

The Role of Interleukin-1 Haplotype in the Association between Atherosclerosis and Periodontitis in a Syrian Population

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ABSTRACT The role of Interleukin-1 haplotype in the association between chronic periodontitis and atherosclerosis has been established in previous studies. However, no studies of this type have been carried out in Syria to assess such a role. Therefore, the purpose of this study is to compare the frequencies of interleukin1 haplotypes in the chronic periodontitis subjects with and without atherosclerosis in a Syrian Arab population. Two hundred Syrian Arab chronic periodontitis patients (184 males, 16 females; mean age 52.61) were divided into two groups: first group was subjects with atherosclerosis; second group was 100 subjects without atherosclerosis. Blood samples were collected from the patients for genotyping analysis of IL-1 α +4845, IL-1 β +3954, IL-1 β -511 and IL-1RN VNTR using PCR-RFLP technique. Chi-square test was used for the statistical study ($P < 0.05$) with SPSS.V17 and EH program for studying interleukin-1 haplotype. Significant differences in the frequencies of interleukin-1 haplotypes between atherosclerosis and non- atherosclerosis groups were shown ($p = 0.0148$). Pattern2 – which includes allele2 at both the IL-1 β -511, and at the IL-1RN VNTR as well as allele1 at both loci of IL-1 β +4845 and IL-1 β +3954 - was significantly higher in atherosclerosis group vs. non-atherosclerosis group (20%-10%). These findings may suggest that Pattern2 is associated with increased susceptibility to atherosclerosis.

INTRODUCTION

Atherosclerosis is considered the most common cause of Coronary Heart Disease (CHD). It is a variable combination of changes of the intima of arteries that lead to the narrowing of the lumen of the coronary arteries (Ross 1999). There is now a convincing body of evidence that atherosclerosis is much more than the simple vascular accumulation of lipids and has a major inflammatory component (Armitage 2000).

Periodontitis is a chronic inflammatory disease of multifactorial etiology initiated by specific bacteria (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium periodontium*, *Eikenella corrodens*, *Bacteroides forsythus*, *Campylobacter rectus*, *Treponema denticola*) that activate host mechanisms which in turn destroy the bone and connective tissues that support the teeth (Kornmann 2008).

Earlier epidemiologic studies showed an association between CHD and periodontitis. The findings of meta-analysis have concluded that periodontitis is a risk factor or marker independent of traditional CHD risk factors with relative risk estimates ranging from 1.24 to 1.35 (Bahekar et al. 2007; Humphrey et al. 2008). Consistent with these findings, it was found in a previous work that the values of alveolar bone loss were significantly higher in the atherosclerosis group. The odds ratio of having atherosclerosis was 1.568 when the alveolar bone loss was (severe + moderate) vs. mild (Bashour et al. 2013).

The epidemiological association between periodontitis and CHD led to the identification of several biological mechanisms that may explain it. One of such observations is the shared genetic predisposition that influences both diseases. One candidate that influences inflammation, IL-1 gene polymorphisms, has been associated with periodontitis and CHD (Kornmann and Duff 2001). IL-1 gene polymorphisms were found to play a significant role in the development of coronary artery disease (CAD), especially myocardial infarction (MI), in patients with *chlamydia pneumoniae* infection (Momiya et al. 2001). Patients with acute coronary syndrome (ACS) or angina were more likely to experience a positive IL-1 polymorphism and severe periodontitis (Goteiner et al. 2008). Allele1 of IL-

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1RN VNTR was associated with the coexistence of CHD and periodontitis in a multiple regression model (Geismar et al. 2008).

IL-1 α +4845 is a nonsynonymous, biallelic polymorphism located on exon 5 of the IL-1 α gene. IL-1 β +3954 is a biallelic, synonymous polymorphism located on exon 5 of the IL-1 β gene. IL-1 β -511 is a biallelic promoter polymorphism of the IL-1 β gene (Nicklin et al. 1994), and IL-1RN VNTR is a variable number of tandem repeats polymorphism located on intron 2 of the IL-1Ra gene (Tarlow et al. 1993). These four polymorphic markers are in significant linkage disequilibrium, indicating that specific groups of alleles are inherited together in at least two distinct patterns (haplotype). One pattern includes allele2 at both the IL-1 α +4845 and at the IL-1 β +3954 loci as well as allele1 at both loci of IL-1 β -511 and IL-1RN VNTR. The other pattern includes allele2 at both the IL-1 β -511, and at the IL-1RN VNTR as well as allele1 at both loci of IL-1 α +4845 and IL-1 β +3954 (Cox et al. 1998). The first pattern (Pattern1) of IL-1 genetic polymorphisms is associated with periodontitis. While the other pattern (Pattern2) is associated with atherosclerotic plaque formation (Kornmann et al. 1999). Similarly, pattern1 of IL-1 Haplotype was found to be associated with severe chronic periodontitis in Yemenis (Al-hebshi et al. 2012). While modifying, the impact of the pattern1 of IL-1 Haplotype failed to be detected even though an association between periodontitis and acute myocardial infarction (AMI) was confirmed (Stein et al. 2009).

