



Association of Matrix Metalloproteinase-1 -1607 1G/2G and C-Reactive Protein -717 C/T Gene Polymorphisms in Iranian Patients with Chronic Periodontitis: A Clinical Trial

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Abstract

Background: An imbalance in the expression of matrix metalloproteinases (MMPs) and tissue inhibitor metalloproteinases initiate the destructive process in chronic periodontitis (CP). C-reactive protein (CRP) is a systemic inflammatory mediator that reflects an acute immune response.

Objectives: The purpose of this investigation was to analyze the association between the MMP-1 -1607 1G/2G (rs1799750) and CRP 717 A/G (rs2794521) gene polymorphisms and chronic periodontitis in Iran.

Methods: This analytical case-control study was performed among 141 participants including 63 CP cases and 78 matched healthy individuals. Five milliliters of peripheral blood was collected for DNA isolation. Restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) was performed for single-nucleotide polymorphism (SNP) analysis. The frequencies were analyzed by chi-squared test (95% CI, $P < 0.05$). In addition, genetic data were assessed by the Hardy-Weinberg principle, linkage disequilibrium, and haplotype analysis.

Results: Our findings presented no significant relationship between genotype/alleles of MMP-1 -1607 1G/2G (rs1799750) (0.73: 0.27-1.95, $P = 0.48$) or CRP 717 A/G (rs2794521) (0.384: 0.104 -1.414, $P = 0.303$) and the presence of CP ($P = 0.47$ and $P = 0.30$, respectively). The analysis of genetic distribution among various severities of CP and controls revealed no significant association between various severities of CP and MMP-1 -1607 1G/2G (rs1799750) ($P = 0.52$) and CRP 717 A/G (rs2794521) ($P = 0.67$).

Conclusions: Our results suggest no association between the occurrence or severity of chronic periodontitis and MMP-1 -1607 1G/2G (rs1799750) and CRP 717 A/G (rs2794521) polymorphisms. Further studies with larger sample sizes may provide a more generalizable evidence-based overview of the relationship between these gene polymorphisms and periodontitis.

Keywords: Chronic Periodontitis, C-Reactive Protein, Genes, Matrix, Metalloproteinase-1, Polymorphism, Risk Factors

1. Background

Chronic periodontitis (CP) is a complex inflammatory disease, which commonly progresses slowly. This disease is initiated by pathogenic microorganisms, the host's response and environmental factors (1) and is estimated to have a prevalence of 5% to 79.6% (2). As an inflammatory disease, CP could not only destroy periodontium due to host immune response and pathological plaques (2, 3) but also it can cause tooth loss and affect systemic health (4-10).

Periodontal tissues in CP are characterized by the accumulation of Gram-negative bacteria and inflammatory cells (11). It has been proposed that the balance in the secretion of cytokines from type 1 T-helper cells and type 2 T-

helper cells in host immune response is an important factor for the determination of inflammatory diseases' consequences (12). In fact, a complex network between cytokines regulates the inflammatory and immune responses in periodontal tissues during a periodontal disease (13).

It has been suggested that the imbalance in the secretion of matrix metalloproteinases (MMPs) and tissue inhibitor metalloproteinase initiates the destructive process in CP (14). These cytokines normally regulate fibrosis formation, and extracellular matrix (ECM) turn over (13). Moreover, the elevated expression of MMPs has been observed in several inflammatory diseases like atherosclerosis (15), osteoarthritis (16) and ovarian cancer (17). Within

MMPs family, MMP-1 is the most common element of the periodontal tissue matrix (18, 19), which adjusts the biodegradation of interstitial collagen fibers (20). Overexpression of MMP-1 in periodontal tissues has been reported in periodontitis (21), and it seems MMP-1 is locally secreted in periodontitis by stimulated fibroblasts (22).

Genetic diversity in MMP genes can affect its expression level (23), and ECM degradation can lead to the development of periodontitis (24-26). The MMP-1 gene has a single nucleotide polymorphic (SNP) region at -1607 base pair (27, 28), and previous studies have shown the association of its 2G/2G genotype with CP in different populations (27, 28).

