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Association of -592 Region of IL-10 Polymorphisms with Asthma in South-Eastern Iranian Patients

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SUMMARY

Background: Cytokines are considered important factors for the pathogenesis of asthma as they play a key role in the regulation of immune responses. The aim of this study was to investigate the association between this disease and polymorphisms in the -592 region of the IL-10 gene.

Methods: This study was carried out on 100 asthmatic patients and 100 healthy controls. PCR-RFLP was applied to examine the polymorphisms in the -592 region of the IL-10 gene.

Results: Our results showed a significant difference between patients and controls in terms of genotypes and alleles of the -592 region of the IL-10 gene.

Conclusions: According to our results, it can be concluded that the IL-10 promoter polymorphisms may play a crucial role in the pathogenesis of asthma.

KEY WORDS
Asthma, IL-10, polymorphism

INTRODUCTION

Asthma is characterized as a multifactorial respiratory disease caused by acute and chronic bronchial inflammation resulting in airway obstructions of various degrees [1]. Bronchial hyper-responsiveness (BHR) and increased total serum IgE levels are the main characteristics associated with the asthma phenotype [2]. The airway inflammation underlying asthma is driven by a hyperactive immune response [3], hence, factors that regulate this system can affect asthma pathogenesis [4]. IL-10, a cytokine expressed by regulatory T lymphocytes, suppresses both Th1 and Th2 responses [5]. Therefore, any changes in IL-10 expression can affect these two arms of immune responses [5,6].

Previous studies showed that IL-10 regulates the homing of eosinophils to the lungs in an animal model [7]. Other studies showed that IL-10 expression was decreased in asthmatic patients [7,8]. Investigators in this field believe that gene polymorphisms may play key roles in the regulation of the expression of cytokines [7,9]. In addition, previous studies have also shown that functional polymorphisms within the promoter region of IL-10 influence the expression of this cytokine [7]. Due to the critical role of IL-10 in asthma, we designed this project to examine polymorphisms of IL-10 in asthmatic patients.

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MATERIALS AND METHODS

Subjects
We studied 100 unrelated asthmatic patients during the period from February to June 2009 in the Molecular-Medicine Research Center of the Rafsanjan University of Medical Sciences. The mean age of the patients was 48 years (ranging from 15 to 79 years) and they were selected by an easy, convenient method based on the aims of this study. Assessment of socio-economic conditions were measured based on two parameters; 1- the level of education (diploma: weak, undergraduate: moderate, and post graduate: high) and 2- the monthly income (under $250.00: weak, $250.00-$1000.00: moderate, and more than $1000.00: high). Healthy control cases were selected from the population living in Rafsanjan city with matched gender, age, and socio-economic status. Pregnant women and patients with a history of cigarette smoking were excluded from the study. Asthma was diagnosed according to the American Thoracic Society (ATS) criteria [10] by an expert infectious disease specialist.

Patients were classified into two groups, allergic and non-allergic, according to their medical history and clinical findings. 100 genetically unrelated controls with normal spirometric values and no respiratory symptoms (refer to Table 1) were also matched with the patients for sex and ethnicity. The study protocol was approved by the ethics committee of the Rafsanjan University of Medical Sciences, and written informed consent was obtained from all participants prior to sample collection. Characteristics of the subjects are summarized in Table 1.

Total serum IgE measurements
Total serum IgE levels were measured by an ELISA kit (Genesis Diagnostics, UK) according to the manufacturer’s guidelines. The minimum detectable concentration of total IgE was 0.9 IU/mL and the range of detection was 1 - 1250 IU/mL.

Genomic DNA extraction
To extract genomic DNA, peripheral blood was collected in tubes containing EDTA and the genomic DNA was prepared using a commercial kit following the manufacturer’s guidelines (Bioneer, South Korea). Extracted DNA was aliquoted for each sample and stored at -20°C for further analysis.