Undeniably, an earlier study by our group found out that allele2 of IL-1 α +4845 may be considered a risk indicator for having both CHD and severe alveolar bone loss, suggesting that linkage disequilibrium or haplotype-based analyses should be used in future studies (Bashour et al. 2013). Therefore, the purpose of this study was to determine whether differences could exist in the frequencies of IL-1 haplotypes at positions (α +4845, β +3954, β -511 and IL-1RN VNTR) between chronic periodontitis patients with and those without atherosclerosis in the investigated Syrian Arab population.

METHODOLOGY

The study protocol was approved by the Research Ethics Committee of the Faculty of Dentistry, Damascus University, Damascus, Syria.

ia. Informed written consent was obtained from participants. The study was conducted as a matched case-control study from 2010 to 2012.

Study Population

The sample consisted of 200 Syrian Arab patients with periodontitis, (184 males, 16 females; aged 40–70 years, mean age 52.61) who were divided into two groups: the cases group consisted of 100 subjects with atherosclerosis (92 males, 8 females; mean age 52.06) from the Cardio surgery Department of Al-Assad University Hospital, Damascus University; and the controls group consisted of 100 subjects without atherosclerosis (92 males, 8 females; mean age 53.16) who were matched for gender and age within two years. They were enrolled in the Department of Periodontology, Faculty of Dentistry, Damascus University. Criteria for exclusion from the study were the presence of diabetes mellitus, hypertension, high cholesterol levels, osteoporosis, pregnancy, and pronounced obesity (body mass index (BMI) > 30 kg/m²). Patients who presented <6 teeth were also excluded from the study. All patients were required to have Syrian Arab parents and grandparents to reduce genetic heterogeneity in the sample.

Medical Examination

All participants had a medical examination, including a questionnaire on CHD and periodontitis risk factors. Hospital case records were reviewed to confirm the diagnosis of individuals with CHD. Diagnosis of CHD was based on coronary artery angiography. In the cases group, patients admitted to the hospital were subject to coronary artery bypass graft, while in the controls group, patients were referred to a specialist in cardiology to conduct an electrocardiogram to determine the absence of CHD.

Oral Examination

The same dentist performed all oral examinations and analyses of radiographs. Diagnosis of periodontitis was based on the existence of one side or more probing depths (PD) $\geq$ 4 mm or clinical attachment loss (CAL) $\geq$ 3 mm. PD and CAL were performed for every patient using UNC dental probe. PD was measured as the distance

from the gingival margin to the bottom of the pocket at four sites per tooth. CAL (distance between the cement-enamel junction and the bottom of the pocket) was obtained by adding the PD values to the gingival recession (GR) values (distance between the gingival margin and the cement-enamel junction) (Newman et al. 2002).

Alveolar bone loss (ABL) was measured using apical digital radiography. ABL was defined as the distance between the cement-enamel junction and the most apical level of the alveolar crest. ABL was stratified into three groups according to the American Dental Association (ADA) classification: ADAII group mean was 3-4 mm, ADAIII group mean was 4-6 mm, and ADAIV group mean was eH6 mm (Haring and Howerton 2005).

