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Original Article

Interleukin-1β rs1143634 Polymorphism and Susceptibility to Periodontitis in the Iraqi Population

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Abstract

Periodontitis is a complex multifactorial inflammatory disease, and its genetic basis has been studied. The Interleukin-1 beta (IL-1β) is a crucial proinflammatory mediator in the pathogenesis of periodontitis with high polymorphism. This study aimed to investigate whether the rs1143634 genetic variant of the IL-1β gene is associated with an increased risk for periodontitis. For this purpose, genotyping of the IL-1β rs1143634 polymorphism was performed using the polymerase chain reaction-restriction fragment length polymorphism method on 90 patients within the age range of 35-60 years old. They were divided into two groups: 64 periodontitis cases (stage 3 and 4 periodontitis according to 2017 classification) and 26 racially matched healthy cases as the control group. Fisher's exact test showed a significant decrease in TT homozygous genotype in periodontitis cases, compared to the control group (P=0.018), suggesting that this genotype is a protective factor in the test population. Allele frequency showed an elevated odd ratio (1.24) and increased risk for periodontitis in subjects with allele C and reduced odd ratio (0.81) and reduced risk for periodontitis in subjects with allele T. Allele T of IL-1β rs1143634 polymorphism could be a protective factor, while Allele C of this polymorphism could be a risk factor for periodontitis in the studied Iraqi population.

Keywords: Association, Interleukin-1β rs1143634 polymorphism, Periodontitis

1. Introduction

Periodontitis is a multifactorial inflammatory disease in response to dental plaque microorganisms. The release of inflammatory mediators within the periodontal tissues stimulates osteoclasts and proteolytic enzymes resulting in the permanent destruction of alveolar bone and periodontal ligament fibers (1). Periodontitis is characterized by a multifactorial etiology, and the rate of disease progression is influenced by many environmental and genetic risk factors (2). The nature of one's inflammatory response determines the disease susceptibility and severity. Individuals susceptible to disease produce excessive inflammatory responses for the same bacterial challenge, compared to those who are non-susceptible or hypo-responsive (3).

Genetic factors influence disease susceptibility by influencing the nature of the individual immune-inflammatory response (4). Polymorphisms of genes involved in the immune response have been related to the susceptibility and severity of periodontitis (5). The pro-inflammatory cytokine Interleukin-1 beta (IL-1β) is significantly involved in the pathogenesis of periodontitis via inducing inflammation, bone resorption, and periodontal tissue destruction (6), as shown in figure 1. Genetic polymorphisms in the IL-1β gene might increase the risk of periodontitis (7).

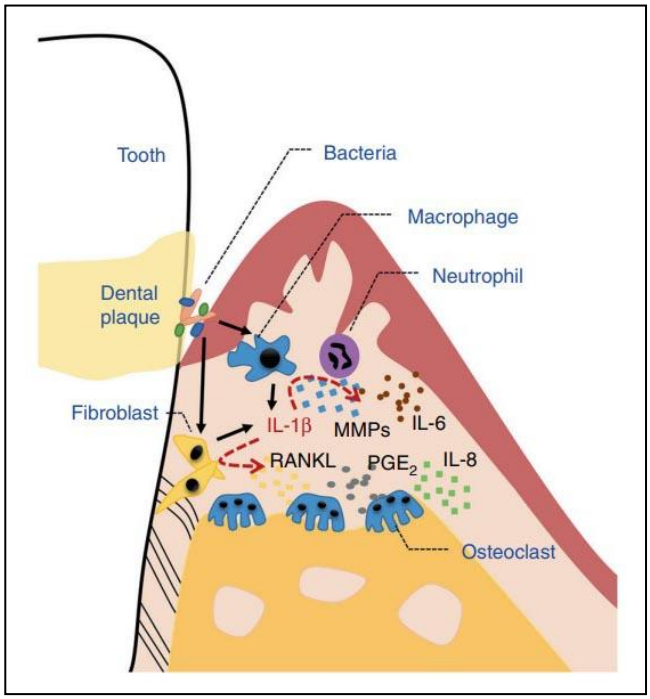

Figure 1. Role of IL-1β in periodontitis. IL-1β promotes the secretion of MMPs, RANKL, PGE2, IL-6, IL-8, etc., which promotes osteoclast genesis

