Gene Polymorphisms of TNF-\( \alpha \)-308(G/A), IL-10-1082(G/A), IL6-174(G/C) and IL1Ra(VNTR) in Egyptian Cases with Adult and Early Onset Periodontitis

A Settin, M Seif, M Al Shahat, R El-Baz, E Abou El-Kheir

Abstract

Background: Periodontitis is an inflammatory destruction of periodontal tissue associated with cytokine dysfunctions.

Objectives: To check for the association of polymorphisms of cytokine genes with the susceptibility and severity of Periodontitis in cases from Egypt.

Subjects: 46 cases with periodontitis in addition to 98 healthy unrelated controls. Cases were classified into aggressive or early onset periodontitis (EOP) (17 cases, 36.9%) and chronic or adult onset periodontitis (AOP) (29 cases, 63.1%).

Methods: DNA was amplified using PCR-SSP for detection of polymorphisms related to TNF-\( \alpha \)-308 (G/A), IL-10-1082 (G/A), IL-6-174 (G/C) and IL-1Ra (VNTR).

Results: Total cases showed high significant frequency of homozygous IL-10-1082 (A/A) (OR=3.97, P<0.05), TNF-\( \alpha \)-308 (G/G) (OR=6.71, P<0.001), IL-6-174 C/C (OR=8.18, P<0.001) and IL-1Ra (A1/A1) (OR=3.42, P<0.05) genotypes. These were considered risk genotypes for disease susceptibility. The same was shown AOP cases while EOP cases showed only high significant frequency of homozygous IL-6-174 (C/C) (OR=8.36, P<0.05) and TNF-\( \alpha \)-308 (G/G) (OR=4.72, P<0.05) genotypes. On the other hand, combined heterozygosity for IL-10-1082 (G/A) with TNF-\( \alpha \)-308 (G/A) showed lowest significant frequency among all cases (P<0.001) and were considered low risk genotypes.

Conclusions: Cytokine gene polymorphisms may be used as a marker for periodontitis susceptibility, and severity helping for early diagnosis and induction of prophylaxis to affected families.

ABBREVIATIONS

TNF: tumor necrosis factor, IL: interleukin, IL-1Ra: IL-1 receptor antagonist, PCR-SSP: polymerase chain reaction with sequence specific primers.

INTRODUCTION

Periodontitis is a chronic bacteriogenic infection leading to rapid destruction of the tissues supporting the teeth in otherwise healthy individuals. The most common form of this disorder has been reported to affect about 30–40% of an adult population and approximately 10% of these subjects exhibit severe disease (1, 2). Genetic factors may account for 50% of the variations seen in periodontal disease expression in humans and also contribute to the variance in clinical symptoms of periodontitis as detected from studies in twins (2, 3). Cytokines play an important role in the pathogenesis of periodontal diseases by promoting periodontal tissue inflammation and destruction through the stimulation of prostaglandin production and the induction of collagenase and other proteases (4).

IL-10 mRNA expression in periodontitis lesions was found significantly higher than that in autologous peripheral blood
mononuclear cells in spite of large variation between patients (\(n\)). These variations of expression may be due to another mechanism, possibly regulatory network between inflammatory and anti-inflammatory cytokines. These led to speculate that the expression of mRNA and subsequent protein production are influenced by the gene polymorphisms, and that specific polymorphisms may be associated with the inflammatory response in periodontal disease (\(\text{IL-1}^{12}\)). One of these polymorphisms of IL-10 at -1082 was shown to affect in vitro IL-10 production in peripheral blood of healthy subjects (\(\text{IL-1Ra}^{13}\)).

Interleukin-1 is known as an important cytokine that has been shown to play a role in the earliest stages of gingival inflammation (\(\text{IL-1}^{14}\)). One of the important polymorphisms at the IL-1 gene cluster is that of IL-IRN-VNTR caused by variable numbers of an 86 bp tandem repeat located in the second intron of the gene. The most common alleles have been termed allele one (A\(_1\), 4 repeats), allele two (A\(_2\), 2 repeats) in addition to other 3 alleles (A\(_3\)).