Purification of DNA and Analyses of Genetic Polymorphisms

DNA Extraction and Genotyping

IL-1 tests were achieved in Genetic Research Laboratory, Faculty of Medicine, Damascus University, Damascus, Syria. Venous blood samples were collected from the patients in vacutainer tubes containing EDTA. The blood samples were stored at -20°C until genomic DNA

was isolated using Genomic DNA purification with NucleoSpin Blood, Macherey-Nagel (MN) kit, Germany. Genotypes of IL-1 α +4845, IL-1 β +3954, and IL-1 β -511 were analyzed by Restriction Fragment Length Polymorphism (RFLP). Genotypes of the IL-1RN VNTR polymorphism were detected by normal Polymerase Chain Reaction (PCR) amplification and fragment size analysis. PCR reactions consisted of 2.5 μ L DNA, 12.5 μ L PCR master mix (PCR Master Mix (2X), Fermentas Life Sciences, Canada), 9 μ L water for PCR, and 0.5 μ L for each primer (VBC Biotech, Austria) (Table 1) in a final volume of 25 μ L. The PCR amplification conditions consisted of 95°C for 2 minutes, followed by 35 cycles of 95°C for 60 seconds and polymorphism-specific annealing temperature (Table 1) for 60 seconds and 72°C for 60 seconds. The PCR was terminated by final elongation at 72°C for 5 minutes. PCR amplification was performed in a thermal cycler (TECHNE-TC-512 thermal cycler, UK). The amplified DNA was digested with restriction enzymes (Fermentas Life Sciences, Canada) as listed in Table 1. Agarose gel electrophoresis and ethidium bromide staining analyzed all PCR products and digested PCR products. Genotypes were determined by comparing the size of the bands with a 100-base pair DNA ladder. The resulting products are listed in Table 1.

Table 1: Genotyping of the four polymorphic variants

Gene / SNP	Primer sequences	Ta (°C)	RE	DT (°C)	DD (h)	PCR product (bp)	HWE P value
IL-1 α +4845 rs17561 G>T	FW: 5' ATGGTTT TAGAAATCATCAAGCCTAGGG - CA-3' Rev: 5' ATGAAAGGAGGGGAGGATGACAGAAA TGT-3'	56	SatI	37	16	153	0.3124
IL-1 β +3954 rs1143634 C>T	FW: 5' CTCAGGTGTCCTCCAAGAAATCAAA-3' - Rev: 5' -GCTTTTTTGCTGTGAGTCCCG-3	57	TaqI	65	2	182	0.0591
IL-1 β -511 rs16944 A>G	FW: 5' -TGGCATTGATCTGGTTCATC-3' Rev: 5' -GTTTAGGAATCTTCCCACTT-3'	55	AvaI	37	16	304	0.547
IL-1 RN VNTR	FW: 5' CTCAGCAACACTCCTAT 3' Rev: 5' TCCTGGTCTGCAGGTAA 3	55				410=4 repeats 240=2 repeats	0.562

SNP = single nucleotide polymorphisms; Ta = Annealing temperature ;HWE = Hardy-Weinberg equilibrium; RE = Restriction Enzyme; DT= Digestion Temperature; DD = Digestion Duration; rs numbers are RefSNP SNP-identification codes as applied in the public nucleic acid polymorphism databases at the national center for biotechnology information. Sherry et al. (2001)

Statistical Analysis

Statistical analysis was performed using statistical software SPSS program V. 10 for Windows, Chicago, IL, USA, with P value < 0.05 considered statistically significant. Using the Pearson chi-square test, the Hardy-Weinberg equilibrium (HWE) was tested for fitness for IL-1 gene polymorphisms at positions (α +4845, β +3954, β -511, and RN VNTR). Haplotype frequencies were calculated on cases and controls by the haplotype frequency estimation (EH) program. The differences between cases and controls in the frequency of haplotypes was also determined by the EH program (Sham 1998).

RESULTS

Descriptive Data and Periodontal Examination

Two hundred Syrian Arab patients with periodontitis (184 males, 16 females; mean age 52.61) were enrolled in the study. Subject characteristics are presented in Table 2. There were no relevant differences between cases and controls for smoking (P = 0.757, Table 2). None of the polymorphisms genotyped deviated significantly from the assumption of the Hardy-Weinberg equilibrium (Table 1).

Haplotype frequencies were calculated on patients and controls by the haplotype frequency estimation (EH) program. Sixteen haplotypes were observed as listed in Figure 1.

