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Analysis of interleukin-10 gene polymorphisms in patients with chronic periodontitis and healthy controls

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ABSTRACT

Background: Interleukin-10 (IL-10) is an anti-inflammatory cytokine that has important roles in the periodontal diseases. The IL10-1082, -819, and -592 polymorphisms in the promoter region of IL-10 gene have been associated with various IL-10 expressions. The aim of this study was to investigate the association between these gene polymorphisms with chronic periodontitis in a sample of Iranian populations from Southeast of Iran.

Materials and Methods: IL-10 single nucleotide polymorphisms were analyzed in 210 patients with chronic periodontitis (CP) and 100 individuals without CP by polymerase chain reaction-restriction fragment length polymorphism method. Statistical analysis of data was performed using the Chi-square test. The risk associated with single alleles, genotypes, and haplotypes were calculated by performing a multiple logistic regression analysis to estimate the odds ratio (OR) and 95% confidence interval (CI). $P < 0.05$ for statistical significance.

Results: The prevalences of AG and GG genotypes of IL10-1082 were significantly different between CP and control groups in comparison to AA genotype (OR = 2.671; CI = 1.482–4.815; $P = 0.001$ for AG vs. AA, OR = 4.151; CI = 2.128–8.097; $P < 0.001$ for GG vs. AA). In addition, subjects with at least one IL10-1082-G allele were significantly had an increased risk for CP (OR = 2.157; CI = 1.531–3.038; $P < 0.001$). The distribution of the IL10-819 and IL10-592 genotypes was not different between CP and control subjects ($P = 0.109$ and $P = 0.139$, respectively). The combination of different genotypes showed that GCC haplotype was significantly different between groups (OR = 4.379; CI = 1.077–17.807; $P = 0.039$).

Conclusion: The results demonstrated that IL10-1082 polymorphism was a putative risk factor for chronic periodontitis and associated with increased susceptibility to CP.

Key Words: Chronic periodontitis, interleukin-10, polymorphism

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INTRODUCTION

Chronic periodontitis (CP) is a destructive inflammatory disease caused by various bacteria. This microbial challenge initiates by bacterial plaque and followed by inflammation and destruction of the tooth-supporting tissues which leads to tooth loss. It seems that more than 15% of the adult people have been affected by severe forms of chronic pancreatitis (CP) in the world. Studies have been shown that bacterial plaque-related inflammation induced innate immune responses

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in the host. These mechanisms affect the levels of immunological elements such as cytokines. In addition, genetic factors such as various polymorphisms can stimulate the production of cytokines.[1] It has been shown that polymorphisms in pro-inflammatory and anti-inflammatory cytokines gene such as interleukin 6 (IL-6),[2] tumor necrosis factor alpha,[3,4] transforming growth factor beta,[5-7] interferon gamma,[8] and IL-10[9,10] are related to the susceptibility to the chronic inflammatory diseases such as chronic periodontitis and chronic hepatitis B virus infection. In addition, stereological and morphological analysis have reported that there is a significant relationship between pro-inflammatory gene polymorphisms and the level of gingival tissue degradation in chronic periodontitis[4-6,11] IL-10 is an anti-inflammatory cytokine which has the vital role in the pathogenesis of periodontal diseases.[1] This cytokine is expressed by various cells especially leukocytes.[12] IL-10 can control viral infections and related tissue damages through stimulating the secretion of immune factors, controlling the phagocytosis and antigen presentation. On the other hand, IL-10 improves the innate and adaptive immunity.[13]

The gene encoded IL-10 is located on chromosome 1q31-q32. Polymorphisms in the promoter region of the IL-10 gene can affect the expression of IL-10 cytokine which leads to changes in inflammatory processes.[9,14] There are some conflicting results regarding the association between IL-10 polymorphisms and CP.[15-17] The IL10-1082, -819, and -592 polymorphisms are in linkage disequilibrium and produce two important haplotypes. The studies revealed that IL10-1082, -819, and -592 polymorphisms were related to the CP in Swedish, Turkish, and Brazilian patients.[15,17] In addition, numerous studies have revealed that these polymorphisms are related to the other chronic diseases such as hepatitis B virus infection,[9] Crohn's disease,[18] and sepsis.[19] Moreover, *in vitro* studies have reported that GCC/GCC genotype is associated with increased the expression of IL-10 cytokine compare to the other genotypes.[20] Given that IL-10 can inhibit the matrix metalloproteinases and reduce the periodontal tissue destruction,[21] the aim of the current study was to reveal the possible relationship between IL-10 polymorphisms (-1082, -819, and -592) and chronic periodontitis.