Furthermore, IL-1 and TNF-\(\alpha\) were reported to stimulate many cells to produce matrix metalloproteinase, prostaglandins and proinflammatory cytokines, as well as affecting bone metabolism, all of which in turn contribute to the pathogenesis of periodontitis. TNF synthesis may be influenced by the presence of certain gene polymorphisms (\(\text{IL-6}^{15}\)).

IL-6 is a multifunctional cytokine that regulates immune responses and its effects overlap those of IL-1 and TNF. It is widely distributed among cells of the periodontium and is thought to play a role in the elevated B-cell response observed in the gingival tissues of patients with chronic periodontitis. A significant higher expression of IL-6 mRNA was reported in tissues of periodontitis patients (\(\text{IL-6}^{16}\)).

Taking into consideration that cytokine gene polymorphisms are population specific, we were interested to test for the association of these polymorphisms with periodontitis among Egyptian cases. In a case control study, we attempted testing the association of susceptibility and severity of periodontitis with polymorphisms of 2 pro-inflammatory cytokines (TNF-\(\alpha\) at position -308 and IL-6 at position -174) and 2 anti-inflammatory cytokines (IL-10 genes at position -1082 and of IL-1Ra VNTR).

**SUBJECTS AND METHODS**

This work included a random sample of 50 cases presenting with generalized form of periodontitis recruited from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, which is the main referral site in the Nile Delta Region of Egypt. Four smoker cases were excluded from the study to nullify the effect of smoking on disease severity and clinical behavior. Studied cases (46) included 26 men and 20 women with a mean age of 40.85 ± 13.22 years (range: 19-65 years). According to clinical presentation, they were classified into aggressive or EOP (17 cases, 36.9%) and chronic or AOP (29 cases, 63.04%). Positive family history was given in 14 (30.4%) of these cases.

Cases genotypes were compared to 98 healthy unrelated adult volunteers with negative family history of the disease from the same locality. These included 52 males and 46 females and their mean age was 44.9 ± 6.7 years.

**DNA EXTRACTION AND PURIFICATION**

After obtaining informed consent from all cases and controls, venous blood samples (3 ml) were collected on EDTA (ethylenediamine tetraacetate) containing tubes, DNA was extracted promptly using DNA extraction and purification kit (Gentra Systems, USA) according to manufacturer's instructions and then stored at -20 \(^\circ\) C till use.

**PCR AMPLIFICATION**

Three single nucleotide polymorphisms (SNPs) were analyzed including promoter sites TNF-\(\alpha\)-308 (G/A), IL-10 -1082 (G/A) and IL-6 -174 (G/C) as well as IL-1Ra VNTR as previously described (\(\text{IL-6}^{17}\)). For TNF-\(\alpha\), IL-6 and IL-10 SNPs identification, PCR with sequence-specific primers (PCR-SSP) in two reactions employing a common forward and 2 reverse primers was used, and for IL-1Ra VNTR polymorphism, a single PCR reaction employing a forward and a reverse primers was used. All primers, Taq polymerase, dNTP, and MgCl\(_2\) were purchased from QiaGene (QiaGene, USA). The assay was performed in Techne-Genius thermal-cycler (England). Briefly, 100-500 ng of genomic DNA was added to 25 \(\mu\)l of reaction mixture containing 1 \(\mu\)M of each common/specific primer, 200 \(\mu\)M of each dNTP, and 1 U of Taq DNA polymerase. We were careful to have master mixes for multiple cases and also for different polymorphisms at the same sitting with confirmation of the negative amplification to obtain accurate subject genotyping.

**DETECTION OF AMPLIFIED PRODUCTS**
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The entire reaction volume plus 5 µl of bromophenol blue track dye were loaded into 2% agarose gel (Boehringer Mannheim) containing ethidium bromide. Gels were electrophoresed for 20 minutes at 200 V, photographed under UV light (320 nm) and then scored for the presence or absence of an allele specific band. Figure 1 shows the amplified PCR products of TNF-α-308 (G/A), IL-10-1082 (G/A) and IL-6-174 (G/C) compared to size marker whereas figure 2 shows amplified alleles of IL-1Ra VNTR region in intron 2 of the gene.

