genomic DNA purification kit (Nucleospin Blood L, Macherey-Nagel, Germany) following the manufacturer's instructions. DNA concentration was determined by a quantitation kit (PicoGreen dsDNA, Molecular Probes Inc., Eugene, OR, USA) according to the manufacturer's instructions and diluted as100 ng/µL

Genotyping of the MCP-1 -2518 gene

Genotyping of MCP-1 gene – 2518 A/G polymorphism in the promoter region was performed by the PCR–RFLP based method as previously described by Rovin et al. (15). Briefly; a 50 ng genomic DNA was amplified in a 25 µl mixture of 1x GeneAmp PCR Buffer II with own 1.5 mM $MgCl_2$ (Applied Biosystems Foster City CA), 200µM each deoxynucleotide triphosphate (dNTP) (Promega, Madison, WI), 5 pmol forward and reverse primers, 0.2µl AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA, USA) and completed with nuclease free water. The sequences of the forward and reverse primers used were: 5'CCGAGATGTTCCCAGCACAG3' and 5'CTGCTTTGCTTGTGCCTCTT3' respectively (TIB MOLBIOL Syntheselabor, Berlin-Germany). PCR amplifications were performed in the GeneAmp 9700 Thermal Cycler (PE Applied Biosystems Foster City, CA). The cycling conditions comprised of a denaturation step at 94° C for 10 min followed by 35 amplification cycles at 94°C for 60s, annealing at 55°C for 60s and

Table 1. Clinical characteristics of the chronic periodontitis (CP) and healthy groups

	All subjects		Non-smoker	
	CP	Healthy	CP	Healthy
No. of subjects	51	48	26	38
Age (age range)	45.94 ± 8.08 (32-65)	46 ± 8.58 (37-51)	45 ± 7.60 (35-63)	47 ± 8.67 (33-51)
Female/Male	18/33	24/24	9/17	17/21
PPD (mm)	4.06 ± 0.87	1.54 ± 0.26	4.19 ± 0.98	1.54 ± 0.26
CAL (mm)	5.47 ± 1.20	0.00 ± 0.00	5.75 ± 1.44	0.00 ± 0.00
BOP (%)	73.12 ± 24.44	15.48 ± 8.49	74.73 ± 20.99	16.89 ± 7.87
Plaque (%)	92.22 ± 15.22	17.08 ± 10.33	92.74 ± 15.79	17.83 ± 11.06

PPD; Probing pocket depth, CAL; Clinical attachment level, BOP; Bleeding on probing.

extension at 72°C for 90 s and a final extension at 72°C for 7 min. Yielded 930bp PCR products were controlled by the ethidium bromide staining 2% agarose gel electrophoresis. Genotyping from positive PCR products were performed using 5U ***Pvu II*** restriction enzyme (New England Biolabs, Beverly, MA), in a total volume of 20 µl at 37°C overnight described as manufacturers. After digestion samples were subjected to electrophoresis on gels containing a mixture of 1.5% Agarose (Sigma, St.Louis, MO) and 0.75% NuSieve GTG (BMA, Rockland, ME) staining with ethidium bromide and run for 1.5 h at 70 V. PCR bands were visualized in the UVP BioDoc-It Biolmaging Systems (Upland, CA). Samples showing only a 930-bp band were assigned as AA wild-type; samples showing two bands of 708 and 222 bp were typed as GG homozygotes; and those with three bands of 930, 708, and 222 bp were assigned as AG heterozygotes.

