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Association between *interleukin-10 (rs1800871) gene* polymorphism and patients with chronic periodontitis

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Abstract

Background: Interleukin-10 (IL-10) is a non-inflammatory cytokine, and has a positive association with periodontitis. IL-10 (rs1800871) polymorphisms in the IL-10 site have been linked with variable expression. **Aim:** Goal of this search was to study a link amidst the polymorphisms of these genes and deep-seated of gum disease in Iraqi patients. **Methods:** 120 blood specimen were collected, 60 of which were from patients with chronic periodontitis and 60 from healthy controls for comparison. Polymerase chain reaction-restriction fragment length polymorphism (PCR) for purpose of analyze IL-10 (rs1800871). Chi-square test was used to statistically analyze the data. Problems related to individual alleles, genotypes, and phenotypes were identified by various logistic regression test to determine odds ratio (OR) and 95% confidence interval (CI). $P < 0.05$ is significant. **Results**: Prevalence of TC, CC genotypes of *IL10-819* were importantly various amidst CP and healthy in simile with TT genotype (OR = 9.72; CI = 3.36–24.4; P = 0.001 for C vs. TT, OR = 5.41; CI = 1.8–8.14; $P < 0.001$ for TC vs. TT). In addendum, themes with at minimum one *IL10-819-C* allele were importantly had high danger for CP (OR = 6.2; CI = 3.52–10.8; $P < 0.001$). **Conclusion:** Our results concluded the IL-10 gene polymorphism (-819T/C) is potential to be a danger agent for chronic periodontitis in Iraqi patients.

Keyword: Advanced gingivitis**,** IL-10, cytokines, PCR, polymorphisms

Introduction

Chronic periodontitis (CP) is a widespread inflammatory disease of the oral cavity that involves persistent inflammation of the periodontal tissue and this is occurs as a result of bacteria. This microbial problem begins with bacterial plaque, and then via inflammation and demolition of tissues protecting the teeth, eventually driving to absence of molars. It turns out extra than 15% of adults have developed sharp

shapes of persistent gingivitis [1]. Symptoms of this problem include bleeding, swelling, redness, persistent inflammation, and tooth loss caused via collapse of the extracellular matrix in gum tissue [2].

Plaque buildup results in damage to the gum tissue surrounding the teeth, and microorganisms multiply and settle, forming biofilms throughout the periodontal pocket. These conditions stimulate the host's immune response [3]. Despite the belief that microbes are the primary player in the development of cerebral palsy, there are many risk factors that also contribute. These include (plastic) environmental factors such as low vitamin D and calcium levels, chronic diseases such as diabetes, and behaviors such as chewing tobacco, smoking, and obesity [4].

Several studies have been conducted in this regard, demonstrating that the inflammation inherent in bacterial plaques activates innate immune responses in the host. This affects cytokines, which are components of the immune system. In addendum, genetic operator, like polymorphisms, can increase cytokine[5]. A single nucleotide polymorphism (SNP) is a variation in DNA sequence patterns that results from a single nucleotide mutation at a specific location in the genomic sequence [6]. Inflammatory cytokines like IL-10 are involve a body's response to persistent inflammatory problems , including CP and persistent HBV[7]. Stereological and morphological test also showed a significant link between the diversity of inflammatory gene expression patterns and the level of gum tissue deterioration in CP[8]. Interleukin-10 is a multifunctional cytokine that regulates immunity, primarily produced by macrophages. Dendritic cells, cytotoxic T cells, B lymphocytes, monocytes, and mast cells also contribute [9]. IL-10 gene, which extent approximately 4.7 kilo bases and consists of 5 exons and 4 introns, is located on chromosome 1 in the 1q31-32 interval. IL-10 gene has a high degree of genetic variation. The three most widely studied SNPs are (G/A, C/T and C/A)[10]. Past surveys indicated that IL10 1082, 819, and 592 polymorphisms were regarding to the CP in Swedish, and Brazilian patients [11][12]. In current study, we investigated the potential associations of *IL-10 (-819T/C)* genes including single-nucleotide polymorphisms (SNP) with CP in Iraqi patients.