MATERIALS AND METHODS

Study population

This study was carried out in Infectious Diseases and Tropical Medicine Research Center, and Zahedan Dental School, Zahedan, Iran. It was approved by the Institutional Ethics Committee of the Zahedan University of Medical Sciences, and written consent forms were signed by all the participants. In the current study, we used blood DNA extracted from 2 ml peripheral blood of 210 CP patients, and 100 healthy individuals were recruited for the Grant No. 6210 in our previous study.[8] All participants were nonsmokers and have at least 20 teeth. Exclusion criteria included subjects with a history of cardiovascular disorders, systemic disorders, immunodeficiency diseases, use of anti-inflammatory drugs, chemotherapy, individuals with previous orthodontic treatment, pregnant women, and smokers. Chronic periodontitis was defined according to the criteria of the International workshop for classification of periodontal diseases and conditions.[22] The control group consisted of unrelated healthy individuals who had no clinical history of periodontal disease. Clinical evidence such as gingival index (GI), periodontal probing depth (PPD), and clinical attachment level (CAL) in healthy subjects included as follows: GI <1 and PPD <3 mm, and CAL = 0 and they had healthy periodontium. Signs of clinical inflammation such as GI >1, PPD >4 mm, and CAL >2 mm and bone loss were clinical evidence for chronic periodontitis.

Genotyping of interleukin-10 polymorphisms (-1082, -819, and -592)

Genomic DNA was isolated from peripheral venous blood using the salting-out method as described previously.[3] IL10 gene polymorphisms were determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method using primers that amplified a short fragment of DNA containing the polymorphism. The primers used for genotyping of IL-10 polymorphisms are listed in Table 1.

Genotyping for IL10-1082, IL10-819, and IL10-592 was performed in volumes of 20 μl containing 1 μl of each primer, 100 ng of template DNA and 10 μl of 2X Prime Taq Premix and 7 μl ddH2O. PCRs were run for 30 cycles: initial denaturation; 5 min at 95°C, denaturation; 30 s at 95°C, annealing; 30 s at 55°C, 57°C, and 60°C IL10-1082, IL10-819 and IL10-592,

Table 1: The primers sequences used for detection of interleukin-10 (-1082, -819, and -592) gene polymorphisms using restriction fragment length polymorphism-polymerase chain reaction

Polymorphisms	Sequence (5'->3')	Restriction enzyme	Product size (bp)
1082 (*G/A*; *rs1800896*)	F: CCAGATATCTGAAGAAGTCCTG R: CTCTTACCTATCCCTACTTCC	MnlI	A allele: 134, 65 G allele: 112, 65, 22
819 (*C/T*; *rs1800871*)	F: CCAGATATCTGAAGAAGTCCTG R: TGGGGGAAGTGGGTAAGAGT	RseI	C allele: 443, 116 T allele: 559
592 (*C/A*; *rs1800872*)	F: GGTGAGCACTACCTGACTAGC R: CCTAGGTCACAGTGACGTGG	RSaI	A allele: 236, 176 C allele: 412

