Interleukin 10 Gene Promoter Polymorphisms in Patients with Chronic Periodontitis

Suhad Jumaa Abd-Alkareem (HD)¹, Ahmed Luaiby Atrooz (HD)², Noor Dhia Hasan(HD)³
¹,²,³ Ministry of Health, Al-Shaab Specialized Dental Health Center, Baghdad, Iraq

Abstract

Background: The anti-inflammatory cytokine, interleukin 10 (IL 10), has a crucial role in periodontal diseases. The gene coding for this cytokine is pleomorphic. In the promoter region of the IL 10 gene, two single nucleotide polymorphisms (SNPs), IL10 1082 and IL 819, have been discovered to affect IL 10 expressions.

Objective: To investigate the association of two single nucleotide polymorphisms in the interleukin 10 gene (IL-10-1082 G/A and IL-10-819 C/T) with CPO.

Patients and Methods: A total of 64 patients with chronic periodontitis (CPO) and another 40 apparently healthy individuals were enrolled in this case-control study. The IL-10 gene was amplified with two specific sets of primers. Genotyping was achieved by allele-specific polymerase chain reaction (AS-PCR).

Results: The frequency of GG, GA and AA genotypes of the SNP IL-10-1082G/A among patients was 48.44%, 42.19% and 9.37% respectively, compared with 32.5%, 35% and 32.5% respectively, among controls. Logistic regression test revealed a significant difference in the frequency of AG (OR=0.19, 95%CI=0.06-0.62, P=0.006) and GG genotype (OR=0.24, 95%CI=0.07-0.77, p=0.016) between patients and controls. Furthermore, the haplotype block GC (G allele of IL-10-1082G/A and C allele of IL-10-819 C/T) was more common in controls than patients (36.25% vs. 33.44%) with a significant difference (OR= 0.54, 95%CI=0.29-0.99, p= 0.048).

Conclusion: These data suggest that the IL-10-1082 G/A gene polymorphism could be a risk factor for CPO in Iraqi patients.

Keywords: Chronic periodontitis, Interleukin 10 gene polymorphism, Haplotype, Linkage disequilibrium

Introduction

One of the most common chronic inflammatory oral diseases is chronic periodontitis (CPO). It is characterized by deregulated inflammatory interactions, involving both innate and adaptive responses, that lead to chronic inflammation in periodontal tissues [1]. The periodontal epithelium is situated where the body's internal connective tissue meets its external environment, similar to other mucosal surfaces. From the outside, it is firmly established that the principal trigger of chronic periodontitis is the presence of...
dysbiotic microbial communities with potential for destructive inflammation [2].

Clinical symptoms of the condition include bleeding and swollen gums, gingival redness, chronic inflammation, and tooth loss brought on by the breakdown of the extracellular matrix in the periodontal tissues [3]. The build-up of dental plaque causes periodontal tissue damage close to the teeth to begin with. The aggregation of microbial flora and the production of biofilm around the periodontal pocket initiates the pathological process. These conditions augment the immune response of the host [4]. Although microbes are thought to be the primary contributors to the development of CPO, other risk factors also play a part. These include environmental (modifiable) factors like obesity, vitamin D and calcium deficiency, and chronic illnesses like diabetes mellitus, as well as behaviors like chewing tobacco, smoking, and obesity. A single-nucleotide polymorphism (SNP), or single-nucleotide variation, is one of the most common heritable variations. An SNP is a DNA sequence polymorphism caused by the alteration of a single nucleotide in a specific position at the genomic level [6]. Such variation can dramatically influence the quality and quantity of protein encoded by the affected gene. When polymorphisms in interleukin genes are found in the promoter region of the gene, they can alter cytokine production [7]. Chronic inflammatory disorders like CPO and chronic hepatitis B virus (HBV) infection have been linked to polymorphisms in anti-inflammatory cytokine genes, such as IL 10 [8]. Interleukin-10 is an immune-regulatory pleiotropic cytokine produced mostly by macrophages. However, dendritic cells, cytotoxic T cells, B lymphocytes, monocytes, and mast cells can also participate in its production [9]. The IL-10 gene, which spans about 4.7 kb and comprises five exons and four introns, is found on chromosome 1 at 1q31-32. The IL-10 gene has several genetic variations. The three single nucleotide polymorphisms (SNPs) 1082(G/A), -819(C/T), and -592 (C/A) that produce three major haplotypes (GCC, ACC, and ATA) are the most studied [10]. Previous studies indicated that IL10 1082, 819, and 592 polymorphisms were related to the CPO in Swedish, and Brazilian patients [11,12]. There aren't enough studies on these relationships among Iraqi patients, and ethnic variation may interfere with them. Therefore, this present study aimed to investigate the possible relationship between IL 10 polymorphisms (1082G/A, rs1800870, and 819T/C, rs1800871) and CPO.