Methods

Participants: The study was conducted at dental clinics, Dentistry college, Al-Qadisiyah University, Diwaniyah, Iraq. Two milliliters of blood were collected from 60 patients with CP and another two milliliters from healthy individuals, from which DNA was extracted. These individuals were non-smokers. Those with persistent cardiovascular problems, systemic disorders, immunodeficiency, inflammatory medication, chemotherapy, orthodontic treatment, and pregnant women -ongoing anti .were excluded While control or healthy, with no history of gum problems. The indicators were (GI), (PPD), and (CAL). For healthy subjects, GII < 1, PPD < 3 mm, CAL = 0. For inflammation, GII >1, PPD >4 mm, CAL >2 mm, and ivory completely destroyed indicators of persistent periodontitis.

Genotyping of interleukin-10 polymorphisms:

Genomic DNA was isolated from venous blood using salting-out method. The IL10 gene polymorphisms were found via restriction fragment length polymorphism (RFLP) PCR with primers that amplify small section of DNA that has polymorphisms. (Table 1) includes the primers used to determine the genotype of IL-10 polymorphisms. IL10-819T/C was genotyped in 20 µl volumes

contains 1 µl of each primer, 100 ng of template DNA, 10 µl of 2X Prime Taq Premix, and 7 µl of ddH2O. PCR were performed for 30 cycles: initial denaturation; denaturation for 5 min at 95°C; annealing for 30 s at 95°C; extension for 30 s at 55°C; final extension for 30 s at 72°C; 5 min at

72°C.IL10-819T/C (10 µl) was digested with MnlI restriction enzyme at 37°C for 16 h, then undergo to electrophoresis in 4% agarose gel (Invitrogen, USA), and stained with EtBr. The IL10-1082 product shows a homozygous pattern to the wild-type (TT), is undigested by the restriction enzyme and remains at 559bp bands. Homozygous lin (CC) mutant shows restriction enzyme digestion to bands of 443 and 116 bp. Heterozygous lin (T/C) mutant, resulting in restriction enzyme digestion to bands of 559, 443, and 116 bp (Fig. 1).

Figure (1): Agarose gel electrophoresis showing RFLP-PCR analysis of the IL-10 gene 819T/C polymorphism (rs1800871) with RSaI restriction enzyme in a 1.5% agarose gel. Where M: marker (2000-100bp). The homozygous wild-type (TT) ligand, the resulting non-restriction enzyme-digested ligand, is a 559bp restriction band. Lin (T/C) heterozygous, the product is digested with restriction enzyme to bands of 559bp, 443bp, and 116bp.

Primer design

The gene polymorphism RFLP-PCR Primers were designed. These primers were provided from (Scientific Reseracher. Co. Ltd. Iraq) as following (Table 1)

Table (1): Primer Sequence with their product size and references.

Primers	**Sequence 5'--3'**	**Amplicon**
IL-10 819T/C (rs1800871)	**CCAGATATCTGAAGAAGTCCTG**	**559bp**
	TGGGGGAAGTGGGTAAGAGT	

Statistical analysis

Data were analyzed using SPSS 20 software package (SPSS Corporation, Chicago, Illinois, USA). Pathological indicators were statistically analyzed using two-specimen t-test. Variation in genotype

frequencies and allele load amidst the two groups were estimated using chi-square test. P were two-sided, and a P value of <0.05 was deem arithmetically significant. Danger linked with single alleles or

genotypes was measured using multivariate logistic regression test to measurement (OR) and 95% (CI). Medical parameters measured as mean ± standard deviation.

Results

Our study included 120 samples, 60 of which were patients and 60 of which were apparently healthy individuals. The demographic information for the two groups is shown in (Table 2). The study groups had a mean age of 29.31 ± 6.21 and 27.22 ± 5.6 years for patients and controls and no significant difference between them (P = 0.412). There were 35 (58.3%) males and 25 (41.7%) females in the patient group, and 30 (50.0%) males and 30 (50.0%) females in the control group. There was no significant difference in gender amidst the two groups (P = 0.360).