Genotyping RANTES −403 gene

The −403G/A genotype of RANTES gene was determined by the PCR-RFLP method(15). PCR amplification were performed with the forward and reverse synthetic oligonucleotide primers 5'-CACAAG AGG ACT CAT TCC AAC TCA-3' and 5'-GTT CCT GCT TAT TCA TTACAG ATC G TA-3'(QIAGEN Operon GmbH, Cologne Germany) respectively. A 100 ng genomic DNA was amplified in the thermalcycler (GeneAmp PCR System 9700, PE Applied Biosystems) in a 25 µl mixture of 1x GeneAmp PCR Buffer II with 2.0 mM $MgCl_2$ (Applied Biosystems Foster City, CA), 200µM each deoxynucleotide triphosphate (dNTP) (Promega, Madison,WI), 5 pmol forward and reverse primers, 0.1 µl AmpliTaq DNA Polymerase; (Applied Biosystems, Foster City, CA, USA) and completed with nuclease free water.

Thermal cycling conditions were 94° C for 10 min, then thirty five cycles of 94°C for 45s, annealing at 61°C for 45s and extension at 72°C for 45 s , followed by 7 min of final extension at 72°C, for yielding 206 bp PCR products , which controlled by the 2% agarose gel electrophoresis. Genotyping from positive PCR products were performed using ***Rsa I*** restriction enzyme (NewEngland Biolabs, Beverly, MA), in a total volume of 20 µl at 37°C overnight described as manufacturers. After digestion samples were subjected to electrophoresis on gels containing a mixture of 1.5% Agarose (Sigma,St.Louis,MO) and 0.75% NuSieve GTG (BMA, Rockland,ME) staining with ethidium bromide and run for 1.5 h at 70 V. PCR bands were visualized in the UVP BioDoc-It Biolmaging Systems (Upland, CA). Digestion with *Rsa* I yield 180 and 26 bp fragments when G is at position -403.

Statistical Analysis

In the present study, Chi-square (χ^2) analysis was used to test for deviation of allele frequencies from Hardy–Weinberg equilibrium. The distribution of the MCP-1 and RANTES genotypes and allele frequencies in healthy and CP groups were also analyzed by a chi-square test. Allele frequencies were calculated from the observed numbers of genotypes. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were also assessed. Differences in clinical parameters between CP patients with homozygous and heterozygous G and A positive genotype and patients with G and A negative genotype were compared by the non-parametric Mann-Whitney U-test. P values <0.05 were considered to be statistically significant. In a subgroup analysis, smokers were excluded and statistical analysis was performed in non-smokers as well. The relationship between genotype and disease status was analyzed by multiple logistic regression analysis while adjusting for potential confounding factors including age, gender and smoking status. Clinical periodontal parameters were comparatively analyzed by use of Repeated Measure ANOVA test before and after periodontal therapy. All data analyses were performed using a statistical package (SPSS Inc., version 14.0, Chicago, IL, USA).

Table 2. Genotype distributions, allele frequencies and G allele carriage rate of MCP-1 gene -2518A/G polymorphism in CP and healthy subjects

	All subjects				Non-smokers			
	CP N=51 (%)	Healthy N=48 (%)	P Value	OR (95% CI)	CP N=26 (%)	Healthy N=38 (%)	P Value	OR (95% CI)
Genotype								
AA	29 (56.9)	26 (54.2)			11 (42.3)	23 (60.5)		
AG	18 (35.3)	20 (41.7)	P=0.612		12 (46.2)	14 (36.8)	P=0.197	
GG	4 (7.8)	2 (4.2)			3 (11.5)	1 (2.6)		
Carriage of G allele	22 (43.1)	22 (45.8)	P=0.787	0.897 (0.40 - 1.98)	15 (57.7)	15 (39.5)	P=0.154	2.091 (0.75 - 5.76)
Allele Frequency								
A	76 (74.5)	72 (75)	P=0.937	1.026	34 (65.4)	60 (78.9)	P=0.091	1.985
G	26 (25.5)	24 (25)		(0.54 - 1.95)	18 (34.6)	16 (21.1)		(0.89 – 4.39)

CP; chronic periodontitis, OR; odds ratio, CI, confidence interval

Results

The clinical parameters of study groups are summarized in Table 1. The frequencies of MCP-1 and RANTES genotypes in the healthy group were found in accordance with those expected by the Hardy–Weinberg equilibrium ($P = 0.978$, $\chi^2 = 0.045$) (25).