respectively, extension; 30 s at 72°C, final extension; 5 min at 72°C. The product of IL10-1082 (10 µl) was digested with MnlI (Fermentas, Vilnius, Lithuania) restriction enzyme at 37°C for 16 h, subjected to electrophoresis in 4% agarose gel (Invitrogen, USA), and stained with ethidium bromide. IL-10 A-1082 allele gives 2 fragments of 134 and 65 bp, and IL-10 G-1082 allele 3 fragments of 112 bp, 65 bp, and 22 bp [Figure 1]. The product of IL10-819 (10 µl) was digested with RseI (Fermentas, Vilnius, Lithuania) restriction enzyme at 37°C for 16 h, subjected to electrophoresis in 2% agarose gel (Invitrogen, USA), and stained with ethidium bromide. IL-10 C-819 allele gives 2 fragments of 443 and 116 bp, and IL-10 T-819 allele a single 559-bp fragment [Figure 2]. The product of IL10-592 (10 µl) was digested with RSaI (Fermentas, Vilnius, Lithuania) restriction enzyme at 37°C for 16 h, subjected to electrophoresis in 2% agarose gel (Invitrogen, USA), and stained with ethidium bromide. IL-10 A-592 allele gives 2 fragments of 236 and 176 bp, and IL-10 C-592 allele a single 412 bp fragment [Figure 3].

Statistical analysis

The analyses were performed using a software package SPSS 20 (SPSS Inc. Chicago, IL, USA). Statistical analysis of the clinical parameters was carried out using the two-sample *t*-test. Differences in frequencies of genotypes and allele carriage between groups were assessed by the Chi-Square test. All *P* values were two-sided and defined as $P < 0.05$ for statistical significance. The risk associated with individual alleles or genotypes was as calculated by performing a multiple logistic regression analysis to estimate the odds ratio (OR) and confidence interval 95% (CI). Demographic data and clinical parameters were expressed as mean ± standard deviation.

RESULTS

There were no significant differences in mean age value, gender, and ethnicity between the chronic periodontitis and control groups ($P > 0.05$) [Table 2.]. The chronic periodontitis group exhibited a significantly greater mean of PD and CAL than the control group. In addition, significant differences in the percentage of sites with BOP were found in chronic periodontitis, compared to the control group ($P < 0.05$). The allele and genotype frequencies of the 3 single nucleotide polymorphisms (SNPs) (-1082, -819, and -592) in the IL-10 gene are shown in Table 3. The genotype frequencies in controls and chronic periodontitis were all in Hardy–Weinberg equilibrium ($P > 0.05$). The Chi-square test found a statistically significant difference of IL10-1082 genotypes between two groups ($P < 0.0001$). The prevalences of AG and GG genotypes were significantly different between chronic periodontitis and control group in comparison to AA genotype. In addition, IL10-1082 G allele was significantly higher in CP subjects compared to controls ($P < 0.0001$) and subjects with at least one G allele (AG, GG genotypes) were significantly had an increased risk for CP (OR = 2.157;

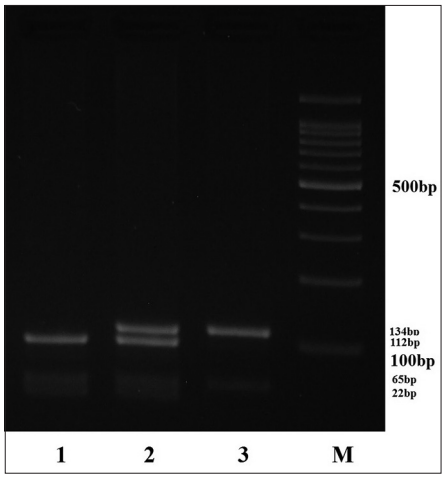

Figure 1: Electrophoretic scheme of RFLP-PCR generated bands for determination of *IL10-1082 A/G* gene polymorphism. Left to right: lane 1, GG homozygous (112 bp, 56 bp and 22 bp), lane 2, AG heterozygous (134 bp, 112 bp, 65 bp and 22 bp), AA homozygous (134 bp and 65 bp), M, DNA marker.