Using RFLP-PCR, the diversity of the IL-10 (rs1800871) polymorphism was calculated. This locus contains three genotypes: the homozygous mutant (CC) which is digested by restriction enzyme to 443bp and 116bp, the wild-type (TT) which is undigested at 559bp and the heterozygous (G/C), which produces a range of 559bp, 443bp and 116bp (Fig. 1). The genotype distribution did not swerve from Hardy-Weinberg equilibrium. CT more prevalent in CP compared to none, 13 versus 8, respectively, where variation was statistically important (P = 0.002). Therefore, CT was found to be a danger parameter for obesity together odds ratio of 5.41 (95% confidence interval 1.8–18.14) and a causal fraction of 0.42. CC was more prevalent in patients than in healthy controls, 35 versus 12, respectively, if the difference was significant (P < 0.001). CC was a risk factor for obesity with an odds ratio of 9.72 (95% confidence interval 3.86–24.4) and a causality fraction of 0.668. The C allele was highly replicated in patients compared to healthy controls, 83 versus 32, respectively, where the contrast was highly significant (P < 0.001). From this, the C allele was considered a risk parameter for obesity with an odds ratio of 6.20 (95% confidence interval 3.52 to 10.8) and a causality fraction of 0.62.

Table (2): Demographic characteristics of CP patients and control subjects

Characteristic	**Patients (n=60)**	**Healthy Control (n=60)**	***P***
Age (years)	29.31 ± 6.21	27.22± 5.6	0.412
Gender			
Male	35 (58.3 %)	30 (50 %)	0.360
Female	25 (41.7 %)	30 (50 %)	

Table (3): Distribution of *IL-10 819T/C (rs1800871)* Genotype and Alleles Frequency

Genotype	Patients (n=60)	Controls (n=60)	OR	95% IC	p	PF	EF
IL-10 819T/C (rs1800871)							
CC	35	12	9.72	3.36 -24.4	0.001	……	0.69

TC	13	8	5.41	1.8 – 18.14	0.002	…..	0.42
TT	12	40	Reference				
Overall P value		0.001					
Alleles Frequency							
C	83	32	6.20	3.52 -10.8	0.001		0.62
T	37	88	0.162	0.09- 0.284		0.62	…..

Discussion

According to our results, no of studied demographic characteristics had an important link with increase in chronic periodontal disease. The results of various studies on this disease have indicated multiple risk factors. For example, age-related changes in innate immunity and inflammatory conditions increase an individual's susceptibility to CP [13]. Males are more likely than females to develop CP [14]. The lack of significance of the association between variables and chronic periodontitis in our study is due to two reasons: First, the control group and the patients were somewhat similar in terms of demographic factors, and second, the study sample size was relatively small compared to previous studies in this regard.

IL-10 mRNA concentrations vary among patients with chronic periodontitis. This has been revealed in previous studies, which has led to variations in clinical outcomes in patients with CP [15]. Genetic diversity in (SNPs) in the IL-10 gene promoters is likely linked with a history of chronic periodontitis. The severity of chronic periodontitis may be closely related to SNP diversity, which may alter expression of the anti-inflammatory cytokine IL-10[7].The results of our study indicated a significant difference between the frequency of IL-10-819 genotypes in both study groups. The distribution of IL-10 genotypes in Iraqis and Swedish Caucasians was somewhat similar, despite the ethnic differences between them [11]. The IL-10 SNP and the IL-10-819C allele increase the risk of developing chronic periodontitis, which is confirmed by our results. The study results also showed that the IL-10-819C alleles are significantly lower in healthy individuals than in patients. CC is more prevalent in patients than in healthy individuals, and IL-10 SNPs at the activator site are important causative agents of the disease under study. These results consistence with Emampanahi *et al.*,[16] It was shown that the C (-819) allele was increased in patients, while another showed that the -819C allele was the most frequently observed [17].