Statistical analysis; Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 10.0). The frequency of studied allelic polymorphisms among cases was compared to that of controls describing number and percent of each and tested for positive association using Fisher’s exact test (modified Chi square test) and Odds ratio with a minimum level of significance of <0.05.

RESULTS

Analysis of IL-10-1082 (G/A) polymorphism among cases compared to controls (table 1), showed that homozygous form A/A was found significantly high in total cases (OR=3.97, P<0.05) and AOP cases (OR=4.29, P<0.05) while the heterozygous form G/A was found significantly low among the same groups (P<0.05 in both groups). Analysis of IL-6-174 (G/C) polymorphism (table 2), showed that homozygous form C/C was found significantly high in total cases (OR=8.18, P<0.001), in EOP (OR=8.36, P<0.05) and AOP cases (OR=8.079, P<0.001), while the heterozygous form G/A was found significantly lower among the same groups (P<0.001 in all).

Analysis of TNF-α-308 (G/A) polymorphism (table 3), showed that homozygous form G/G was found significantly high in total cases (OR=6.71, P<0.001), in EOP cases (OR=4.7, P<0.05) and AOP cases (OR=4.0, P<0.05), while the heterozygous form G/A was found significantly low among the group of AOP cases only (P<0.05). Analysis of IL-1Ra VNTR polymorphism (table 4), showed that homozygous form A1/A1 was found significantly high in total cases (OR=3.42, P<0.05) and AOP cases (OR=2.7, P<0.001), while the heterozygous form A1/A2 was found significantly low among total cases and AOP cases (P<0.05 and <0.001 respectively).

Figure 1
Table 1: Frequency of IL-10 (G→A) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
<th>G/G</th>
<th>G/A</th>
<th>A/A</th>
<th>G</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(96)</td>
<td>%</td>
<td>6(5.2)</td>
<td>10(10.4)</td>
<td>80(84.4)</td>
<td>95(95.8)</td>
<td>4(4.2)</td>
</tr>
<tr>
<td>Total cases(46)</td>
<td>%</td>
<td>4(8.4)</td>
<td>30(60.0)</td>
<td>12(23.9)</td>
<td>*36(72.0)</td>
<td>4(8.0)</td>
</tr>
<tr>
<td>Clinical types</td>
<td>%</td>
<td>1.56</td>
<td>0.29</td>
<td>0.9 (15.9)</td>
<td>2.0 (6.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>G051</td>
<td>%</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Clinical types</td>
<td>%</td>
<td>3.8</td>
<td>2.0</td>
<td>0.9</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>A0 Ra VNTR region in intron 2 of the gene.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2
Table 2: Frequency of IL-6 (G/C) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
<th>G/G</th>
<th>G/C</th>
<th>C</th>
<th>G</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(98)</td>
<td>%</td>
<td>56(57.1)</td>
<td>87(88.8)</td>
<td>6(6.1)</td>
<td>97(98.9)</td>
<td>9(9.1)</td>
</tr>
<tr>
<td>Total cases(46)</td>
<td>%</td>
<td>6(13.0)</td>
<td>24(62.2)</td>
<td>*16 (4.3)</td>
<td>36(80.0)</td>
<td>7(18.0)</td>
</tr>
<tr>
<td>Clinical types</td>
<td>%</td>
<td>2.7</td>
<td>0.14</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>G051</td>
<td>%</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Clinical types</td>
<td>%</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>A0 Ra VNTR region in intron 2 of the gene.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of TNF-α-308 (G/A) polymorphism (table 3), showed that homozygous form G/G was found significantly high in total cases (OR=6.71, P<0.001), in EOP cases (OR=4.7, P<0.05) and AOP cases (OR=4.0, P<0.05), while the heterozygous form G/A was found significantly low among the group of AOP cases only (P<0.05). Analysis of IL-1Ra VNTR polymorphism (table 4), showed that homozygous form A1/A1 was found significantly high in total cases (OR=3.42, P<0.05) and AOP cases (OR=2.7, P<0.001), while the heterozygous form A1/A2 was found significantly low among total cases and AOP cases (P<0.05 and <0.001 respectively).
Gene Polymorphisms of TNF-α-308(G/A), IL-10-1082(G/A), IL-6-174(G/C) and IL1Ra(VNTR) in Egyptian Cases with Adult and Early Onset Periodontitis