Detection of polymorphisms
Polymorphisms within the IL-10 gene promoter were analyzed by polymerase chain reaction-restriction length polymorphism (PCR-RFLP) method which we have described elsewhere [9]. PCR of this gene was performed using 5 μL of Taq DNA polymerase buffer (10X), 1.5 μL of MgCl2 (stock concentration 1.5 mM), 1 μL of each dNTP ([dATP, dCTP, dGTP, dTTP] stock concentration of 10 mM], 2 μL of each primer (stock concentration of 25 ng/μL), 1 μL of prepared DNA and sterile double distilled water to a final volume of 50 μL. The sequence of the forward primer was 5'-GTAATAT CTCGTGCTTC-3' and the sequence of the reverse primer was 5'- CATTCCGAATACATAAGG -3'. The amplification was performed using the following program: one cycle of 95°C for 5 minutes (denaturation), 50 seconds at 53°C for annealing, 72°C for 40 seconds (elongation) followed by 30 cycles of 95°C for 50 seconds, 50 seconds at 53°C for annealing and 72°C for 40 seconds using thermal cycler (Mastercycler, Eppendorf, Germany). During the last 45 seconds of the first stage 0.3 μL of Taq DNA polymerase was added to the reaction. The amplified PCR product of the promoter region of the IL-10 gene covers the -592 region and has a molecular size of 437 bp. The Rsa-1 (Fermentase, Finland) has a unique restriction site covering the -592 region, thus, the fragment will be digested into two fragments of 236 and 201 bp following digestion with Rsa-1. In the case of the A/C genotype 3 different fragments of 201, 236, and 437 bp are visible. In the case of the C/C genotype, the Rsa-1 enzyme will not cut and the product will resolve as a single 437 bp fragment (C/C genotype). In the case of the A/A genotype two bands of 201 and 236 bp were observed (A/A genotype). Contol DNA of known genotype; C/C, A/C and A/A, were used for quality controls and as internal references for the PCR-RFLP analyses [9]. The digested products were run on a 2.5% agarose gel (Cinnagen-Iran) and analyzed using a chemi-doc-XRS system (Bio-Rad, USA) after staining with ethidium bromide.

Statistical Analysis
Hardy-Weinberg equilibrium was assessed using genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analysis of the differences between groups was determined by χ² test using EPI 2000 and SPSS software version 13. A p value of less than 0.05 was considered significant.

RESULTS
Analysis of the patient groups showed no significant differences between patient and controls regarding age, sex, and socio-economic condition (Table 1). Our results indicated significantly elevated IgE levels in asthmatic patients (287.2 ±407 IU/mL) in comparison to controls (50.2 ±32 IU/mL) (p=0.04). Evaluation of polymorphisms at the -592 position of the IL-10 gene by Rsa-1 restriction analysis showed that the prevalence of the C/C genotype was 69 (69%) in patients and 22 (22%) in controls, the frequency of the A/C genotype was 21 (21%) and 56 (56%) in patients and controls, respectively, and the frequency for the A/A genotype was 10 (10%) and 22 (22%) in patients and controls, respectively (Table 2). Statistical analysis of data also revealed that the difference between groups regarding these genotypes was significant (p=0.001).
Table 1. Clinical and socio-economic parameters of asthma patients and healthy controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/M/F ratio</td>
<td>27/73</td>
<td>31/69</td>
</tr>
<tr>
<td>Age/ys</td>
<td>43 ±14</td>
<td>48 ±12</td>
</tr>
<tr>
<td>Assessment of at least 2 symptoms (cough, wheeze, and shortness of breath)</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>% of group with allergy</td>
<td>N/A</td>
<td>70.3%</td>
</tr>
<tr>
<td>Positive family history of asthma</td>
<td>N/A</td>
<td>43.2%</td>
</tr>
<tr>
<td>Smoking history</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>87.3 ±14</td>
<td>79.2 ±21</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>88.1 ±11</td>
<td>82.3 ±9</td>
</tr>
<tr>
<td>PEF (% predicted)</td>
<td>91.6 ±21</td>
<td>74.6 ±19</td>
</tr>
<tr>
<td>FEF25-75 %</td>
<td>84.7 ±17</td>
<td>62.6 ±34</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>93.4 ±8</td>
<td>79.9 ±19</td>
</tr>
<tr>
<td>socio-economic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>weak</td>
<td>24 (24%)</td>
<td>21 (21%)</td>
</tr>
<tr>
<td>moderate</td>
<td>44 (44%)</td>
<td>49 (49%)</td>
</tr>
<tr>
<td>high</td>
<td>32 (32%)</td>
<td>30 (30%)</td>
</tr>
</tbody>
</table>