During the past decades, the association between periodontal diseases and systemic health has been reported due to local bacterial mediators or systemic host immune mediators (29). C-reactive protein (CRP) is a systemic inflammatory mediator that reflects an acute immune response (30). CRP is produced and released by the liver due to fever, infection and hypoxia, and is one of the most sensitive markers used to assess the inflammatory status (31, 32). There is an association between increased level of CRP in the peripheral blood and periodontal ligament, and this relationship seems to be dose-dependent (CRP level ranging from 1 to 4 mg/L indicates healthy periodontal condition to severe periodontitis) (33). In addition, previous studies demonstrated a reduction in plasma levels of CRP after periodontal treatments and decreased inflammation (34). Different studies among diverse populations showed a variation in increased levels of CRP within patient and control groups (34, 35), which suggests the impact of other factors including genetic variations in host responses in periodontitis. Only a few studies have studied CRP gene polymorphisms in CP, which reported an association between CRP 717 heterogeneous type within patients and normal subjects (36).

2. Objectives

Thus, the purpose of the current investigation was to analyze the association between the MMP-1 -1607 1G/2G (rs1799750) and CRP 717 A/G (rs2794521) gene polymorphisms and chronic periodontitis in Iranian patients.

3. Methods

3.1. Sample Preparation and Examinations

In the present case-control investigation that was performed during 2016 - 2017 in Zanjan, Iran, 170 individuals were examined. All the procedures were approved by the Ethics Committee of Shahid Beheshti University of Medical

Sciences and the ethical guidelines of the Helsinki Declaration. The inclusion criteria for both groups (patients and matched healthy individuals) were being over 21 years old, being Iranian, having more than 20 teeth, and not having any systemic health problems (except chronic periodontitis). The exclusion criteria, on the other hand, were the presence of systemic health problems such as diabetes, HIV infection, hepatitis and various malignancies. Moreover, we excluded pregnant patients or those who were taking antibiotics in the past three months. After obtaining written informed consent, the individuals were assessed by an experienced periodontist using a standard Williams probe (grading 1-2-3-5-7-8-9-10 mm).

After that, chronic periodontitis cases were determined and divided into mild, moderate and severe groups according to the American Academy of Periodontology (AAP) classification guideline (37). The control group consisted of gingivitis cases and healthy individuals. For each patient, probing depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL) were determined at six sites (i.e., mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) of each tooth. Finally, the mean PD, BOP and CAL of each individual was calculated. In this study, CP cases who had 30% > affected teeth with ≥ 5 mm CAL, 3 - 4 mm CAL and 1 - 2 mm CAL were considered as severe, moderate and mild CP, respectively.

3.2. DNA Extraction and Genotyping

We obtained 5 mL of peripheral blood samples with standard venipuncture and collected the samples in tubes containing EDTA (Sarstedt, Numbrecht, Germany). DNA was extracted by a DNA extraction kit (CinnaGen Inc., Tehran, Iran) and modified salting-out method previously used by Miller et al. (37). Restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) was applied to determine genotypes and single-nucleotide polymorphisms. At first, 50 ng of DNA produced by PCR was exposed to restriction enzymes (TaqI, SacII and Bsp restrictive enzymes [New England Bio Labs, Beverly, MA, USA]) and purged by silica gel membrane (QIAquick PCR Purification Kit, Quiagen, Hilden, Germany). The analysis of the single-nucleotide polymorphisms of MMP-1 -1607 1G/2G (rs1799750) and CRP 717 C/T (rs2794521) were performed (Table 1). For the PCR reactions, at first the specimens were denatured at 95°C for 15 minutes, followed by 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and 72°C for 10 minutes. Then, all the remaining sequences were analyzed by electrophoresis on 2% agarose gel stained by Gel Red (Biotium Inc., Hayward, CA, USA).