Figure 3
Table 3: Frequency of TNF-α (G→A) genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
<th>Cases (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cases (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>6.0 (2.77)**</td>
<td>5.1 (10.95)</td>
<td>6.4 (11.1)</td>
</tr>
<tr>
<td>N(%)</td>
<td>10 (21.7)**</td>
<td>9 (20)</td>
<td>10 (21.7)**</td>
</tr>
<tr>
<td>(95%C)</td>
<td>0.9 (0.2-2.9)</td>
<td>0.9 (0.2-2.9)</td>
<td>0.9 (0.2-2.9)</td>
</tr>
</tbody>
</table>

Clinical types

- EOP(17) N(%) 4 (21.5)% OR 3.4 (1.4-8.1) P<0.001
- AOP(20) N(%) 6 (30.7)% OR 1.5 (0.5-4.5) P<0.001

Figure 4
Table 4: Frequency of IL-1Ra VNTR genotype and allelic polymorphisms among periodontitis cases compared to controls with their statistical significance.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
<th>Cases (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cases (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>2.4 (1.2-2.6)</td>
<td>2.4 (1.2-2.6)</td>
<td>2.4 (1.2-2.6)</td>
</tr>
<tr>
<td>N(%)</td>
<td>6 (20.7)%</td>
<td>6 (20.7)%</td>
<td>6 (20.7)%</td>
</tr>
<tr>
<td>(95%C)</td>
<td>0.4 (0.2-1.2)</td>
<td>0.4 (0.2-1.2)</td>
<td>0.4 (0.2-1.2)</td>
</tr>
</tbody>
</table>

Clinical types

- EOP(17) N(%) 4 (21.5)% OR 3.4 (1.4-8.1) P<0.001
- AOP(20) N(%) 6 (30.7)% OR 1.5 (0.5-4.5) P<0.001

Figure 5
Table 5: Frequency of combined genotypes of different cytokines among cases compared to controls with their statistical significance.

<table>
<thead>
<tr>
<th>Combined Genotype</th>
<th>Cases N(%)</th>
<th>Control N(%)</th>
<th>OR 95%CIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α-308G with IL-10-1082A</td>
<td>6 (13.6)%</td>
<td>3 (6.6)%</td>
<td>2.0 (1.0-2.9)</td>
</tr>
<tr>
<td>IL-10-1082G with IL-6-174C</td>
<td>5 (10.8)%</td>
<td>4 (8.7)%</td>
<td>1.0 (1.0-2.9)</td>
</tr>
<tr>
<td>IL-10-1082G with IL1Ra A1/A2</td>
<td>4 (2.0)%</td>
<td>2 (0.4)%</td>
<td>1.0 (1.0-2.9)</td>
</tr>
<tr>
<td>IL-10-1082G with TNF-α-308G</td>
<td>2 (0.4)%</td>
<td>1 (0.2)%</td>
<td>1.0 (1.0-2.9)</td>
</tr>
</tbody>
</table>

*P<0.05 significant ** P<0.01 extremely significant OR (95%CIB) = Odds ratio (95% Confidence interval)

Interestingly, no significant difference was found in the frequencies of all studied alleles except for IL-1Ra A1, that showed significant higher frequency among total and AOP cases, while the other allele A2 has shown significant lower frequency among same groups.

DISCUSSION

The observation of a familial occurrence of periodontitis is indicative of its genetic basis (21). Similarly, positive family history was obtained in approximately one third of the currently studied cases supporting this concept.

It has been suggested that individual cytokine gene polymorphism may be considered a useful indicator or a diagnostic marker for inflammatory disorders including periodontal diseases among affected families (22, 23).

Polymorphisms in the IL-1 gene cluster are correlated with the severity of adult periodontal disease (24). On the other hand, IL-1RN gene showed 5 allelic polymorphisms that have been found associated with a variety of human diseases, primarily of epithelial cells or tissues. A1 allele containing four repeats, was the most common allele and was found in 73.6% of the population, while A2 allele containing two repeats, was found in 21.4% of the population (25). The same ratio was nearly found in our studied controls. Thus, A1 and A2 alleles were found in 79.6% and 20.4% of controls respectively. Analysis of IL-1Ra VNTR polymorphism among our studied cases showed the presence of high significant frequency of homozygous form A1/A1 with a significant low frequency of heterozygous form A1/A2 in total cases and AOP cases. These results were different from that reported previously of association of variable number tandem repeat (VNTR) polymorphisms in IL-1 receptor antagonist with generalized early-onset periodontitis (26).

