Association of CD14 gene Polymorphisms with Asthma

1Ahmed H.K. Al-Hachamy, 2Ali H. Al-Saadi, 3Dr. Moshtak Wtwtt

1University of Kufa - College of Sciences
2University of Babylon, College of Sciences

ABSTRACT
To verify the association of CD14 (C-159T) gene polymorphisms with asthma severity in a sample of patients with atopic asthma. Aim of the study: This study aimed to verifying the association of CD14 (C-159T), gene polymorphisms with asthma.

METHODS: A clinical, laboratory, prospective study was performed in patients with atopic asthma, compared to a control group at allergy and asthma center also at Marjan teaching hospital between April and October 2014 in Babylon province/Iraq. CD14 C-159T gene polymorphism was detected by PCR and restriction enzyme.

RESULTS: This study included 58 patients with persistent atopic asthma and 30 healthy blood donors. When distribution of C-159T polymorphism genotype frequency (CD14) in asthma was compared with the control group, there was a result with the TT genotype.

Conclusion: Our results indicate that C-159T (CD14) polymorphism might be involved in modulation of asthma.

INTRODUCTION

Asthma is the most common chronic disease in childhood and adolescence. It is caused by genetic and environmental factors, and many genes have been identified in its pathogenesis. Some studies, also including twins, have shown that anumber of genes and their polymorphisms influence immune and pulmonary development and response to environmental factors, contributing to asthma occurrence and/or severity. The CD14 gene is located on chromosome 5q. The C-159T polymorphism has been associated with changes in CD14 and IgE levels in many populations of different ethnicities [2]. CD14 is a multifunctional receptor expressed in the surface of monocytes, macrophages and neutrophils or serum soluble.16 It is the main receptor of lipopolysaccharides (LPS) or inhaled endotoxins, which are potent inducers of pulmonary inflammation and may activate the immune system and cause Th1 differentiation and/or Th2 suppression. It has been proposed that altered CD14 expressions, more increased in asthmatics after LPS inhalation,18 can change the balance of Th1-Th2 cells, influencing IgE levels and inflammation in allergic diseases such as asthma [2]. Thus, changes in CD14 expression seem to be important, especially in allergic asthma, and such expression is regulated, at least partially, by the gene.

METHODS:

A clinical, laboratory and prospective study was carried out at DNA laboratory in the departments of biology, sciences college, university of Babylon.

Genomic DNA was extracted from 200 μl of whole blood using the geneaid Blood Kit in accordance with manufacturer’s instructions.

Method, primer sequence and restriction enzyme, as well as size of fragments generated by CD14, described in Table1.

Statistical analysis:

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means with their 95% confidence interval.

Corresponding Author: Ahmed H.K. Al-Hachamy, University of Kufa - College of Sciences
E-mail: Ahmedhachamy@yahoo.com
(CI). Independent sample t-test was used to compare means between two groups. A p-value of $\leq 0.05$ was considered as significant.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Method</th>
<th>Primers</th>
<th>Amplified fragment (pb)</th>
<th>Restriction enzymes and fragments (pb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14 gene (C-159 T)</td>
<td>PCR + RE</td>
<td>5'-gtgccacagatagttcac-3' 5'-gcctctgacagttttatgtaatc-3'</td>
<td>497</td>
<td>Ava II, 144 and 353</td>
<td>(Kedda 2005)</td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction; RE = restriction enzyme.

**Results:**

**DNA extraction:**

DNA extracted after collecting blood samples from the asthma patients and control individuals. Where we used gel electrophoresis device and 1% agarose substance also used 5 micro liter of DNA and 3 micro liter from loading dye for each well and we achieved the electrophoresis process with 75 V, 20 Am for 1 hour.

**Cluster of differentiation (CD14) genotyping:**

The polymorphism of CD14 gene (C-195T) gene was determined by polymerase chain reaction technique and agarose gel electrophoresis devise. Where used 1% agarose substance, 75 V, 20 Am for 120 min. (10 µl in each well) and appeared one band sized 495 bps. As shown in figure (3).

**RFLP-PCR for CD 14 gene:**

Genotyping of CD14 (C-159T) gene was determined by RFLP-PCR technique. Where used Ava II enzyme and appeared different bands sized 497, 353 and 144 bps. As shown in figure (4).
Fig. 4: Electrophoresis pattern of RFLP-PCR for PCR product (497 bps) with restriction enzyme Ava, 1% agarose, 75 V, 20 Am for 120 min. (10 µl in each well).

Lane 1: DNA ladder 100 bp.
Lane 2,4,5: showing mutant homozygote (TT) genotype.
Lane 3: showing mutant heterozygote (CT) genotype.

The Genotype of CD14 gene polymorphism with allele frequency between the two group (patient vs control):

The frequencies of CC, CT and TT for CD14 gene polymorphism were 12.06%, 43.10% and 44.82% for the patient with asthma group, and 23.33%, 36.66% and 40%. As shown in table (5).

Table 5: CD 14 gene polymorphism characterization in patients groups and control group.

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>P</th>
<th>95% CI</th>
<th>OR</th>
<th>Control group</th>
<th>Patient group</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.169</td>
<td>0.62-7.57</td>
<td>2.17</td>
<td>7(23.33%)</td>
<td>7 (12.06%)</td>
<td>CC</td>
</tr>
<tr>
<td>C(41.66%)</td>
<td>C(33.61%)</td>
<td>2.17</td>
<td>11 (36.66%)</td>
<td>25 (43.10%)</td>
<td>CT</td>
<td></td>
</tr>
<tr>
<td>T(58.33%)</td>
<td>T(66.37%)</td>
<td>0.184</td>
<td>0.64-8.05</td>
<td>2.27</td>
<td>12 (40%)</td>
<td>TT</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>58</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (P ≤0.05).
** CI 95%: confidence interval at 95% level.

Discussion:

From table (5), one can tell that homozygous genotype was more frequent in control groups, while heterozygous genotype was the abundant genotype in asthmatic group. In order to evaluate the significance of these results, T test was used to investigate the odds ratio (O .R.) and significance of genotyping and allele frequency.

In the present study, when genotype distribution of the C-159T polymorphism in asthma was compared with the control group, there was a difference with the TT and CT genotype in relation to asthma. It was verified that the TT and CT genotype represented a risk factor for severe asthma.

A longitudinal study including white individuals from Australia aged between 8-25 years, showed that those with the CC genotype were probably the same that had earlier atopy, suggesting that the influence of the -159C polymorphism in atopy could be age specific.

Another study showed that there are no publications showing association between the C-159T polymorphism and asthma severity, although it has been suggested that this polymorphism changes severity of air flow obstruction in asthmatic [3].

REFERENCES