Distribution of MCP-1 -2518 genotype, allele frequency and carriage of rare allele:

The distribution of MCP-1 genotypes among the study groups is presented in Table 2. There was no significant difference among the groups in the distribution of MCP-1 A/G genotypes ($P=0.612$). The G allele was found in 25.5% of the CP patients as compared with 25% in the healthy group. There was no significant difference among the study groups in allele frequencies ($P = 0.937$). The frequency of G allele carriage of the MCP-1 gene was similar between CP and healthy control ($P = 0.787$; OR = 0.897 95% CI = 0.40–1.98; Table 2). In a subgroup analysis when the smokers were excluded, identical genotype distribution was observed among non-smoker study groups and no significant difference was found between non-smoker CP and healthy subjects for overall allele frequencies ($P=0.197$, $P=0.091$ respectively).

Distribution of RANTES -403 genotype, allele frequency and carriage of rare allele:

The distribution of RANTES gene polymorphism among the study groups is presented in Table 3. No significant difference was detected between CP and healthy groups in the distribution of RANTES G/A genotypes ($P=0.942$). The A allele was found in 30.4% of CP patients as compared with 29.2% in the healthy group. There was no significant difference among the study groups in allele frequencies ($P = 0.851$). The frequency of A allele carriage of the RANTES gene was similar between CP and healthy subjects ($P = 0.770$; OR = 1.125, CI = 0.51–2.47; Table 3. In a subgroup analysis when the smokers were excluded, there were no significant differences in the distribution of RANTES genotypes and allele frequencies between non-smoker CP and non-smoker healthy subjects. (P=0.603, P=0.792, respectively; Table 3).

Carriage of rare allele in relation to susceptibility to periodontitis:

In order to investigate whether carriage of rare allele is associated with clinical parameters, differences in clinical parameters between CP patients with G positive (GG and AG) and A positive (AA and GA) genotype were compared by non-parametric Mann Whitney U-test. No significant associations were observed between clinical parameters and MCP-1 gene G positive genotypes in CP patients (P>0.05) (Table 4).There was no significant difference in probing depth, clinical attachment loss and in sites with plaque (%) between CP patients with RANTES A positive and A negative genotypes (P>0.05). When only non-smoker CP patients were taken into consideration, clinical periodontal parameters were found to be similar between RANTES A positive and negative genotype except the percentage of sites with bleeding on probing. BOP was found to be significantly higher in RANTES gene A negative genotypes compared to A positive genotypes (P=0.034; Table 4).

Influence of genotypes on response to non-surgical periodontal therapy:

In order to investigate whether MCP-1 G positive/ negative genotypes and RANTES A positive/negative genotypes affect the response to the non-surgical periodontal therapy were analyzed by Repeated Measure of ANOVA.

The clinical parameters of CP group markedly improved during 6-month study period. Significant

Table 3. Genotype distributions, allele frequencies and A allele carriage rate of RANTES gene -403G/A polymorphism in CP and healthy subjects

	All subjects				Non-smokers			
	CP N=51 (%)	Healthy N=48 (%)	P Value	OR (95% CI)	CP N=26 (%)	Healthy N=38 (%)	P Value	OR (95% CI)
Genotype								
GG	24 (47.1)	24 (50.0)			11 (42.3)	16 (42.1)		
GA	23 (45.1)	20 (41.7)	P=0.942		14 (53.8)	18 (47.4)	P=0.603	
AA	4 (7.8)	4 (8.3)			1 (3.8)	4 (10.5)		
Carriage of A allele	27 (52.9)	24 (50.0)	P=0.770	1.125 (0.51 - 2.47)	15 (57.7)	22 (57.9)	P=0.987	0.992 (0.36 - 2.72)
Allele Frequency								
G	71 (69.6)	68 (70.8)	P=0.851	1.06	36 (69.2)	50 (71.4)	P=0.792	1.11
A	31 (30.4)	28 (29.2)		(0.57 – 1.95)	16 (30.8)	20 (28.6)		(0.50 -2.43)