The polymorphisms of the IL-1β gene that have been studied for their association with periodontitis risk include 3954/3 C/T (rs1143634), −511C/T(rs16944), and −31T/C (rs1143627) (8-12). The most frequently reported polymorphism associated with increased risk for periodontitis progression is IL-1β rs1143634 (13, 14). This polymorphism has also been associated with elevated levels of IL-1β in the gingival crevicular fluid (15). However, other studies have not supported such an association (16, 17). Since genetic polymorphisms and their association with disease risk differs among populations, the present study aimed to genotype IL-1β rs1143634 polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of periodontitis in Iraq.

2. Materials and Methods

2.1. Sampling

This case-control study was performed on 90 subjects who were recruited from the Periodontology Department in the College of Dentistry, Baghdad University, Baghdad, Iraq. The study group included 64 periodontitis patients (stage 2 and 3 periodontitis, based on the classification) (1), and the control group included 26 healthy individuals. It should be mentioned that case and control groups had similar racial backgrounds.

2.2. DNA Isolation

Blood samples were used for DNA isolation; accordingly, 3 ml of venous blood was obtained from each participant in ethylenediamine tetraacetic acid-containing tubes. A gSYNC™ DNA extraction kit (Geneaid, Taiwan) was used for DNA isolation according to the instructions of the manufacturer. After being checked for purity and concentration, DNA samples were stored at -80 °C.

2.3. Polymerase Chain Reaction

The PCR was performed using a mixture that was supplied in 0.2 ml tubes. The DNA sample and forward primer 5'-CTCAGGTGTCCTCGAAGAAATCAAA-3' and reverse primer 5'-GCTTTTTTGCTGTGAGTCCCG-3' were added to the contents of the PCR PreMix tubes according to the instructions of the manufacturer. Afterward, the tubes were transferred to a thermal cycler to complete the PCR. The thermal cycling program is summarized in table 1. The anticipated PCR product (194bp) was visualized using agarose gel electrophoresis.

Table 1. Thermal cycling program for PCR of Interleukin-1β rs1143634 gene

Program	Temperature	Time	Cycle
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	30 sec	35 cycles
Annealing	54°C	35 sec	
Extension	72°C	30 sec	
Final elongation	72°C	5 min	1

2.4. Restriction Fragment Length Polymorphism (RFLP)

The TaqI restriction enzyme (5 U) was used at 65 °C to digest the PCR product to differentiate among genotypes of rs1143634. The resulting products were separated by agarose gel electrophoresis using a concentration of 3% gel stained with ethidium bromide and observed under UV light. In the case of allele 1

(C), the PCR product was digested into three fragments (97+85+12 bp), while in the case of allele 2 (T), the PCR product was digested into two fragments (182 +12bp). Uncut fragments were heterozygous genotypes (CT) (Figure 2).