The differences between cases and controls in the frequencies of haplotypes were also determined by the EH program. This program was used three times: on cases alone to determine the frequency of haplotypes and the log likelihood in cases only (in L_{cases}) (Table 3); once on controls to calculate the frequency of haplotypes

and the log likelihood in controls only (in $L_{controls}$) (Table 4). Thereafter, it was performed on cases and controls pooled together to obtain the frequency of alleles, haplotypes and log likelihood in cases and controls (in $L_{case-controls}$) (Table 5). Considering N as a degree of freedom (df), which is the number of haplotypes in the target population (df=15), a Chi-square test with N-1 was obtained using the following formula:

Haplotype frequency estimates = $2[(\log \text{likelihood (in } L_{cases}) + \log \text{likelihood (in } L_{controls}) - (-\log \text{likelihood [in } L_{combined}])}] = \text{Chi squared with } n-1$.

$$\chi^2 = 2 \times [-371.26 - 331.20 - (-717.10)] \\ = 2 \times 14.64 = 29.28$$

After calculating the value of Chi-square test, assuming there were 16 haplotypes, then there would be 15 degrees of freedom and the P value would be 0.0148, which was calculated using Microsoft office excel.

Since P value < 0.05, there was a significant difference between cases and controls in the frequency of haplotypes. This means that, there is a significant difference between periodontitis patients with and those without atherosclerosis in the distribution of IL-1 haplotypes at positions (α +4845, β +3954, β -511 and IL-1RN VNTR) in the investigated Syrian Arab population.

Table 6 and Figure 2 showed that pattern2 was significantly more frequent in the atherosclerosis group (20% vs. 10%), while pattern1 was significantly more frequent in chronic periodontitis patients without atherosclerosis (15% vs. 11%).

DISCUSSION

The study was very carefully designed to induce the statistical power. The sample size was

Table 2: Descriptive parameters and periodontal parameters in patients with and without atherosclerosis

Variable	Atherosclerosis	Non-atherosclerosis	P value
	n=100	n=100	
Age (years; mean $\pm$ SD)	52.06 $\pm$ 6.899	53.16 $\pm$ 5.832	† 0.225
Male (%)	92%	92%	† 1.00
Smokers (%)	68%	63%	† 0.757
BMI (Kg/m ² ; mean $\pm$ SD)	26.17 $\pm$ 2.03	25.87 $\pm$ 1.91	† 0.005

† Unpaired T test.

‡ Mann Whitney U test

* χ^2 test.

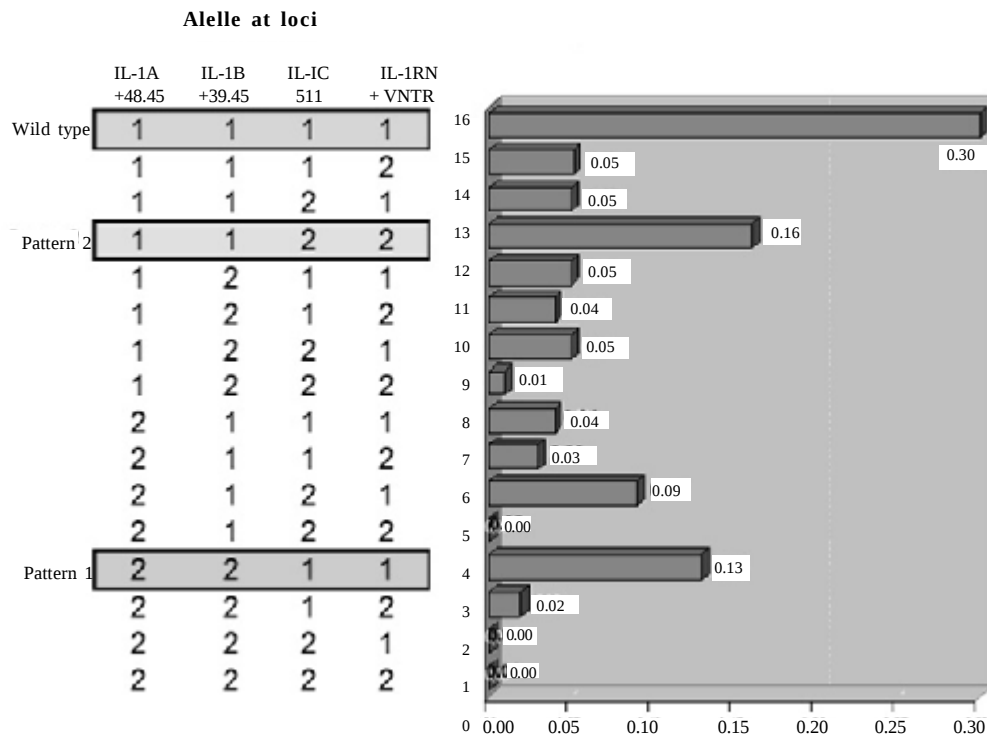


Fig. 1. Frequencies of haplotype patterns calculated using EH program

Table 3: Frequencies of IL-1 haplotypes in atherosclerosis group (cases) as determined by EH program