N/A: Not assessed
FEV1: Forced Expiratory Volume in 1 Second
FVC: Forced Vital Capacity
PEF: Peak Expiratory Flow
FEF25-75 %: Forced Expiratory Flow or average flow of air coming out of the lung during the middle portion (25 - 75%) of the expiration
L/% predicted: data are measured by liter and are shown as percentage of predicted volume

Table 2. Frequency of polymorphisms within the -592 region of the IL-10 gene in asthmatic patients and controls.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genotype</th>
<th>Patients</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/C n (%)</td>
<td>69 (69%)</td>
<td>22 (22%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/C n (%)</td>
<td>21 (21%)</td>
<td>56 (56%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A n (%)</td>
<td>10 (10%)</td>
<td>22 (23%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C n (%)</td>
<td>159 (79.5%)</td>
</tr>
<tr>
<td>A n (%)</td>
<td>41 (20.5%)</td>
</tr>
</tbody>
</table>

frequency of the C allele was 159 (79.5%) and 100 (50%) in patients and controls, respectively. This value for A allele in patients and controls was 41 (20.5%) and 100 (50%), respectively, with a significant difference of p=0.001 (Table 2).
DISCUSSION

Several studies reported divergent crucial modulatory roles for IL-10 on the immune system, especially its immunosuppressive effects in autoimmunity [11,12]. These findings suggest that factors that can influence IL-10 expression will have a downstream effect on the regulation of autoimmune responses. Interestingly, a previous study reported that the polymorphisms in the promoter of the IL-10 at position -592 have an impact on IL-10 expression [13]. In this current study, we demonstrated that there is an association of RsA-1 evaluated polymorphisms in the promoter of the IL-10 gene at position -592 and asthma. We based this conclusion on the finding that the frequency of the C/C genotype at position -592 of the IL-10 gene promoter was higher in the asthmatic patient group than in the control group (69% versus 22%, respectively). Our results also showed that RsA-1 evaluated genotypes are associated with asthma which is possibly due to the altered IL-10 expression levels.

Karjalainen J et al. showed that the A allele is associated with higher expression of IL-10 than the C allele [7]. Therefore, based on our results, it may be concluded that the higher frequency of the C allele as well as the C/C genotype in asthmatic patients leads to a decreased IL-10 expression by immune cells in the tissues of the patients, hence, type I hypersensitivity (allergy) occurred. Chatterjee R et al. reported that the A allele was positively correlated and the C allele negatively associated with asthma in the Indian population [14] which was in total contradiction to the results presented in this report. However, a study by Lyon SH et al. [15] reported a positive association of asthma with SNPs in the -592 region (referred to as -627C/A in their manuscript). Iranian investigators also reported a significant difference between asthmatic patients and controls regarding polymorphisms in the promoter of IL-10 [16]. Another study demonstrated that the A allele at -627 region of the IL-10 gene (equivalent to the -592 polymorphism reported here) is associated with atopic bronchial asthma [17]. In addition, Hobbs K et al. reported that polymorphisms in the promoter of IL-10 are associated with asthma in the American population [18]. These findings were entirely consistent with the results reported elsewhere [7,19-22]. Therefore, it may be concluded that IL-10 promoter polymorphisms are important in the development of asthma due to their effects on the rate of IL-10 expression. On the other hand, Zhang J et al. demonstrated that there is no link between polymorphisms in the promoter region of the IL-10 gene and susceptibility to asthma among Chinese population [23]. The discrepancy between our findings and the results from the aforementioned studies may be related to the differences in the studied populations in terms of genetic background or their exposure to environmental factors. Lyon et al (2004) suggested that other polymorphisms in the promoter of the IL-10 gene (i.e. -1117A/G and -854C/T) may have accumulative suppressive effects on IL-10 transcription and therefore other haplotypes within the promoter may need to be studied in parallel with the -592 SNP reported here.

In summary, our results suggest that the frequency of the C allele at position -592 of the IL-10 promoter is significantly higher in asthma patients and we hypothesize that this mutation influences transcriptional activity of the IL-10 promoter, causing a reduction in IL-10 expression in patients. As a consequence of reduced IL-10 expression the Th2 immune responses are expanded in asthmatic patients.

Acknowledgments:
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Declaration of Interest:
The authors of this manuscript have no invested interests in products described or used in this article. The Authors have no conflicts of interest.

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