Patients and Methods

This case-control study included a total of 64 patients with confirmed chronic periodontitis. From December 2018 to May 2019, these patients were enrolled at Al-Imamain Al-Kadhhumain Medical City, the Department of Dentistry, and the Teaching Hospital / Al-Israa University College.

Clinical characteristics such as probing depth (PD> 3 mm), clinical attachment loss (CAL> 3 mm), plaque index (PI>1), gingival index (GI>1), tooth loss, bleeding on probing (BOP> 30%), color and texture of gums were used to diagnose CPO (red and swollen) were used for clinical diagnosis for patients enrolled in the present study. These clinical evaluations were performed on six sites per
tooth and on all of the patient's remaining teeth.

The control group consisted of 40 people whose ages and genders matched those of the patients. Those subjects had less than 3 mm of PD and CAL had no history of tooth loss in the previous five years. The PI was less than 1 and the GI ranged from 0 to 1. Furthermore, local bleeding on probing was less than 30%. The gum had a solid texture and was pink in color.

Patients or controls with systemic diseases like arthrosclerosis, hypertension, diabetes, and osteoporosis were excluded from the study. After describing the study's purpose, each participant signed a consent form prior to sample collection. Each patient was given the unrestricted right to withdraw at any moment. Throughout the trial, data confidentiality was ensured, and patients were told that their information would only be used for research purposes. Through a direct interview with each participant, demographic and clinical information such as age, gender, smoking status, and family history of periodontal disease were gathered.

**DNA Extraction and Gene Amplification**

In an EDTA tube, 3.0 ml of venous blood was obtained from each individual. A ready kit (Geneaid/Korea) was used to extract the nuclear DNA from leukocytes. The company's instructions were followed precisely. The target SNPs were amplified and genotyped using the allele-specific polymerase chain reaction (AS-PCR) assay. For this approach, two sets of primers shown in Table (1) (Bioneer Company/Korea) were obtained in lyophilized form. A stock solution was made with a concentration of 100 pmol/L using deionized water. The stock solution was then mixed with 90 litres of distilled water to make a working solution with a concentration of 10 pmol/L. For gene amplification, a pre-packaged 50 l PCR master mix (Bioneer/Korea) was employed.

PCR reaction was achieved in 20 µl reaction which contains 2 µl 10× PCR buffer, 2 mmol/l MgCl2, 0.2 mmol/l dNTP's mix, 1.5 µmol/l of both primers, 1 U recombinant Taq DNA Polymerase (Bioneer/Korea) and 100 ng genomic DNA. The tubes were placed in thermocycler (Hybaid/ UK). The PCR conditions for -1082G/A polymorphism involved an initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 45 sec, 63°C for 30 sec and 72°C for 45 sec. A final elongation was performed at 72°C for 7 min. The same conditions were applied for -819 C/T except for annealing step which was 53°C for 45 sec. PCR products were stained with ethidium bromide (Biobasic/Canada)(0.5 µg/ml) for 20 min and undergone electrophoresis. A commercial 1000 bp DNA marker (Kappa Biosystem/USA) was used to measure the size of the amplified products.
Primers, restriction enzymes and fragment length of digestion for IL-10-1082A/G and IL-10-819C/T polymorphisms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primers (5’ to 3’)</th>
<th>Product size</th>
</tr>
</thead>
</table>
| IL-10-1082A/G | (A) Upstream: ACTACTAAGGCTTTTTGGA A  
           | (G) Upstream: CTACTAAGGCTTTTGGAAG  
           | Downstream: CAGTGCCAACAGAATTTGG     | 258          |
| IL-10-819C/T | (C) Upstream: CCCCCCTGTACAGGTGATGTAAC  
           | (T) Upstream: ACCCTTGTACAGGTGATGTAAT  
           | Downstream: AGGATGTGTTCCAGGCTCTT     | 233          |

Statistical Analysis

For data analysis and graph generation, the SPSS software (version 25.0) was utilized. Categorical variables were presented as numbers and percentages, whereas numerical variables were presented as a mean as well as standard deviation (SD). The statistical significance of the association between different genotypes and alleles of the -1082G/A and -819C/T polymorphisms with the development of CPO was determined using a bivariate logistic regression test. This test yielded odds ratios (OR) with their corresponding 95 percent confidence intervals (CI). The haplotype analysis was conducted using SHesis Software [13]. The statistical tests were considered significant when p-values were less than 0.05.