	Allels at loci				Haplotype frequency	
	IL-1a+4845	IL-1B+3954	IL-1B-511	IL-1RA VNTR	Independent	w/Association
Wild Type	1	1	1	1	0.255095	0.337788
	1	1	1	2	0.122824	0.039725
Pattern2	1	1	2	1	0.134363	0.052682
	1	1	2	2	0.064693	0.200962
Pattern1	1	2	1	1	0.091973	0.054240
	1	2	1	2	0.044283	0.051117
	1	2	2	1	0.048444	0.043486
	1	2	2	2	0.023325	0.005000
	2	1	1	1	0.069867	0.032778
	2	1	1	2	0.033640	0.028196
	2	1	2	1	0.036800	0.042869
	2	1	2	2	0.017719	0.000000
	2	2	1	1	0.025190	0.111157
	2	2	1	2	0.012129	0.000000
	2	2	2	1	0.013268	0.000000
	2	2	2	2	0.006388	0.000000

log-likelihood(InL_{cases})= -371.26

Table 4: Frequencies of IL-1 haplotypes in non- Atherosclerosis group (controls) as determined by EH program

	<i>Allels at loci</i>				<i>Haplotype frequency</i>	
	<i>IL-1a+4845</i>	<i>IL-1B+3954</i>	<i>IL-1B-511</i>	<i>IL-1RA VNTR</i>	<i>Independent</i>	<i>w/Association</i>
Wild type	1	1	1	1	0.197068	0.263168
	1	1	1	2	0.076638	0.067362
Pattern2	1	1	2	1	0.108464	0.043839
	1	1	2	2	0.042180	0.104376
	1	2	1	1	0.088538	0.037840
	1	2	1	2	0.034431	0.032920
	1	2	2	1	0.048730	0.065493
	1	2	2	2	0.018951	0.000001
	2	1	1	1	0.123368	0.041403
	2	1	1	2	0.047976	0.028562
Pattern1	2	1	2	1	0.067900	0.117463
	2	1	2	2	0.026406	0.023827
	2	2	1	1	0.055426	0.150794
	2	2	1	2	0.021555	0.022951
	2	2	2	1	0.030506	0.000000
	2	2	2	2	0.011863	0.000000

log-likelihood (InLcontrols)= -331.20

Table 5: Frequencies of IL-1 haplotypes in (cases and controls) as determined by EH program

	<i>Allels at loci</i>				<i>Haplotype frequency</i>	
	<i>IL-1a+4845</i>	<i>IL-1B+3954</i>	<i>IL-1B-511</i>	<i>IL-1RA VNTR</i>	<i>Independent</i>	<i>w/Association</i>
Wild Type	1	1	1	1	0.226121	0.300986
	1	1	1	2	0.098067	0.052186
Pattern2	1	1	2	1	0.121757	0.047279
	1	1	2	2	0.052805	0.157881
	1	2	1	1	0.091242	0.047249
	1	2	1	2	0.039571	0.039323
	1	2	2	1	0.049130	0.048839
	1	2	2	2	0.021307	0.006257
	2	1	1	1	0.096909	0.035449
	2	1	1	2	0.042029	0.031588
Pattern1	2	1	2	1	0.052182	0.087128
	2	1	2	2	0.022631	0.000003
	2	2	1	1	0.039104	0.127957
	2	2	1	2	0.016959	0.015262
	2	2	2	1	0.021056	0.002612
	2	2	2	2	0.009132	0.000000

log-likelihood (InLcase-controls)= -717.10

Table 6: Percentage frequencies of pattern1 and pattern2 of IL-1 haplotypes in atherosclerosis and non- atherosclerosis groups

		<i>Atherosclerosis group</i>		<i>Non- athero-sclerosis group</i>		<i>Sample</i>		<i>P value</i>
		<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	
Haplotype	Pattern1	11	11%	15	15%	13	13%	0.0148*
	Pattern2	20	20%	10	10%	16	16%	

relatively large in comparison to other studies (Stein et al. 2009; Prakash and Victor 2010; Drozdziak et al. 2006; Kaarthikeyan et al. 2009),

where the relatively small sample sizes limit their robust conclusions. Second, stringent diagnostic criteria for cases were used in which only