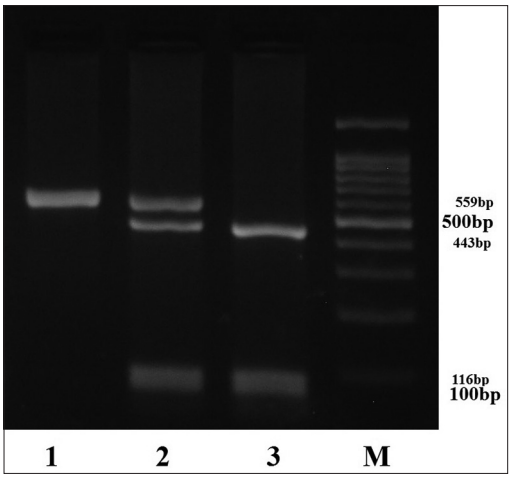

Figure 2: Electrophoretic scheme of RFLP-PCR generated bands for determination of IL10-819 T/C gene polymorphism. Left to right: lane 1, TT homozygous (559 bp), lane 2, TC heterozygous (559 bp, 443 bp and 116 bp), CC homozygous (443 bp and 116 bp), M, DNA marker.

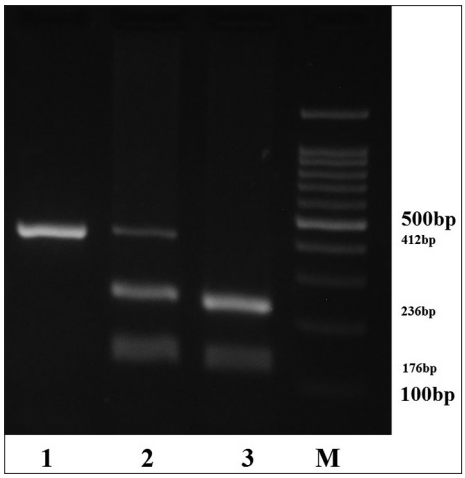

Figure 3: Electrophoretic scheme of RFLP-PCR generated bands for determination of IL10-592 A/C gene polymorphism. Left to right: lane 1, CC homozygous (412 bp), lane 2, AC heterozygous (412 bp, 236 bp and 176 bp), AA homozygous (236 bp and 176 bp), M, DNA marker.

Table 2: Demographic parameters of chronic periodontitis patients and control group

Parameters	CP (*n*=210)	Control (*n*=100)	*P*
Age (years) (mean±SD)	28.33±5.76	29.22±3.60	0.159
Gender			
Male	115 (54.8)	48 (48.0)	0.265
Female	95 (54.2)	52 (52.0)	
Ethnicities			
Sistani	82 (39.2)	42 (42.0)	0.186
Baluch	71 (33.8)	24 (24.0)	
Others	57 (27.1)	34 (34.0)	
Clinical parameters (mean±SD)			
BOP index (%)	85.86±3.68	-	
Mean PD (mm)	5.58±0.63	1.50±0.86	
CAL (mm)	5.44±0.58	-	

CP: Chronic periodontitis; SD: Standard deviation; BOP: Bleeding on probing; PD: Probing depth; CAL: Clinical attachment level

CI = 1.531–3.038). Statistical analysis showed that the distribution of the IL10-819 and IL10-592 genotypes and alleles were not different between CP and control subjects (P = 0.139 and P = 0.109, respectively) [Table 3]. Haplotype frequencies of IL-10 polymorphisms were significantly different between patients and controls (P < 0.0001). Combination of different genotypes of IL-10 polymorphisms (-1082, -819, and -592) generates 7 haplotypes as shown in Table 4. As indicated in Table 4 and compared with the control group, the findings showed that GCC haplotype was significantly different between subjects with CP and controls (P = 0.039; OR = 4.379; CI = 1.077–17.807).