IL-10 is also an inflammatory signal is remarkable in most infections, as shown by previous studies. This relationship is embodied in examples including HBV, SL erythematosus, Silk Rod disease, so IL-10 promoter-819 G ([18]. In the same consideration, Berglundh *et al.*,[11], They studied linked IL-10-819 SNP with CP and finished C was elevated in patients compared to controls. This led them to confirm the association of the IL-10-819 SNP with chronic periodontitis in Caucasians. Berglundh *et al.*, [11], Their study showed a higher CC in non-smoking patients with CP compared to the control group. In 1997,

Kornman *et al.,*[19] Their study showed the distribution of five alleles in patients with CP and studied the association between this disease and allelic groups.

Our search revealed that lung cancer (CC) genotype ratio (in both study groups) was OR = 9.72, CI = 3.36–24.4, which is consistent with the results reported above. From this, we conclude that the lung cancer genotype may be a genetic biomarker for predisposition to chronic periodontitis. Our results are consistent with those of Berglundh *et al.,*[11] They found an important high in IL-10 CC-819

levels in Chinese patients compared to healthy controls. Patients with at least one C allele were also more likely to develop CP. Moreover, Schaefer *et al.,*[20], Their study proposition that CC was increased in patients than in others. otherwise, Smith et al [21], Their study proposition that 819C is linked with high IL-10 expression, while 819T is linked with deficiency in the mentioned gene. In a second search, Donati *et al.,*[22], They demonstrated that when comparing the expression of IL-10 in volunteers with CC and those with TC and TT, those with CC had higher levels. They also confirmed that the existence of IL-10 in CP lesions was strongly related to the severity of deep-seated gingivitis. As mentioned above, our portents results were among IL-10 variants, particularly SNP 819, which was strongly link with chronic gingivitis.

Several searches have appeared that the diversity of IL-10 gene variants affects IL-10 secretion, which in turn affects the body's immune response to deep-seated diseases like hepatitis B virus infection. These variations result in personage variations in immune responses then differences in host immune function. Furthermore, elevated IL-10 levels have been reported in deep-seated inflammatory problems, suggesting that individuals carrying the 819 C allele are at greater risk of developing inflammation [23]. In our study, IL-10-819 showed that individuals with at least one IL-10-819C allele were at greater danger of CP compared to those with the IL-10-819T allele. Reuss *et al.,*[24], They reported that IL-10-819 CC generated more IL-10, which reduces the immune response to infection. The frequency of IL-10-819 TT was also significantly reduced, while IL-10-819 CC and TC were increased in patients compared to healthy controls.

In a meta-test which was done by Albuquerque *et al.,*[25] It has been suggested that there is a link between the SNP IL-10-819 and deep-seated periodontitis in Caucasians. In addendum, Scarel-Caminaga *et al.,*[26] They proposition SNP IL-10-819 was link with severity (OR = 3.04, 95%CI = 1.34–6.91). The discrepancies between different studies are likely due to genetic variations in cytokine genes in variant ethnic origins of variant populations. Environmental factors and variables, as well as technical errors such as cigarette smoking, may cause variations in results. On the other hand, the interaction of IL-10 with other cytokines, ethnic differences, and other associated genetic markers are the reason for the varying results.

Conclusion

Finally, our results demonstrate a relationship amidst IL-10 isoform diversity at activator site and CP in patients with CP. The diversity of the effects of this disease and the complexity of its mechanism require a lot of explanation. From the above, we conclude that the diversity of IL-10 isoforms leads to important risk factors for CP, which may help in studying the potential trends in the development of chronic periodontitis.