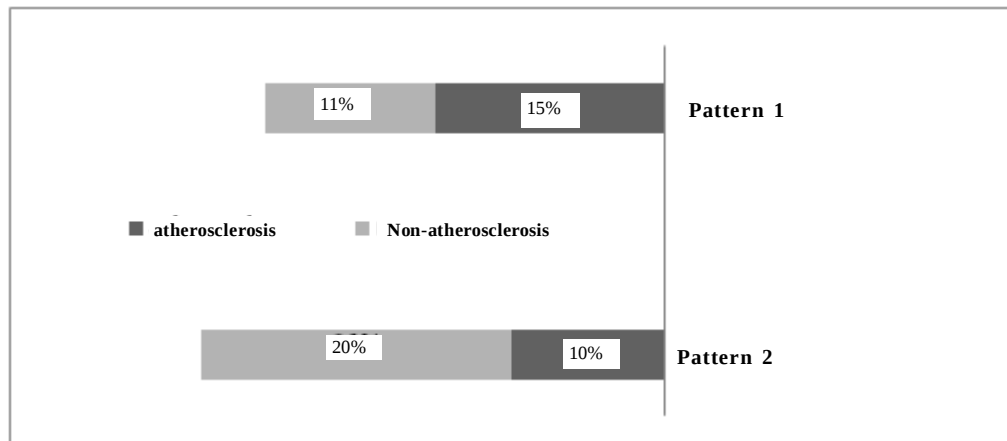


Fig. 2. Observed frequencies of IL-1 haplotypes in atherosclerosis and non-atherosclerosis groups

subjects with coronary artery bypass graft were included. In fact, this case-enrichment approach is recommended for minimizing phenotypic heterogeneity and improving statistical power (Schafer et al. 2011). Third, controls were matched for age and gender to avoid the need for statistically adjusting their effects. This is actually an advantage over a number of previous studies in which controls were much younger than cases (Anusakathien et al. 2003; Sakellari et al. 2003). Fourth, subjects with diabetes mellitus, blood hypertension, hyperlipidemia, and BMI > 30 kg/m² were excluded, eliminating the possibility of interactions and the need for data stratification. Finally, the modifiable effect from environmental agents, including smoking, could differ between phenotypes, but in this study there was no relevant difference between cases and controls for smoking ($P > 0.05$). However, because patients with atherosclerosis were examined before controls and most of them were males of 92%, this resulted in a small number of females of about 8% in the study population.

When data collection started and cases with atherosclerosis were recruited, it was recognized later on that those patients had had different degrees of periodontitis. This finding is in line with the results of (Czerniuk et al. 2004). Accordingly, the sample was divided into two groups; the comparison was made between those with moderate to severe alveolar bone loss with those with mild degrees of alveolar bone loss in a similar way to what was done in Czerniuk et al.'s study. In elderly patients greater than 50 years

of age, it is difficult to find subjects with no periodontal pathology since this kind of periodontal inflammation increases with age (REF). In addition, there is a general trend of poor oral hygiene among the recruited patients at our department (REF). Furthermore, cardiovascular disease prevalence is higher among elderly patients exceeding fifty years of age (REF). Smoking is very common among those referred patients at the Department of Periodontology (REF). It is well established that smoking has been considered as a risk factor for both periodontal and cardiovascular diseases which increases their occurrence (REFs). The control group was matched to the 'cases' group according to age and sex. The proportion of primary periodontitis in the control group was higher than that of the 'cases' group. Therefore, the control group was considered suitable for the comparisons made. Also, it was deemed too difficult to construct a third group of healthy volunteers with no periodontal involvement and cardiovascular problems with an average age of fifty years because of the previously mentioned reasons; therefore the study was confined to two groups based on the presence or absence of atherosclerosis.

The association between the IL-1 gene polymorphisms, both CHD and periodontitis was first reported in white Caucasians by (Kornmann and Duff 2001). Since then, many attempts have been made to reproduce the results in the same way as those of other races/ethnic populations (Karimbux et al. 2013). To the best of our knowledge, the current study is the first to involve a

Syrian population who are usually classified under the Caucasian race (Risch et al. 2002).

Recently, haplotype analysis has become a powerful tool in gene-disease studies as it is easier to determine the association between a particular region of the genome with the disease rather than using a single marker where by some single-nucleotide polymorphisms, from which haplotypes are constructed, may be closely linked. (Geismar et al. 2008) suggested that future research should focus on the haplotypes when studying genetic interaction in the association between periodontitis and coronary heart disease. Therefore, in the current study, four SNPs at positions $\alpha+4845$, $\beta+3954$, $\beta-511$ and IL-1RN VNTR, which form IL-1 haplotypes, have been analyzed and sixteen haplotypes were observed.