DISCUSSION

Previous researches showed that amounts of IL-10 mRNA were varied between different CP patients and it seems that caused the different clinical condition in their CP outcomes.[21] Variation in the natural history of CP disease may be due to genetic polymorphisms in the IL-10 gene promoter SNPs. These SNPs are related to the CP severity and can affect the expression of IL-10 cytokine against the inflammation.[9,15,21,23,24] The current study analyzed the IL-10 gene polymorphisms (-1082, -819, and -592) in CP patients. The study findings revealed that there is a significant difference in the frequency of IL-10-1082 genotypes among CP patients and controls. The distribution of the IL-10-1082 genotypes in Iranians is similar to the Swedish Caucasians,[15] although these two population are not racially close to each other. Another result of the current study was that IL-10-1082 SNP associated with CP and IL-10-1082G allele increase the susceptibility to CP. IL-10-819 and IL-10-592 SNPs were not related to the CP susceptibility in our population, but IL-10-819C and IL-10-592C alleles were slightly higher in CP patients compared to healthy subjects. GCC haplotype was predominant in CP patients compared to controls and these results suggested that IL-10 SNPs in the promoter region are important risk factors for chronic periodontitis.

Previous studies reported that IL-10 was an important inflammatory marker in certain inflammations and the significant association was shown between chronic

Table 3: The frequency of genotypes and alleles of interleukin-10-*592* (*rs1800872*, *A/C*), *-819* (*rs1800871*, *T/C*) and *-1082* (*rs1800896*, *A/G*) polymorphisms gene in chronic periodontitis patients and control subjects

IL-10 polymorphisms	Chronic periodontitis, *n* (%)	Control, *n* (%)	OR (95% CI)	*P*
-592 (*rs1800872*, *A/C*)				
AA	46 (21.9)	29 (29.0)	Reference=1	-
AC	152 (72.4)	61 (61.0)	1.571 (0.905-2.727)	0.109
CC	12 (5.7)	10 (10.0)	0.757 (0.290-1.974)	0.569
AC + CC	165 (78.6)	71 (71.0)	1.498 (0.870-2.579)	0.145
Allele				
A	244 (58.1)	119 (59.5)	Reference=1	-
C	176 (41.9)	81 (40.5)	1.060 (0.752-1.492)	0.740
-819 (*rs1800871*, *T/C*)				
TT	58 (27.6)	37 (37.0)	Reference=1	-
TC	138 (56.7)	54 (54.0)	1.630 (0.971-2.739)	0.065
CC	14 (6.7)	9 (9.0)	0.992 (0.390-2.524)	0.987
TC + CC	152 (72.4)	63 (63.0)	1.539 (0.928-2.554)	0.095
Allele				
T	254 (60.5)	128 (64.0)	Reference=1	-
C	166 (39.5)	72 (36.0)	1.162 (0.820-1.647)	0.399
-1082 (*rs1800896*, *A/G*)				
AA	34 (16.2)	38 (38.0)	Reference=1	-
AG	98 (46.7)	41 (41.0)	2.671 (1.482-4.815)	0.001
GG	78 (37.1)	21 (21.0)	4.151 (2.128-8.097)	0.000 <0.001
AG + GG	176 (83.8)	62 (62.0)	3.173 (1.838-5.476)	0.000 <0.001
Allele				
A	166 (39.5)	117 (58.5)	Reference=1	-
G	254 (60.5)	83 (41.5)	2.157 (1.531-3.038)	0.000 <0.001

IL: Interleukin; OR: Odds ratio; CI: Confidence interval

Table 4: Haplotype frequencies in chronic periodontitis (case) and normal subjects (control)

Haplotypes	CP group, *n* (%)	Control group, *n* (%)	*P*	OR
GCC	104 (49.5)	19 (19.0)	0.039	4.379 (1.077-17.807)
ATC	4 (1.9)	10 (10.0)	0.203	0.320 (0.055-1.847)
GCA	21 (10.0)	18 (18.0)	0.926	0.933 (0.217-4.010)
GTC	31 (14.8)	17 (17.0)	0.608	1.459 (0.345-6.168)
GTA	20 (9.5)	7 (7.0)	0.303	2.286 (0.475-11.003)
ACC	25 (11.9)	25 (25.0)	0.759	0.800 (0.192-3.333)
ATA	5 (2.4)	4 (4)	Reference=1	-
Total	210 (100.0)	100 (100.0)		