Table 1. Primers and Nucleotide Polymorphisms for CRP and MMP-1 Which Were Utilized in This Study

Gene (Genebank code)	Primer Sequence	Size (bp)	Restriction Enzyme (bp)	Annealing Temperature (°C)
CRP -717C/T (rs2794521)	5'-TCAATTGGCTGAGAAAATGTGC-3'	502	BstUI (Bsh1236I); 44 + 458	60
	5'-AATGGGAAATGGTAACATATTAAC-3'			
MMP1 -1607 1G/2G (rs1799750)	5'-TCGTGAGAATGTCTCCCAT-3'	118	AluI; 28 + 90 (1G)	58
	5'-TCTTGGATTGATTGAGATAAGTCATAgC-3'			

Abbreviations: CRP, C-reactive protein; MMP1, matrix metalloproteinase 1; SNPs, single nucleotide polymorphisms.

3.3. Statistical Analysis

PD, BOP and CAL were analyzed descriptively and the distribution of the means was compared between the groups. For testing normality of the data, Kolmogorov-Smirnov test was performed and paired and unpaired t tests were run to compare the groups (CP and control). In addition, the chi-squared test was used for phenotypic analysis. The statistical procedures were performed by SPSS version 20.0 at the significance level of 5% with 95% confidence interval. Haploview 4.2 software (Cambridge, Massachusetts, USA) was used to evaluate the deviation from Hardy-Weinberg principle, multiple inheritance models, and linkage disequilibrium and haplotype conditions.

4. Results

4.1. Demographic and Clinical Data

Twenty-nine individuals were excluded due to incomplete cooperation, and 141 participants (77 healthy individuals and 64 CP patients) were enrolled. Table 2 displays the demographic and clinical data. The comparison of demographic and periodontal status between the groups showed a non-significant difference between the groups in terms of gender and age ($P = 0.31$ and $P = 0.09$, respectively), but PD and CAL were significantly higher in the patient group ($P = 0.001$).

4.2. Genetic Data

All the genotypes and allelic distribution within the groups are presented in Table 3. We found no significant association between genotype/phenotype of MMP-1 -1607 1G/2G (rs1799750) and the presence of CP ($P = 0.479$). Moreover, no statistically significant association was observed between chronic periodontitis and CRP -717 C/T gene polymorphism (rs731236) ($P = 0.303$). The comparison of genetic distributions (genotypes and alleles) among various severities of CP and healthy individuals (Table 4) revealed a significant relationship among various severities of CP and

MMP-1 -1607 1G/2G ($P = 0.52$) or CRP -717 C/T gene polymorphism ($P = 0.67$). None of the investigated SNPs met the Hardy Weinberg principle (38).

5. Discussion

Previous studies have shown that genetic factors may increase the risk of periodontitis, one of the most prevalent inflammatory conditions, through potential interactions that are not still completely described (39). In the current study, we aimed to assess the relationship between the MMP-1 -1607 1G/2G (rs1799750) and CRP 717 A/G (rs2794521) single-nucleotide gene polymorphisms and CP in Iranian patients for the first time. Our findings showed no significant relationship between genotype/phenotype of MMP-1 -1607 1G/2G (rs1799750) or CRP 717 A/G (rs2794521) and the occurrence or severity of CP.

Li et al. in 2016 conducted a review to evaluate the role of various MMPs in the risk of periodontal diseases (40). In accordance with our results, they could not find any significant relationship between MMP-1 -1607 1G/2G SNP and the risk of periodontal disease in several studies that assessed this association among CP patients. In addition, they failed to associate MMP-1 -519 A/G and -422 A/T SNPs with susceptibility to periodontitis. A possible reason for this finding is that the presence of high diversity in the expression of MMP-1 among periodontitis patients could be due to the additional impact of specific pathogens and cytokine stimulation (41). Moreover, it has been shown that the combination of several significant gene variants can synergistically influence susceptibility to the disease (42). In addition, an increase in mRNA transcription caused by these MMP-1 promoter SNPs may not necessarily intensify the impact of MMP-1 on the surrounding periodontal tissues, and many other factors such as bacterial metabolites, cytokines, and other gene variants are supposed to be involved in the regulation of MMP-1 expression and functionality.