Although this study showed that ILRN allele A1 was found
Gene Polymorphisms of TNF-α-308(G/A), IL-10-1082(G/A), IL6-174(G/C) and IL1Ra(VNTR) in Egyptian Cases with Adult and Early Onset Periodontitis

protective in cases of periodontitis, other authors reported increased severity of other immune conditions like systemic lupus erythematosus and Sjögren’s syndrome in Japanese populations carrying the same allele. Others reported that the IL-1Ra intron 2 polymorphism did not influence the susceptibility to or severity of rheumatoid arthritis (37, 38).

Besides the immunoregulatory effects of IL-10, the ability to enhance proliferation of B cells and autoreactive B cells, in particular, is of interest. Regarding the association between the -1082 IL-10 gene polymorphism and severe AOP, it was suggested that IL-10 related functions on autoreactive B cells are involved in the pathogenesis of periodontal disease (39, 20, 30).

The association of an IL-10 gene polymorphisms, with periodontitis in a Japanese population was studied and showed that none of the haplotypes could be linked to subjects with periodontitis and that the occurrence of the G allele at position -1082 in such patients was in relative terms low (33). However, in the current study, a significant higher frequency of A IL-10 -1082 A/A genotype was found in cases compared to controls that may be considered a risk genotype for periodontitis susceptibility. On the other hand, significant lower frequency of G/A heterozygosity genotype was found among total cases compared to controls that may be considered a protective genotype against susceptibility to periodontitis. These results were in contrast with what previously reported that the proportion of subjects that exhibited the G/G genotype was significantly larger in patients with severe periodontitis than in periodontally healthy individuals (34). This conflict can be explained on the basis of racial difference or association with other interactive types of cytokines or genetic markers predisposing for the disease in the studied cases.

Conflicting results have been reported regarding the association of -308 TNF-α gene polymorphism and periodontitis. Authors reported no differences in the distribution of TNF-α alleles between patients adult periodontitis and in healthy matched controls or between patients with different disease severity (40, 23, 20). Our studied cases however showed a significant higher frequency of homozygous (G/G) genotype compared to controls that may be considered a risk genotype for periodontitis susceptibility.

In the current study, total cases showed a significant higher frequency of homozygous genotype IL-6 -174 (C/C) compared to controls. Thus, IL-6 -174 C/C genotype may be considered a risk genotype for periodontitis susceptibility. On the other hand, significant lower frequency was observed with heterozygous genotype G/C that was considered a low risk or protective genotype. This result was in contrast to what was reported in caucasian Brazilians that genotype G/G was statistically associated with susceptibility to AOP (33). The finding that the frequency of heterozygous individuals (G/C) decreases the degree of severity of the disease was conforming with the current study although it was speculated to be due to a protective function of allele C, whose presence may reduce IL-6 production. Similarly, a reduction in the frequency of the C/C genotype in patients with systemic-onset juvenile arthritis was found suggesting that this genotype confers a protective influence against the development of the disease (35).

Analyzing studied Egyptian cases for combined genotypes, a certain pattern could be found to play a role in periodontitis disease susceptibility and/or severity. These included (IL-10 -1082 A/A with TNF-α-308 G/G; OR= 31.62), (TNF-α-308 G/G with IL-6 -174 C/C; OR= 20.85), (IL-10 -1082 A/A with IL-6 -174 C/C; OR= 11.83) and (IL-6 -174 C/C with IL-1Ra VNTR A/A; OR = 10.28). On the other hand, maximum protection genotypes included heterozygous genotypes (IL-10 -1082 G/A with TNF-α-308 G/A; OR= 0.29).

CONCLUSION

Cytokine gene polymorphisms may be used as a marker for periodontitis susceptibility, clinical behavior and severity helping for early diagnosis and induction of prophylaxis to other family members of affected probands against disease progression.

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References

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