Results

Demographic and Clinical Characteristics of the Study Population: The mean age of the patients was 51.72±11.64 which did not differ significantly from that of the controls (48.18±10.43 years). Similarly, there was no significant difference in the distribution of males and females between the two groups. Although patients had a slightly higher mean BMI (27.16±6.32 kg/m²) than controls (25.68±6.11 kg/m²), the difference was not significant. Ex/current smokers were almost doubled in patients compared to controls (20.31% vs. 10%), although the difference was not significant. Patients, per se, had a far higher mean of BI, PD, CAL, GI, and PI (82.14±12.98%, 6.12±1.48 mm, 7.42±1.64 mm, 2.71±1.09 and 2.86±1.01, respectively) than controls (10.8±1.23%, 0.62±0.52 mm, 0.04±0.23 mm, 0.02±0.07 and 0.04±0.2, respectively) as shown in Table (1).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients(64)</th>
<th>Patients(40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>51.72±11.64</td>
<td>48.18±10.43</td>
<td>0.115</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34(53.33%)</td>
<td>20(50%)</td>
<td>0.138</td>
</tr>
<tr>
<td>Female</td>
<td>30(46.67%)</td>
<td>20(50%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.16±6.32</td>
<td>25.68±6.11</td>
<td>0.140</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>51(79.68%)</td>
<td>36(90%)</td>
<td>0.166</td>
</tr>
<tr>
<td>Ex/current</td>
<td>13(20.31%)</td>
<td>6(10%)</td>
<td></td>
</tr>
<tr>
<td>Bop index (%)</td>
<td>82.14±12.98</td>
<td>10.8±1.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean PD (mm)(full mouth)</td>
<td>6.12±1.48</td>
<td>0.62±0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAL (mm)(full mouth)</td>
<td>7.42±1.64</td>
<td>0.04±0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GI</td>
<td>2.71±1.09</td>
<td>0.02±0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PI</td>
<td>2.86±1.01</td>
<td>0.04±0.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*BMI: body mass index; PD: Probing depth; CAL: Clinical attachment loss; GI: Gingival index; PI: Plaque index
Molecular Assays
The distribution of genotypes in both polymorphisms was in good accordance with Hardy Weinberg Equilibrium (HWE) in patients and controls.

IL-10- 1082A/G
According to AS-PCR, this polymorphism appeared in three genotypes in patients and controls, which were AA, GA and GG as shown in Figure (1).

Figure (1): Gel electrophoresis of PCR product for the polymorphism -1082A/G. Lanes M: molecular marker

The frequency of these genotypes among patients was 48.44%, 42.19% and 9.37% respectively, compared with 32.5%, 35% and 32.5% respectively, among controls (Table 2). Statistically, there was a significant difference in the frequency of AG (OR=0.19, 95%CI=0.06-0.62, P=0.006) and GG genotype (OR= 0.24, 95%CI=0.07-0.77, p=0.016) between patients and controls. At allelic level, the mutant allele (allele G) was more common among controls than patients (50% vs. 30.47%) with a highly significant difference (OR= 0.44, 95%CI=0.25-0.78, p=0.005).

Table (2): Genotypes and alleles of SNPs IL-10-1082A/G

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients N=64</th>
<th>Control N=40</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10-1082A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>31(48.44%)</td>
<td>13 (32.5%)</td>
<td>0.017</td>
<td>1.0</td>
</tr>
<tr>
<td>AG</td>
<td>27(42.19%)</td>
<td>14(35%)</td>
<td>0.006</td>
<td>0.19(0.06-0.62)</td>
</tr>
<tr>
<td>GG</td>
<td>6(9.37%)</td>
<td>13(32.5%)</td>
<td>0.016</td>
<td>0.24(0.07-0.77)</td>
</tr>
<tr>
<td>HWE</td>
<td>0.972</td>
<td>0.057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>89(69.53%)</td>
<td>40(50%)</td>
<td>0.005</td>
<td>1.0</td>
</tr>
<tr>
<td>G</td>
<td>39(30.47%)</td>
<td>40(50%)</td>
<td></td>
<td>0.44(0.25-0.78)</td>
</tr>
</tbody>
</table>

IL-10- 819C/T
Similar to IL-10- 1082G/A, this polymorphism had three genotypes in patients and controls. These genotypes were CC, CT and TT Figure (2).
Table (3) shows the frequencies of these genotypes in patients and controls. Although CC genotypes were more frequent among controls (20%) than patients (12.5%) the difference was not significant. Allele T had a slightly higher frequency among patients than controls (66.41% vs. 62.5%) with no significant difference.