OR: Odds ratio; CP: Chronic periodontitis

inflammatory diseases such as hepatitis B virus infection,[9] systemic lupus erythematous,[25] Behcet's disease,[26] and IL-10 promoter-1082 G allele. In this regard, Berglundh *et al*.[15] analyzed the association of IL-10-1082 SNP with chronic periodontitis and concluded that the frequency of G allele was significantly higher in CP patients than in control subjects and they resulted that IL-10-1082 SNP was associated with CP in Caucasian subjects. Berglundh *et al*. revealed the higher frequency of GG genotype in nonsmokers CP patients in comparison to the controls with OR = 6.11 and CI = 2.03–18.37. In 1997, Kornman *et al*.[27] have reported the distribution of 5 alleles in subjects with the different clinical condition of CP and investigated the significant relationship between CP and a number of allele combinations such as IL1B + 3953 A2 allele and IL1A − 889 A2 allele (OR = 6.8, CI = 1.01–45.95). In the current study, the ratio for the GG genotype (CP patients vs. controls) was OR = 4.251, CI = 2.128–8.097 which is same to the abovementioned results. It means that GG genotype is a useful factor as a genetic biomarker for CP susceptibility. The present results are in agreement with findings reported by Berglundh *et al*.,[15]

Kornman *et al*.[27] and Loo *et al*.,[28] Gore *et al*.,[29] Galbraith *et al*.,[30] and Schaefer *et al*.[31] Loo *et al*. found that -1082 IL-10 AA genotype frequencies were lower in Chinese CP patients than healthy subjects and volunteers who had at least one G allele had increased risk to CP. Furthermore, Schaefer *et al*. suggested that GG genotype was more prevalence in subjects with CP than in subjects without CP. On the other hand, Smith *et al*.[32] suggested that -1082 G allele related to the increased expression of IL-10 cytokine gene while the -1082 A allele was related to reduced expression of this gene. In another study, Donati *et al*.[33] found that expression of IL-10 and proportion of IL-10 positive cells of peripheral area in gingival biopsies of volunteers with GG genotype were significantly larger than those with AG or AA genotypes. They suggested that IL-10 expression in chronic periodontitis lesions was associated with CP severity. As mentioned above, our strongest findings were among variants of IL-10, especially the SNP 1082 (A/G; rs1800896), which had a strong association with CP.

Studies have shown that IL-10 gene polymorphism affects the secretion of IL-10 which can affect the body's immune response to the chronic disease such as hepatitis B virus infection.[34] Therefore, these polymorphisms cause individual differences in immune response and lead to different host immune function. In the current study, -1082 G allele was significantly represented in CP patients. The -1082 G allele corresponds to higher IL-10 protein expression *in vitro*. Furthermore, the -1082 GG, GA, and AA genotypes were related to high, intermediate and low IL-10 production in patients with HBV, respectively.[34] On the other hand, increased levels of IL-10 have been showed in chronic inflammatory diseases,[34] indicating that subjects carrying -1082 G allele have a risk for development of inflammation. In this study, analysis of IL-10-1082 genotypes showed that subjects with at least one -1082 G allele are more susceptible to CP compared to subjects with -1082 A allele. Reuss *et al*. noted that the -1082 GG allele produced higher levels of IL-10 that may compromise the immune response to the infection.[24] The study results were in accordance with previous reports.[15,27-31] The study findings presented reduced frequency of the IL-10-1082 A/A genotype and increased frequency of IL-10-1082 GG and AG genotypes in CP patients than the controls.

