Original Article

Tumor Necrosis Factor-Alpha and Interleukin-6 Gene Polymorphisms in Iranian Patients with Ischemic Heart Failure

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Abstract

Background: Proinflammatory cytokines have been known to be elevated in patients with Chronic Heart Failure (CHF). Given the importance of proinflammatory cytokines in the context of the failing heart, the prevalence of Tumor Necrosis Factor-α (TNF-α), Interleukin (IL)-6 polymorphisms in patients with CHF was studied due to ischemic heart disease.

Methods: Forty three patients with ischemic heart failure were enrolled in this study and compared with 140 healthy individuals. The allele and genotype frequency of four Single Nucleotide Polymorphisms (SNPs) within the IL-6 (-174, nt565) and TNF-α (-308, -238) genes were determined, using Polymerase Chain Reaction with Sequence-Specific Primers (PCR-SSP) assay.

Results: The frequency of the TNF-α (-238) A/A genotype was significantly higher in patients comparing to controls (p=0.043), while TNF-α G/A genotype at the same position decreased significantly, in comparison with controls (p=0.018). The most frequent haplotype for TNF-α was A/A in the patient group in comparison with controls (p=0.003). There was no significant difference in allele and genotype frequencies of IL-6 at positions -174 and nt565, and TNF-α at position -308.

Conclusion: Certain alleles, genotypes, and haplotypes in TNF-α, but not IL-6, gene were overrepresented in patients with ischemic heart failure, which may, in turn, predispose individuals to this disease.

Keywords: Genes, Heart failure, Interleukin-6, Tumor necrosis factor-alpha

Introduction

Chronic Heart Failure (CHF) is among the leading causes of mortality worldwide, with an incidence rate of 10 per 1000 population after the age of 65.1,2. Given the increasing incidence of the disease, identifying groups of patients who may be genetically more susceptible to developing CHF would be essential. Thus...
Association of TNF and IL-6 Gene Polymorphisms with Heart Failure

Written informed consent was obtained from all participants prior to blood sampling. This study was approved by the Ethical Committee of Tehran University of Medical Sciences.

Genotyping
Genomic DNA was isolated from peripheral blood leukocytes, using salting-out method. Cytokine typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as previously described in details. Briefly, amplification was carried out using a thermal cycler Techni Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. All individuals were genotyped for polymorphic sites of the following cytokine genes: IL-6, -174 G/C and nt565 G/A; TNF-α, -308 G/A and -238 G/A.

Statistical analysis
Statistical analyses were performed with EPI info software. Allele, genotype, and haplotype frequencies for all cytokine gene polymorphisms were calculated by direct counting. Frequencies of alleles, genotypes, and haplotypes were compared between the patient and control groups using the Fisher’s exact test. The odds ratio with 95% confidence intervals was calculated.

Results

Alleles and genotype frequencies
Allelic and genotype frequencies in patients with ischemic heart failure and healthy controls are presented in table 1. It should be noted that among 140 controls enrolled in the original study, results of three controls were not conclusive for TNF-α, while no result was detected for IL-6 of a control.

A significant positive association with the A/A genotype was found for TNF-α at position -238 in our patients compared to controls (7 vs. 0.7%, p=0.003), while TNF-α G/A genotype at the same position decreased significantly in patients compared to controls (20.9 vs. 41.6%, p=0.018).

The allele and genotype frequencies of IL-6 at positions -174 and nt565, and TNF-α at position -308 were similar in two groups of patients and controls.

Haplotype frequencies
Haplotype frequencies in patients with ischemic heart failure and healthy control subjects are shown in table 2. The most frequent haplotype for TNF-α (positions -308, -238) was A/A in the patient group in comparison with controls (4.7 vs. 0%, p=0.003).

Discussion
There is a paucity of data in the literature on the association of TNF-α -238G/A polymorphism with CHF. Bruggink et al reported increased frequency of TNF-α -238/A allele in patients suffering from dilated cardiomyopathy on left ventricular assisted device compared.
In the present study, the TNF-α association was reported in patients with ischemic heart failure. The number of patients with the A/A genotype and TNF-α (-308, -238) A/A haplotype was higher in patients compared to controls. Moreover, the frequency of TNF-α (-308, -238) A/A haplotype was higher in patients compared to controls. However, this might be a chance finding, the results need to be replicated in other, preferably larger, population with greater haplotype diversity. Moreover, given the small number of patients in this study, any conclusions can only be interpreted with caution.

There are conflicting reports on the influence of TNF-α -238G/A polymorphism on the expression level of TNF-α. The TNF-α -238A allele has been reported to be associated with both increased and decreased TNF-α expression.\(^{9,22}\), whilst other investigators reported no significant association between TNF-α -238G/A polymorphism and the cytokine expression level.\(^{24,25}\)

A single SNP, together with other SNPs, may only be able to modify the disease phenotype in an appropriate environmental context. Therefore, circulating TNF-α levels might reflect combined effects of multiple SNPs, in addition to environmental factors. This might in part explain the conflicting reports regarding the influence of TNF-α -238G/A polymorphism on the expression level of TNF-α.