A previous study had defined two haplotype patterns: pattern1 includes allele2 at both the IL-1 $\alpha+4845$ and at the IL-1 $\beta+3954$ loci and allele1 at both loci of IL-1 $\beta-511$, and IL-1RN VNTR. Pattern2 includes allele2 at both the IL-1 $\beta-511$, and at the IL-1RN VNTR and allele1 at both loci of IL-1 $\alpha+4845$ and IL-1 $\alpha+3954$ (Cox et al. 1998). According to this definition, the frequency of pattern1 was (13%), and the frequency of pattern2 was (16%) of the population investigated in the current study. While a previous study on Caucasians found that pattern1 was estimated at 17.4% of the chromosomes, and pattern2 was estimated at 13% (Kornmann et al. 1999). Another study found that the frequency of pattern1 was 18% of the subjects (Geismar et al. 2008).

The two genetic patterns are associated with specific differences in the functional biology of IL-1. For example, the SNP at IL-1 $\alpha-889$ has been associated with different levels of IL-1 α in human gingival tissue fluid and the SNP at IL-1 $\alpha+4845$ codes for an altered amino acid sequence in the IL-1 α protein (Shirodaria et al. 2000). In addition, Allele 2 of IL-1 $\beta+3954$ is associated with a 4-fold increase in IL-1 β production (Pociot et al. 1992). Conversely, IL1 α and IL-1 β production upregulates prostaglandin E2 and matrix metalloproteinase, and together with these components, promote the loss of connective tissue and bone in periodontitis lesions (Dinarello 1998). Moreover, allele 2 of IL-1 RA VNTR is more likely to be observed in association with an acute endothelial injury as in thrombosis (Witkin et al. 2002).

In the current study, pattern1 was significantly more frequent in chronic periodontitis patients without atherosclerosis (15% vs. 11%). This is in agreement with previous studies indicating that pattern1 is associated with periodontitis (Kornmann et al. 1999). For example, pattern1 of IL-1 haplotype is associated with severe chronic periodontitis in Yemenis (Al-hebshi et al. 2012). IL1 α and IL1 β genetic variations are significant contributors to chronic periodontitis in Caucasians (Kornmann et al. 1997).

Pattern2 was significantly more frequent in the atherosclerosis group (20% vs. 10%) in the current study, which is in agreement with previous studies indicating that pattern2 is associated with atherosclerotic plaque formation (Kornmann et al. 1999). For example, the individuals who carried two copies of IL-1 $\alpha+4845$ allele 2 were four times more likely to have a CHD event during an 11-year monitoring period than were individuals with the same level of cholesterol who do not carry out this IL-1 genotype (Kornmann 2006). Also, the -511C/T IL-1 beta gene polymorphism affects the risk of MI and ischemic stroke at a young age as well as the response of mononuclear cells to inflammatory stimulation (Iacoviello et al. 2005).

In this study, significant differences between periodontitis patients with and those without atherosclerosis in the frequencies of IL-1 haplotypes at positions ($\alpha+4845$, $\beta+3954$, $\beta-511$ and IL-1RN VNTR) were found ($P=0.0148$). These findings are in agreement with previous studies indicating that allele1 of IL-1RN VNTR may be associated with the coexistence of coronary heart disease and periodontitis in a multiple regression mode (Geismar et al. 2008) and patients with acute coronary syndrome (ACS) or angina were more likely to experience pattern1 of IL-1 haplotypes and severe periodontitis (Goteiner et al. 2008). Nevertheless, the findings of the current study were in contrast with another study which failed to detect the modifying impact of the pattern1 of IL-1 genotype even though an association between periodontitis and acute myocardial infarction (AMI) was confirmed (Stein et al. 2009).

CONCLUSION

Significant differences between periodontitis patients with and those without atherosclerosis in the frequencies of IL-1 haplotypes at

positions (α +4845, β +3954, β -511 and IL-1RN VNTR) were found, which may suggest that Pattern2 of IL-1 haplotype is associated with increased susceptibility to atherosclerosis.

RECOMMENDATIONS

Further studies on the role of interleukin-1 haplotype in atherosclerosis and periodontitis are required.

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