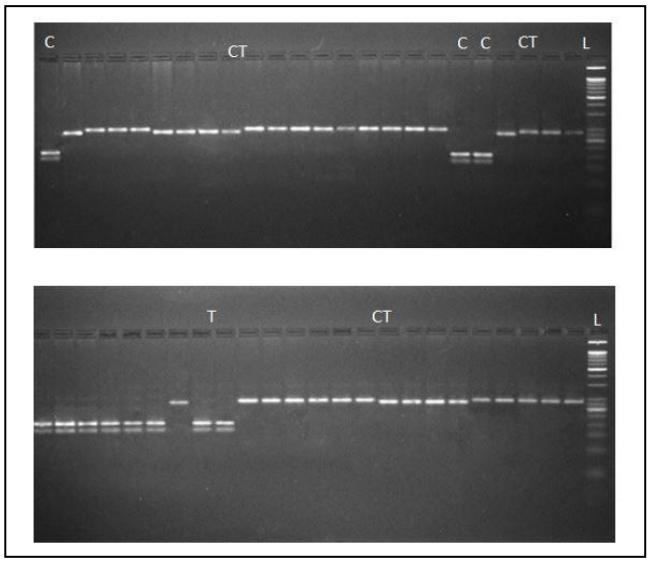

Figure 2. RFLP forInterleukin-1β rs1143634 polymorphism, L (DNA ladder), C (3 fragments:97+85+12 bp), T (2 fragments:182 +12bp), CT (uncut fragments were heterozygous genotypes)

2.5. Statistical Analysis

The SPSS software (version 20) was used to perform statistical analysis. The frequency of genotype distribution between groups was compared using Fisher's exact test. The strength of the relationship between genotypes and case group was assessed using the Odds ratio. It should be mentioned that a *P* value of ≤ 0.05 was considered statistically significant.

3. Results

There were 26 subjects in the control group (13 males and 13 females) and 64 subjects in the periodontitis group (36 males and 28 females). The age range was 25-50 years old in controls and 25-60 years old in cases. Mean plaque index values for the control and periodontitis groups were 0.52±0.23 and 2.03±0.17, respectively. Moreover, the mean gingival index values for the control and periodontitis groups were 0.43±0.16 and 2.35±0.21, respectively. The mean CAL of the periodontitis cases was 5.33±0.93 mm (Table 2).

Table 2. Characteristics of control and the periodontitis group (PLI: plaque index, GI: gingival index, CAL: clinical attachment loss)

		Control group	periodontitis group
Number		26	64
Age Range		25-50 years	25-60 years
Gender	Male no (%)	13 (50%)	36 (56.25%)
	Female no (%)	13 (50%)	28 (43.75%)
Mean PLI (±SD)		0.52 (±0.23)	2.03 (±0.17)
Mean GI (±SD)		0.43 (±0.16)	2.35 (±0.21)
Mean CAL (±SD)		0	5.33mm (±0.93)

The distribution of the rs1143634 genotypes in the study subjects is summarized in table 3. Accordingly, CT heterozygous was the most frequent genotype (44.5%), followed by TT homozygous and CC homozygous. Besides, allele frequency was 63.4% for Allele T and 36.6% for Allele C.

Table 3. The distribution of the rs1143634 genotypes and allele frequency in the study subjects

Genotypes	Frequency in the study population	Total
CC homozygous	13 (14.4%)	n=90
TT homozygous	37 (41.1%)	
CT heterozygous	40 (44.5%)	
Alleles		
C (Allele 1)	66 (36.6%)	2n=180
T (Allele 2)	114 (63.4%)	

Table 4 tabulates the distribution of the rs1143634 genotypes and alleles in the periodontitis cases and controls. The results showed a significant decrease in TT homozygous genotype in periodontitis cases, compared to controls, while the distribution of CT and TT genotypes showed a non-significant increase in cases, compared to controls ($P \leq 0.05$). Odd ratios (ORs) of CC and CT genotypes were high (OR=2.49 and OR=2.25, respectively), indicating that these genotypes are associated with a higher risk for periodontitis. However, the OR of the TT genotype was less than 1

(OR=0.31), indicating that this genotype is associated with a reduced risk for periodontitis. Allele frequency also showed a high OR with an increased risk of periodontitis in the case of the C allele and reduced OR and risk for periodontitis in the case of allele T.

Table 5 summarizes the distribution of the rs1143634 genotypes and alleles between males and females. The results showed a non-significant difference in genotype and allele distribution between males and females in both cases and controls. However, the comparison of the elevated odd ratio of CC and CT genotypes of male and female periodontitis cases indicated that these genotypes could increase the risk for periodontitis in males. The TT genotype was associated with an odds ratio of less than 1, indicating that this genotype was associated with a reduced risk for periodontitis in males.