Increased levels of IL-6 have been described repeatedly in patients with CHF with a positive correlation with disease severity. Previous studies have also reported the association between the polymorphisms in the promoter region of the IL-6 gene and altered cytokine production.\(^{10,26}\) However, to the best of our knowledge, the association between IL-6 gene polymorphisms and risk of ischemic heart failure has not been reported previously. In the present study, no association between -174G/C and +565A/G polymorphisms in the IL-6 gene and ischemic heart failure was found.

### Table 2. Comparisons of allele and genotype frequencies between patients with ischemic heart failure and controls

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Position</th>
<th>Alleles/Genotypes</th>
<th>Patients (n=43) N (%)</th>
<th>Controls (n=140) N (%)</th>
<th>p-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>-308</td>
<td>A</td>
<td>14 (16.3)</td>
<td>39 (14.2)</td>
<td>0.606</td>
<td>1.17 (0.60 to 2.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>72 (83.7)</td>
<td>235 (85.8)</td>
<td>0.606</td>
<td>0.85 (0.44 to 1.66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>1 (2.3)</td>
<td>0 (0)</td>
<td>0.239</td>
<td>9.71 (0.39 to 242.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>12 (27.9)</td>
<td>39 (28.5)</td>
<td>1.000</td>
<td>0.97 (0.45 to 2.09)</td>
</tr>
<tr>
<td></td>
<td>-238</td>
<td>GG</td>
<td>30 (69.8)</td>
<td>98 (71.5)</td>
<td>0.848</td>
<td>0.92 (0.43 to 1.94)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>15 (17.4)</td>
<td>59 (21.5)</td>
<td>0.449</td>
<td>0.77 (0.41 to 1.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>71 (82.6)</td>
<td>215 (78.5)</td>
<td>0.449</td>
<td>1.30 (0.69 to 2.43)</td>
</tr>
<tr>
<td>IL-6</td>
<td>-174</td>
<td>C</td>
<td>3 (7)</td>
<td>1 (0.7)</td>
<td>0.045*</td>
<td>10.20 (1.03 to 100.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>9 (20.9)</td>
<td>57 (41.6)</td>
<td>0.018*</td>
<td>0.37 (0.17 to 0.83)</td>
</tr>
<tr>
<td></td>
<td>+565</td>
<td>A</td>
<td>31 (72.1)</td>
<td>79 (57.7)</td>
<td>0.108</td>
<td>1.90 (0.90 to 4.01)</td>
</tr>
</tbody>
</table>

### Table 2. Comparisons of haplotype frequencies of TNF-α and IL-6 between patients with ischemic heart failure and controls

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Haplotype</th>
<th>Patients (n=43) N (%)</th>
<th>Controls (n=140) N (%)</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (-308, -238)</td>
<td>GG</td>
<td>61 (70.9)</td>
<td>176 (64.2)</td>
<td>1.36 (0.80 to 2.30)</td>
<td>0.298</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>10 (11.6)</td>
<td>39 (14.2)</td>
<td>0.79 (0.38 to 1.66)</td>
<td>0.594</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>11 (12.8)</td>
<td>59 (21.5)</td>
<td>0.53 (0.27 to 1.07)</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>4 (4.7)</td>
<td>0 (0)</td>
<td>29.95 (1.6 to 562.4)</td>
<td>0.003*</td>
</tr>
<tr>
<td>IL-6 (-174, n565)</td>
<td>GG</td>
<td>57 (66.3)</td>
<td>173 (62.2)</td>
<td>1.19 (0.72 to 1.98)</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>13 (15.1)</td>
<td>55 (19.8)</td>
<td>0.72 (0.37 to 1.4)</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>13 (15.1)</td>
<td>46 (16.6)</td>
<td>0.90 (0.46 to 1.76)</td>
<td>0.868</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>3 (3.5)</td>
<td>4 (1.4)</td>
<td>2.51 (0.55 to 11.45)</td>
<td>0.361</td>
</tr>
</tbody>
</table>
and IL-6 genes and IHD, as one of the most common causes of CHF, has been reported but remains controversial. Bennet et al reported no significant association between five SNPs in the TNF-α promoter region (-238G/A, -308G/A, -857C/T, -863C/A, and -1031T/C) and risk of Myocardial Infarction (MI) 27. Similarly, allele frequencies, and genotype and haplotype distributions of the TNF-α promoter polymorphisms -863C/A and -308G/A were not related to the risk of CAD and MI 28. In another study, none of the four TNF-α SNPs (-806C/T, -308G/A, -238G/A, and +467G/A) investigated reached statistical significance in the total sample of patients; however, a significant interaction between 238G/A polymorphism and risk of CHD was reported among nonsmokers in Chinese Han population 29. Regarding the role of IL-6 gene polymorphisms in CHD, in a very recent meta-analysis, it was shown that the IL-6 -174G/C polymorphism is not significant in CHD, in a very recent meta-analysis, it was shown that the IL-6 -174G/C polymorphism is not significant- 

In conclusion, this study demonstrates the association between certain allele, genotype, and haplotype frequencies in TNF-α gene with ischemic heart failure. Further investigation, using a larger sample size, to obtain more conclusive data regarding the role of TNF-α genotype in the pathogenesis of ischemic heart failure and influence on TNF-α level is warranted.

Acknowledgement

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References