CP; chronic periodontitis, OR; odds ratio, CI, confidence interval

Table 4. Clinical parameters (mean±SD) of chronic periodontitis (CP) patients distributed by G positive/negative and A positive/negative genotype

MCP-1 -2518 A/G	All CP subjects (n=51)			Non-smoker CP subjects (n=26)		
	AA (n=29)	AG + GG (n=22)	P Value	AA (n=11)	AG + GG (n=15)	P Value
Age (age range)	45.48 ±7.67	46.55 ± 8.72	P=0.646	45.55 ± 8.68	44.60 ± 6.98	P=0.761
PPD (mm)	3.79 ± 0.51	4.23 ± 1.12	P=0.354	4.16 ± 0.70	4.21 ± 1.17	P=0.516
CAL (mm)	5.13 ± 0.79	5.30 ± 0.97	P=0.850	5.77 ± 0.94	5.73 ± 1.75	P=0.452
BOP (%)	68.67 ± 29.17	78.57 ± 25.00	P=0.705	77.18 ± 21.22	72.94 ± 21.38	P=0.533
Plaque (%)	89.89 ± 16.79	96.29 ± 7.52	P=0.113	87.95 ± 22.77	96.26 ± 6.59	P=0.485
RANTES -403 G/A	GG (n=24)	GA + AA (n=27)	P Value	GG (n=11)	GA + AA (n=15)	P Value
Age (age range)	46.71 ±7.87	45.26 ± 8.34	P=0.528	46.09 ± 9.01	44.20 ± 6.59	P=0.542
PPD (mm)	4.24 ± 1.04	3.89 ± 0.66	P=0.159	4.55 ± 1.21	3.93 ± 0.71	P=0.114
CAL (mm)	5.75 ± 1.43	5.22 ± 0.91	P=0.120	6.20 ± 1.83	5.41 ± 1.00	P=0.172
BOP (%)	81.27 ± 21.58	65.88 ± 24.91	P=0.023 *	84.76 ± 15.24	67.38 ± 22.00	P=0.034*
Plaque (%)	92.60 ± 14.34	91.89 ± 16.23	P=0.869	91.17 ± 14.86	93.89 ± 16.85	P=0.673

CP; Chronic Periodontitis, PPD; Probing pocket depth, CAL; Clinical attachment level, BOP; Bleeding on probing.

* Significant difference between RANTES A positive genotypes (Mann-Whitney test, $P < 0.05$)

Table 5. Clinical parameters (mean±SD) of chronic periodontitis (CP) patients' in baseline and 6 month distributed by G positive/negative and Apositive/negative genotype