Table (3): Genotypes and alleles of SNPs IL-10-819 C/T

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patient(n=64)</th>
<th>Controls(n=40)</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>29(45.32%)</td>
<td>18 (45%)</td>
<td>0.963</td>
<td>1.0</td>
</tr>
<tr>
<td>CT</td>
<td>27(42.18%)</td>
<td>14(35%)</td>
<td>0.876</td>
<td>1.07(0.44-2.63)</td>
</tr>
<tr>
<td>CC</td>
<td>8(12.5%)</td>
<td>8(20%)</td>
<td>0.877</td>
<td>0.92(0.32-2.63)</td>
</tr>
<tr>
<td>HWE</td>
<td>0.663</td>
<td>0.109</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Patient(66.41%)</th>
<th>Controls(62.5%)</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>85</td>
<td>50</td>
<td>0.566</td>
<td>1.0</td>
</tr>
<tr>
<td>C</td>
<td>43(33.59%)</td>
<td>30(37.5%)</td>
<td></td>
<td>0.84(0.47-1.51)</td>
</tr>
</tbody>
</table>

Haplotype Analysis

Table (4) the different haplotypes in patients with CPO and controls. The GC haplotype (G allele of IL-10-1082G/A and C allele of IL-10-819 C/T) was more common in controls than in patients (36.25% vs. 33.44%) with a significant difference (OR= 0.54, 95%CI= 0.29-0.99, p= 0.048). Other haplotype blocks were very close between the two groups with no significant differences.

Table (4): Different haplotype blocks of IL-10 gene in CPO patients and controls

<table>
<thead>
<tr>
<th>Haplotype blocks</th>
<th>Patients (128)</th>
<th>Controls (80)</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>76(59.37%)</td>
<td>39(48.75%)</td>
<td>0.135</td>
<td>0.154(0.87-2.7)</td>
</tr>
<tr>
<td>GC</td>
<td>30(23.44%)</td>
<td>29(36.25%)</td>
<td><strong>0.048</strong></td>
<td><strong>0.54(0.29-0.99)</strong></td>
</tr>
<tr>
<td>AC</td>
<td>13 (10.16%)</td>
<td>5(6.25%)</td>
<td>0.334</td>
<td>1.69(0.58-4.95)</td>
</tr>
<tr>
<td>GT</td>
<td>9(7.03%)</td>
<td>7(8.75%)</td>
<td>0.810</td>
<td>0.88(0.32-2.42)</td>
</tr>
</tbody>
</table>

Linkage Disequilibrium (LD)

The results of the LD analysis are presented in the figure 3. LD plot was constructed using combined genotype data from all patients. The SNP rs1800870 was in a strong LD with rs1800871 (the measure D’ was 0.71).
Discussion

According to the results of the present study, none of the included demographic factors had a significant association with the development of CPO. Different studies worldwide indicate different risk factors for CPO. For instance, age-related changes in innate immunity and inflammatory states may enhance periodontitis susceptibility [14]. Men are more likely than women to develop CPO [15,16]. CPO is predicted by socioeconomic status, which can be measured in terms of education, occupation, or income. However, education has a priority over income or occupation in this regard [17]. Furthermore, current and previous smokers are at a higher risk of CPO than non-smokers, according to several studies. There is a dose–response relationship between smoking and periodontitis [18]. Some literature has shown that the prevalence of CPO is greater among obese people. It is assumed that an increased BMI may be a potential risk factor for periodontitis [19]. The non-significant association between these factors and CPO in the present study is attributed to two main factors: firstly, the selection of the control group to be similar to patients in most demographic characteristics; and secondly, the relatively small sample size of the present study compared with other studies.

The most interesting finding in the present study is the protective role of AG, GG genotypes, and G alleles of the SNP IL-10-1082 G/A against CPO. Furthermore, the haplotype block GC (G allele of IL-10-1082G/A and C allele of IL-10-819 C/T) has a protective role against. Such results are in accordance with many previous studies. In an Iranian study, Moudi et al. [20] analyzed the role of three SNPs (IL10 1082, IL10 819, and IL10 592) in the IL-10 gene with the susceptibility of
CPO in 210 patients and 100 healthy controls. The study revealed a protective role of the G allele against CPO. Almost similar results were obtained by Emampanahi et al. [21] in the same population. Several other Asian studies [22-25] also indicated such a role of the G allele of IL-10-1082 G/A polymorphism against CPO. Emampanahi et al. [21] revealed that the AGC haplotype (-592/-1082/-819) haplotype was higher in healthy subjects, while AAC (-592/-1082/-819) was markedly higher among patients with CPO.