As to the IL-10-819 and -592 gene polymorphisms, we did not find any significant association between these SNPs and susceptibility to CP. In a meta-analysis conducted by Albuquerque *et al*.[35] suggested that IL-10-819 SNP was associated with chronic periodontitis inflammation in Caucasian population with regard to the ethnic variations. In addition, Scarel-Caminaga *et al*. suggested that IL-10-819 SNP related to the severity of disease (OR = 3.04, 95%CI = 1.34–6.91).[16] By contrast, many studies did not investigate any positive results. Our findings were in agreement with studies conducted by Sumer *et al*.,[17] Reichert *et al*.,[36] Hu *et al*.,[37] Tang *et al*.,[38] and Kobayashi *et al*.[39] For the relationship between IL-10-819 SNP and chronic periodontitis, these studies presented no effect of the IL-10-819 SNP on the disease susceptibility. In this polymorphism, we found an evidence of IL-10-819 C allele versus T allele in CP patients versus controls alleles comparison. The results indicated that subjects with C allele carriers had a risk of CP, but it was not significant. Regarding the IL-10-592 SNP, the findings of some studies revealed that the frequency of subjects with A allele was significantly larger in CP patients than in controls.[16,17,36,37] By contrast, our current study[9] did not show any significant different in allele distribution between hepatitis B virus-infected patients and healthy controls which is in agreement with the results of this study. However, the capacity for IL-10 production depends on genetic polymorphisms, but polymorphism at position -592 and -819 of the candidate gene in patients with inflammation does not emerge as a probable biomarker for determining the disease. However, according to the OR, it seems that heterozygosity of genotypes −592 A/C and −819 T/C is associated with liver inflammation and increased risk of persistent chronic infection. Anyway, in regard to the IL-10-592 SNP, our findings did not confirm the related studies. For this SNP, we investigated evidence of IL-10-592 C allele versus A allele in CP patients versus controls alleles comparison, but it was not significant. The IL-10-819 and -592 gene polymorphisms were in linkage disequilibrium, and no association with CP disease was observed. Furthermore, it is believed that IL-10 production is independent of -592A/C and -819T/C polymorphisms. The differences between various studies could be due to the genotypic differences in cytokine genes in the different ethnic origin of the different populations. Moreover, environmental condition or technical errors, such as smoking and type 1 errors might explain contradictory results. In addition, interactions between IL-10 and other types of cytokines, racial

difference and other related genetic markers might explain the incongruous results.

In the current study, combination of different alleles of the IL-10 SNPs-592 A/C, -819 T/C, -1082 A/G generates 7 haplotypes which GCC haplotype had increased frequency among CP patients in comparison to healthy subjects. Our findings were in agreement with results reported by Moudi *et al*.[9] and Turner *et al*.[34] Analysis of IL-10 haplotype in HBV patients and controls indicated that the frequency of the GCC variant was higher among HBV patients than healthy subjects.[9] Turner reported that GCC haplotype was related to increased level of IL-10 expression *in vivo*. The level of transcriptional activation of a gene depends on the binding of regulatory factors to specific recognition sequences in the promoter. A number of putative recognition sites are present in the IL-10 promoter. Mutations in cytokine gene promoter sequences may alter specific transcription factor recognition sites and consequently affect transcriptional activation and cytokine production. The presence of the specific alleles of bi-allelic polymorphisms at position -592 A/C, -819 T/C, -1082 A/G in the IL-10 promoter has been shown to change the transcription in a reporter gene assay. These polymorphism lies within an ETS-like recognition site and may, therefore, affect the binding of transcription factors, which has been shown to act as a regulator of inflammations.[34] Luciferase study by Crawley *et al*.[20] revealed that GCC haplotype had increased transcriptional activity than ATA. Moreover, the activity of the GCC haplotype did not differ from that of ACC or ATA haplotypes and subjects with ATA haplotype had lower IL-10 production. In another study, Suarez *et al*.[40] have reported that GCC haplotype had a lower frequency in healthy population compared to the ATA and ACC haplotypes. In addition, increased levels of IL-10 mRNA expression were observed in subjects with GCC/GCC genotype compared to ATA/ACC and/or ATA/ATA genotypes.[40]

CONCLUSION

In summary, the results of the current study indicate the existence of an association between IL-10 polymorphisms in the promoter region and chronic periodontitis in CP patients. Based on the multifactorial character of CP and complexity of disease mechanism, more clarification of CP pathogenesis is necessary. It can be concluded that IL-10 polymorphisms are important risk factors for CP and can useful to understand the pathways of CP susceptibility.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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