MCP-1 -2518 A/G	All CP subjects (n=51)				tNon-smoker CP subjects (n=26)			
	AA		AG+GG		AA		AG+GG	
	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
PPD (mm)	3.79 ± 0.51	4.23 ± 1.12*	4.23 ± 1.12	3.05 ± 1.06*	4.16 ± 0.70	3.09 ± 0.75*	4.21 ± 1.17	3.07 ± 1.08*
CAL (mm)	5.13 ± 0.79	5.30 ± 0.97*	5.30 ± 0.97	4.27 ± 1.40*	5.77 ± 0.94	4.70 ± 0.95*	5.73 ± 1.75	4.48 ± 1.54*
BOP (%)	68.67 ± 29.17	78.57 ± 25.00*	78.57 ± 25.00	22.55 ± 16.15*	77.18 ± 21.22	38.37 ± 23.97*	72.94 ± 21.38	24.94 ± 18.34*
Plaque (%)	89.89 ± 16.79	96.29 ± 7.52*	96.29 ± 7.52	29.44 ± 22.47*	87.95 ± 22.77	40.65 ± 33.24*	96.26 ± 6.59	30.77 ± 25.78*
	GG		GA+AA		GG		GA+AA	
RANTES -403 G/	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
PPD (mm)	4.24 ± 1.04	3.13 ± 1.08*	3.89 ± 0.66	2.85 ± 0.71*	4.55 ± 1.21	3.24 ± 1.16*	3.93 ± 0.71	2.96 ± 0.75*
CAL (mm)	5.75 ± 1.43	4.45 ± 1.30*	5.22 ± 0.91	4.04 ± 1.02*	6.20 ± 1.83	4.87 ± 1.56*	5.41 ± 1.00	4.36 ± 1.09*
BOP (%)	81.27 ± 21.58	24.08 ± 14.86*	65.88 ± 24.91	25.78 ± 22.11*	84.76 ± 15.24	26.25 ± 17.35*	67.38 ± 22.00	33.82 ± 24.24*
Plaque (%)	92.60 ± 14.34	31.15 ± 18.42*	91.89 ± 16.23	32.40 ± 26.77*	91.17 ± 14.86	29.95 ± 23.50*	93.89 ± 16.85	38.62 ± 32.71*

*Significant difference from baseline ($P < 0.05$).

Table 6. Logistic regression analysis for susceptibility to chronic periodontitis

	OR	95% CI	P value
Age	0.166	0.08-1.15	0.217
Gender	0.511	0.18-1.38	0.188
Smoking	4.031	1.34-12.11	0.013
G allele carriage	1.420	0.51-3.88	0.495
A allele carriage	1.058	0.37-2.97	0.914

CP; chronic periodontitis, OR; odds ratio, CI, confidence interval

improvements in clinical parameters were found after non-surgical periodontal therapy in both MCP-1 and RANTES genotypes (P<0.05; Table5). These significant differences were not associated with MCP-1 gene G positive and RANTES gene A positive genotype. The effect of treatment during the 6-month study period was similar in the MCP-1 gene G positive and RANTES gene A positive genotype. There were no interactions between both MCP-1 and RANTES genotypes and treatment effect (P >0.05).

Multiple logistic regression model

Multiple logistic regression analysis was used to evaluate the association of the G and A positive genotype with periodontal susceptibility, while adjusting for significant confounders. In this model, smoking was found to be significant confounders (*P*=0.013). On the other hand, gender, G allele carriage and A allele carriage were not statistically significant (*P*=0.188, *P*=0.495 and 0.914, respectively; Table 6).

Discussion

The primary role of the inflammatory response is to protect the host against bacterial invasion as the first line defence (26,28). Recent years have witnessed a great deal of interest in the role that some chemokines might play initiation and perpetuation of inflammation by facilitating selective leukocyte recruitment to inflammatory sites and directly activating leukocytes to release inflammatory mediators (7,15). The present study evaluated MCP-1 and RANTES gene polymorphisms in patients with CP and healthy controls. Results of the present study revealed that the distribution of MCP-1 and RANTES genotypes and allelic frequencies of CP patients were similar to those of periodontally healthy group. Although MCP-1 and RANTES polymorphisms were widely investigated in several diseases with chronic inflammation such as atopic dermatitis, atherosclerosis, systemic lupus erythematosus and inflammatory renal disease (16,19,20,21,22), there is no data regarding MCP-1 and RANTES genotypes in periodontitis up to date. This report shows the effect of MCP-1 and RANTES polymorphisms on non-surgical periodontal therapy outcomes. Thus, our data could provide useful reference and serve to represent Turkish population and CP for future studies.