The protective mechanism of the G-allele and its associated genotypes is related to the position of this polymorphism in the promoter region of the gene. The homozygous GG allele augmented serum levels of IL-10, according to Reuss et al. [26], which could weaken the immunological response to the infection. In comparison to -1082A, -819T, and -592A, another study found that -1082G, as well as -819C and -592C, were significantly expressed alleles [27]. An in vitro investigation found that stimulation of lymphocytes led to reduced IL-10 production in the presence of allele -1082A than in allele -1082G cells [28]. Increased serum IL-10 levels are linked to decreased expression of IL-1, tumor necrosis factor alpha (TNF-), P-selectin, and other pro-inflammatory cytokines, as well as reduced neuronal apoptosis [29]. As a result, the G allele is linked to a lower inflammatory response and, as a result, a lower risk of CPO.

Another interesting finding in the present study was the strong linkage disequilibrium between IL-10 1082 and IL-10 819. In accordance with this result is the study of Zhang et al. [30] among the Chinese population with primary open angle glaucoma, where there was a complete LD between IL-10 1082 and IL-10 819. In another study, Shin et al. [31] demonstrated a tight link between their SNPs among Korean patients with tuberculosis.

Conclusions
These data indicate the protective role of GA, GG genotypes, and G alleles of the SNP IL-10-1082-G/A against CPO. Although the SNP IL-10-819C/T had no significant association with CPO in the present study, the haplotype block GC (G allele of IL-10-1082G/A and C allele of IL-10-819 C/T) does have a protective role.

Recommendations
Carriers of the A allele of IL-10-1082G/A and the T allele of IL-10-819 C/T should be aware that they have an increased risk of CPO. They should avoid modifiable risk factors when possible. Further studies, including other SNPs IL-10 gene, are required for more reliable conclusions.

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Ethical clearance: The study was approved by Ministry of Health, Al-Shaab Specialized Dental Health Center Committee.

Conflict of interest: Nil

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التغايرات الجينية في منطقة البروموت للجين الانترلوكين 10 في مرضى التهاب ماحول اللثة المزمن

سهاد جمعة عبد الكريم، احمد لعيبي عطروز، نور مويه حسين

الملخص

خلفية الدراسة: للسیتکین المضاد للالتهابات (IL-10) دور حاسم في أمراض اللثة. يعد الجين الذي يشفر لهذا السیتکین من الجینات المتعددة التغاير. هناك تغايران جینيان وهما IL-10-1082 و IL-10-819 في منطقة البروموت للجين IL-10، تؤثران على التعبير الجيني لهذا السیتکین.

أهداف الدراسة: لكشف عن ارتباط أشيئين من أشكال النوكليوتيدات المنفردة في جين إنترلوكين 10 و هما (IL-10-1082 G/A و IL-10-819 C/T) مع التهاب ماحول اللثة المزمن.

الطريقة: مجموعتين محددتين من الباحثات، من خلال تفاعل البلمرة المتسلسل النوعي للأليل.

النتائج: كان معدل تكرار الأنماط الجينية GG و GA و AA في مجال IL-10-1082 G/A لل터غایر AA و GG و GC على التوالي مقارنة بـ 32.5٪ و 35٪ و 32.5٪ على التوالي بين المرضى، بينما كانت النمط الفردی GC كألف G للتراگیر IL-10-1082 G/A و الگل C للتراگیر IL-10-819 C/T بالذات (OR=0.24، 95%CI=0.07-0.77، P=0.016) كانت أكثر شيوعًا في مجموعة السيطرة من المرضى (36.25٪ مقابل 33.44٪) مع اختلاف معنوي OR=0.54، 95%CI=0.29-0.99، p=0.048.

الاستنتاجات: تشير هذه البيانات إلى أن تعدد الأشكال الجينية IL-10-1082 G/A يمكن أن يكون عامل خطر لالتهاب ماحول اللثة المزمن في المرضى العراقيين.

الكلمات المفتاحية: التهاب دواعم السن المزمن، تعدد الأشكال الجينية للإنترلوكين 10، النمط الفردی، عدم توازن الارتباط

البريد الالكتروني: um_zeina@yahoo.com

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