References

1. Wadia R. and Chapple I. Periodontal care in general practice: 20 important FAQs- Part two: BDJ Team. (2020); 7(1):26–32.
2. Liu J, Zhao J, Li C, Yu N, Zhang D. and Pan Y. Clinical and microbiologic effect of nonsurgical periodontal therapy on patients with chronic aggressive periodontitis: Quintessence Int. (2013); 44(8):575-583.
3. Moura da Silva G, Coultinho SB, Piscoya MD, Ximenes RA, Jamelli SR. Periodontitis as a risk factor for preeclampsia: J Periodontol. (2012); 83:1388-1893.
4. Albandar JM. Global risk factors and risk indicators for periodontal diseases: Periodontol. (2002); 29(1):177-206.
5. Cardoso EM, Reis C, Manzanares. and Céspedes MC. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases: Postgrad Med. (2018); 130 (1):98-104.
6. Jian and Ying L. A narrative review of single-nucleotide polymorphism detection methods and their application in studies of Staphylococcus aureus: J Bio-X Res. (2021); 4(1):1-9.
7. Moudi B, Heidari Z. and Mahmoudzadeh-Sagheb H. Impact of host gene polymorphisms on susceptibility to chronic hepatitis B virus infection: Infect Genet Evol. (2019); 44:94-105.
8. Heidari Z, Mahmoudzadeh-Sagheb H, Hashemi M, and Rigi-Ladiz MA. Stereological analysis of interdental gingiva in chronic periodontitis patients with tumor necrosis factor alpha (-308G/A) gene polymorphisms: Gene Cell Tissue. (2018); 1:e18315.
9. Trifunović J, Miller L, Debeljak Ž. and Horvat V. Pathologic patterns of interleukin 10 expression—a review: Biochem Med (Zagreb). (2015); 25(1):36-48.
10. Sun ZL, Feng Y, Zou ML, Zhao BH, Liu SY, Du Y, Yu S, et al. Emerging Role of IL-10 in Hypertrophic Scars: Front Med (Lausanne). (2020); 27;7:438.
11. Berglundh T, Donati M, Hahn Zoric M, Hanson LA, et al. Association of the 1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians: J Clin Periodontol. (2003); 30:249 54.
12. Scarel Caminaga RM, Trevilatto PC, Souza AP, Brito RB, et al. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis: J Clin Periodontol. (2004); 31:443-448.
13. Hajishengallis G. Aging and its impact on innate immunity and inflammation: implications for periodontitis: J Oral Biosci. (2014); 56:30–7.
14. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO. et al. Update of prevalence of periodontitis in adults in the United States: NHANES 2009-2012: J Periodontol. (2015); 86:611–22.
15. Garlet GP, Martins W, Fonseca BA, Ferreira BR, Silva JS. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease: J Clin Periodontol. (2016); 31:671-9.
16. Emampanahi M, Masoudi R., Saghaeian JM, Mansour SN, et al. Association between interleukin-10 gene polymorphisms and severe chronic periodontitis: Oral Dis. (2019); 25(6):1619-1626.

17. Hoffmann SC, Stanley EM, Darrin CE, Craighead N, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated blood lymphocytes: peripheral Transplantation. (2001); 72:1444-1450.
18. Stanilova SA, Miteva LD, Karakolev ZT and Stefanov CS. Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility: Intensive Care Med. (2006); 32:260-6.
19. Kornman KS, Crane A. and Wang HY. The interleukin-1 genotype as a severity factor in adult periodontal disease: J Clin Periodontol. (1997); 24:72-7.
20. Schaefer AS, Bochenek G, Manke T, Nothnagel M, et al. Validation of reported genetic risk factors for periodontitis in a large-scale replication study: J Clin Periodontol. (2013); 40:563-72.
21. Smith AJ and Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality: Cytokine Growth Factor Rev. (2009); 20:43-59.
22. Donati M, Liljenberg B, Padyukov L, and Berglundh T. Local expression of interleukin-10 and mCD14 in relation to the -1087 IL-10 and -159 CD14 gene polymorphisms in chronic periodontitis: J Periodontol. (2008); 79:517-24.
23. Turner DM, Williams DM, Sankaran D, Lazarus M, et al. An investigation of polymorphism in the interleukin-10 gene promoter: Eur J Immunogenet, (1997); 24:1-8.
24. Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, et al. Differential regulation of interleukin-10 production by genetic and environmental factors – A twin study: Genes Immun. (2012); 3:407-13.
25. Albuquerque CM, Cortinhas AJ, Morinha FJ, Leitão JC, et al. Association of the IL-10 polymorphisms and periodontitis: A meta-analysis. Mol Biol Rep. (2012); 39:9319-29.
26. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, et al. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis: J Clin Periodontol. (2024); 31:443-8.