Our results indicate that the distribution of MCP-1 genotype in CP patients was similar to those of the periodontally healthy group. The G allele carriage rate of CP patients was 25.5% while healthy subjects had a carriage rate of 25.0%. Szalai et al. suggested that individuals homozygous for the MCP-1 -2518G alleles are at increased risk of developing coronary artery disease (CAD) and the MCP-1 -2518G allele frequency was 23.9% in CAD versus control group (29.9%) (21). Simeoni et al. mentioned that MCP-1 -2518 G homozygosity was also associated with arteriosclerosis (22). Present study show that the distribution of RANTES -403 A positive and A negative genotypes were not significantly different in both CP and healthy groups. RANTES -403A allele frequency was not different between the CP (30.4%) and healthy groups (29.2%).

Although the frequencies of MCP-1 -2518 G and RANTES -403A alleles in our population was very similar to those reported previously in other populations (15,19,22), MCP-1 and RANTES gene polymorphisms are not associated with the susceptibility to chronic periodontitis in Turkish population.

Simeoni et al. aimed to determine the association of RANTES -403A gene polymorphism with increased risk of coronary arteriosclerosis and specified that RANTES -403A may increase genetic susceptibility to arteriosclerosis (22). It has been reported that RANTES -403A allele was not significantly frequent in atopic eczema/dermatitis syndrome (19).

Different factors could influence increased susceptibility to periodontitis, which is a multifactorial periodontal disease (26). Smoking is known to be a well-established risk factor for periodontitis that influences host inflammatory immune response by suppressing the activity of several mediators (29-32). Therefore, in the present study, MCP-1 and RANTES gene polymorphisms were evaluated in non-smoker subjects as well. Even in the group of non-smokers, there was no association between CP and MCP-1 and RANTES gene polymorphisms.

CP is a multifactorial disease whose manifestation and progression is influenced by a variety of factors such as genetic factors, smoking, age and gender (23,33,34). It has been suggested that increasing age and being male is associated with the prevalence, extent and severity of periodontitis (33). In the present study, logistic regression analysis was performed to evaluate the genotype effects on an increased severity of CP while adjusting these confounding factors. In this model, smoking was found to be significant confounders to the increased susceptibility to CP. On the other hand, the MCP-1 -2518G and RANTES -403A allele positivity was not associated with susceptibility to CP.

Although we found similar genotype distributions and allele frequencies in the MCP-1 and RANTES genes in CP and healthy subjects, no other study was found that is investigating the MCP-1 and RANTES genotypes in periodontitis. Thus, our data could provide useful reference and representation of a Turkish population for studies to come. It was previously shown that BOP, which is a significant sign of periodontal inflammation and an early indicator of disease progression in periodontal tissues, was found to increase in IL-1 genotype-positive subjects (32,35). Our data have shown that the percentage of BOP was significantly higher in RANTES -403A allele positive subjects. Therefore, this significant higher percentage of bleeding shows that A allele carriage might be related to the susceptibility to inflammation.

It is well known that the efficacy of non-surgical therapy at an individual site is related to the baseline PPD and deeper pockets have more potential of PPD reduction and CAL gain (36). As one might expect, significant improvements in clinical parameters were observed following non-surgical therapy at 6-month, in both MCP-1 G positive/negative and RANTES A positive/ negative genotypes.

To our best knowledge, this is the first study investigating MCP-1 and RANTES gene polymorphisms in CP patients in the literature. The current study of relative small sample size suggests that there were no association between the MCP-1 -2518G and RANTES -403A alleles in chronic periodontitis patients in Turkish population. Although the sample size is low and has low power for a genetic study, non-surgical periodontal therapy was applied to CP patients. In the present study we aimed to analyze the potential impact of MCP-1 and RANTES genotypes in response to the non-surgical periodontal therapy. Moreover, the responses to the non-surgical periodontal therapy during the 6-month study period were not different in the MCP-1 gene G positive and RANTES gene A positive genotype. In other words, there were no relation between both MCP-1 and RANTES specific alleles and treatment effect.

Periodontal disease should be viewed as a polygenic disease in which many interacting gene variants contribute to disease susceptibility. The novel results of this study need to be confirmed in future studies in different populations and genetic variations in periodontal disease.

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