CRPs are commonly produced in immediate inflammatory conditions, infections, and trauma in reaction to

Table 2. Demographic Data and Periodontal Parameters of the Subjects Recruited to This Study^a

Variable	Study Groups		P Value
	Control (N = 78)	Chronic Periodontitis (N = 63)	
Age, y	34.22 ± 11.07	37.61 ± 12.63	0.091 ^b
Gender, No. (%)			0.317 ^c
Male	27 (34.6)	27 (42.8)	
Female	51 (65.3)	36 (57.1)	
PD, mm	1.59 ± 0.21	2.9 ± 1.34	0.001 ^b
CAL, mm	0.21 ± 0.31	2.8 ± 1.53	0.001 ^b
BoP, %	9.83 ± 15.32	16.48 ± 17.71	0.040 ^b
Frequency of periodontitis, No. (%)			-
Mild	-	20 (31.7)	
Moderate	-	24 (38)	
Severe	-	20 (31.7)	

^a Values are expressed as mean ± SD unless otherwise indicated.

^b t test.

^c Pearson chi-square.

Table 3. Distribution of Genotypes and Alleles for Matrix Metalloproteinase-1 (MMP-1) -1607 1G/2G and C-Reactive Protein (CRP) -717 C/T Gene Polymorphisms Among Controls and Chronic Periodontitis Group

Genotypes and Alleles	Controls	CP Cases	OR (95% CI)	P Value
CRP				
T/T	6	10	1	0.303
T/C	43	32	0.382 (0.111 - 1.313)	
C/C	27	19	0.384 (0.104 - 1.414)	
T	52	55	1	0.319
C	70	97	1.310 (0.8041 - 2.135)	
Hardy-Weinberg equilibrium	0.049	0.571		
MMP1				
2G/2G	19	14	1	0.479
1G/2G	29	19	0.730 (0.273 - 1.954)	
1G/1G	29	30	1.292 (0.505 - 3.303)	
1G	87	79	1	0.328
2G	67	47	1.294 (0.7997 - 2.095)	
Hardy-Weinberg equilibrium	0.040	0.004		

other proinflammatory cytokines (43). D'Aiuto et al. reported that CRP gene polymorphism was an independent predictor of CRP expression in intensive periodontal treatment (44). They showed specific genotypes could elevate the serum level of CRP up to its intermediate level at 30 mg/L after periodontal instrumentation. Furthermore, contrary to our results, Auerkari et al. found a relationship between CRP (717) SNP and the risk of periodontal disease in the Indonesian population (36). However, they did

not find any significant relationship between CRP (1444) single-nucleotide polymorphism and CP. These discrepancies may be due to different ethnicities or our study's limited sample size which could have influenced our results. Larger sample size and using sequencing methods may help address these issues.

In conclusion, our results suggested no association between the occurrence or severity of CP and MMP-1 -1607 1G/2G (rs1799750) and CRP 717 A/G (rs2794521) polymor-

Table 4. Distribution of the Matrix Metalloproteinase-1 (MMP-1) -1607 1G/2G and C-Reactive Protein (CRP) -717 C/T Genotypes in the Study Groups Based on the Periodontitis Severity

Genotype	Controls		CP Cases			OR (95% CI)	P Value
	Healthy	Gingivitis	Mild CP	Moderate CP	Severe CP		
MMP1							0.521
2G/2G	15	4	2	6	6	REF	
2G/1G	19	9	6	6	8	0.774 (0.303 - 1.97)	
1G/1G	23	19	20	24	20	0.565 (0.238 - 1.34)	
CRP							0.67
TT	4	1	4	3	4	REF	
TC	31	12	8	14	10	2.605 (0.707 - 90.59)	
CC	21	6	6	7	6	0.994 (0.437 - 2.25)	

phisms. According to our literature review, this study was the first investigation of the association between gene polymorphisms of CRP and MMP-1 and the risk of periodontal disease among the Iranian population.

Footnotes

Authors' Contribution: Surena Vahabi designed the study, worked on it and supervised. Bahareh Nazemiasman did the literature review and work on it. Mahsa Kalantari and Sepanta Hosseinpour coordinated the study and collected the data.

Conflict of Interests: The authors have no conflicts of interests.

Ethical Considerations: This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and all the procedures were performed under the supervision of the committee and according to the 1964 Helsinki Declaration.

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Patient Consent: Written informed consent was obtained from all the participants (cases and controls).

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