Table 4. Distribution of rs1143634 genotypes and alleles between periodontitis cases and controls

rs1143634		**Controls**	**periodontitis cases**	**OR**	**Fishers' exact probability**
Genotypes		n=26	n=64		
CC	No (%)	2 (7.7%)	11 (17.2%)	2.49	0.333 NS
TT	No (%)	16 (61.5%)	21 (32.8%)	0.31	0.018
CT	No (%)	8 (30.8%)	32 (50.0%)	2.25	0.108 NS
Allele frequency		**2n=52**	**2n=128**		
C (Allele 1)	No (%)	12 (23%)	43 (34%)	1.24	0.606 NS
T (Allele 2)	No (%)	40 (77%)	85 (66%)	0.81	0.606 NS

Table 5. Distribution of the rs1143634 genotypes and alleles between males and females

rs1143634			**Controls**	**periodontitis cases**	**OR**	**p-value**
Genotypes			**n=26**	**n=64**		
CC	No (%)	M	1 (3.9)	10 (15.6)	18.19	0.295 NS
		F	1 (3.9)	1 (1.6)		
TT	No (%)	M	8 (30.8)	7 (10.9)	0.50	0.336 NS
		F	8 (30.8)	14 (21.9)		
CT	No (%)	M	4 (15.3)	19 (29.7)	1.46	0.702
		F	4 (15.3)	13 (20.3)		
Allele frequency			**2n=52**	**2n=128**		
C (Allele 1)	No (%)		12 (23%)	43 (34%)	1.24	0.606 NS
T (Allele 2)	No (%)		40 (77%)	85 (66%)	0.81	0.606 NS

4. Discussion

Periodontitis is a multifactorial inflammatory disease caused by bacteria in plaque deposits under the gums. The bacteria cause inflammation of the gums and can turn into periodontitis in untreated patients. Many factors and molecules are involved in this complex disease (5). Risk factors determine one's susceptibility to the development of periodontitis. Among these risk factors are genetic factors, which determine how each individual responds to bacterial challenges.

Genetic polymorphisms of many inflammatory mediators, cytokines, enzymes, receptors, and other molecules involved in the pathogenesis of periodontitis have been studied in different populations for their association with disease susceptibility. The IL-1β stimulated inflammatory reactions and activated osteoclastogenesis and collagenoly (16, 18). This polymorphism was also associated with susceptibility to other diseases and cancers (19). Genetic polymorphisms and their association with disease susceptibility vary among different populations.

The present study investigated the association of this polymorphism with periodontitis susceptibility in a sample of the Iraqi population. Based on the results, the CT heterozygous was the most frequent genotype in the

studied Iraqi population, followed by TT homozygous genotype, while CC homozygous genotype was the least frequent. Hence, Allele T of IL-1β rs1143634 polymorphism was the most frequent allele in this population.

According to the results, allele T could be protective and associated with less periodontitis risk in the Iraqi population, which is consistent with the results of the study performed by Sharma, Joseph (20). However, it is inconsistent with the findings of previous studies on Indian and Brazilian populations (21, 22).

In the present study, the frequency of the TT genotype was significantly decreased in periodontitis cases, compared to controls. Allele T of IL-1β rs1143634 polymorphism seemed to be a protective factor, while Allele C of this polymorphism could be a risk factor for periodontitis in the studied Iraqi population.

Authors' Contribution

Study concept and design: S. A. D.
Acquisition of data: L. K. H.
Analysis and interpretation of data: S. A. D.
Drafting of the manuscript: S. A. D.
Critical revision of the manuscript for important intellectual content: L. K. H.
Statistical analysis: L. K. H.
Administrative, technical, and material support: S. A. D.

Ethics

Permeation of participants was obtained using informed consent, and their dental and medical histories and background information were collected. Furthermore, a clinical examination was performed, and the following periodontal parameters were recorded: plaque index, gingival index, bleeding on probing pocket depth, and clinical attachment level. Ethical approval was obtained from the Ethics Committee of the College of Dentistry, University of Baghdad.

Conflict of Interest

The authors declare that they have no conflict of interest.

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