CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND PARAOXONASE-1 192 AND 55 GENE POLYMORPHISMS

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ABSTRACT
Chronic obstructive pulmonary disease (COPD) is a leading cause of chronic morbidity and mortality. The oxidative stress is increased in COPD patients. Paraoxonase (PON1) in the lung may have a role to protect from oxidative stress. We have investigated a possible relationship between PON1 55 and PON1 192 gene polymorphisms in COPD patients and control subjects.
A Total of 62 inpatients of COPD, 45 non-smokers and 35 smokers without COPD were included in the study. The serum levels of PON1 were measured. The PON1 genotypes were determined by PCR amplification of the region containing the polymorphism followed by restriction enzyme digestion.
The serum levels of PON1 were significantly low in the COPD patients group (p<0.001). There were no statistical differences between the COPD and control groups for PON1 55 polymorphism. The PON1 192 QQ and QR genotypes occurred with similar frequencies in the COPD and control groups with no significant differences while a significant difference was found between the PON1 192 RR allele frequencies (p<0.05) of all groups.
PON1 192 gene polymorphism may be considered associated with COPD. PON1 polymorphisms and low PON1 activity levels might be considered as an independent risk factor for COPD.

Keywords: COPD, PON1, PON1 192 gene polymorphism, PON1 55 gene polymorphism, spirometry

Introduction
Chronic obstructive pulmonary disease (COPD) is a leading cause of chronic morbidity and mortality worldwide. Cigarette smoking is considered the most important risk factor for the development of COPD (7). Projections for 2020 indicate further increase in global COPD mortality, making COPD the third leading cause of death (19). It is known that oxidative stress is increased in COPD patients compared with either healthy subjects or smokers with similar smoking history but who have not developed COPD (15, 16).
The free radicals and lipid peroxidation have many roles in the pathogenesis of COPD (3). The oxidative burden produced by cigarette smoke can be further enhanced in the lungs by the release of oxygen radicals from neutrophils and macrophages (13, 15).
The importance of oxidative stress in both lung and systemic circulation is well established in the pathogenesis of COPD (4). In healthy subjects there is a balance between oxidants and antioxidants, keeping the extracellular environment in a reduced state. Cigarette smoke contains massive amounts of oxidants in both the gas phase and particulate matter (approximately 10¹⁴ free radicals per puff) (20). Oxidative stress occurs when the burden of oxidants is not well counterbalanced by the antioxidant defense system (16).
Human serum paraoxonase (PON) is a polymorphic enzyme initially characterized as an organophosphate hydrolase, and its name derives from one of its most commonly used substrates, paraoxon (14).
PON1 in the lung may have a protective role from oxidative stress. PON1 is expressed in Clara cells, endothelial cells, and type 1 cells of the alveolar epithelium. However, smoking, which causes vital damage at the airspace epithelium, may also cause reduction in the levels of PON1 (5).
The PON1 gene is located on the long arm of chromosome 7 at q21-q22 regions (1, 14). The PON1 gene has two common polymorphisms in the coding region, which lead to a glutamine/arginine substitution at position 192 and leucine/methionine substitution at position 55 (8). The variants are designated as PON1 M/L55 and R/Q192. More attention has been given to the 192 polymorphism which appears to be major determinant of the well known biochemical polymorphism in serum PON activity towards number of substrates. The frequency of the PON1 alleles varies considerably across human population (8).
In the recent years, several studies have showed that extracts of cigarette smoke inhibited the activity of PON1 (2, 9, 21).
In this study, we investigated the possible relationship between serum PON levels, PON1 55 and 192 gene polymorphisms in the COPD patients, smokers without COPD and the non-smoker healthy subjects.
Materials and Methods

Diagnosis of COPD

All pulmonary function testing was performed according to the criteria of the American Thoracic Society/European Respiratory Society task force on standardization of lung function testing (17, 18) using the same spirometer. Three technically acceptable measurements were performed in each patient, and the highest value was included in the analyses. We performed spirometric assessments on all subjects and early reversibility testing on those who had FEV1/FVC < 70%.

The diagnosis of COPD in subjects with symptoms compatible with COPD was made with post-bronchodilator FEV1/FVC, less than 0.7. COPD was diagnosed in accordance with the GOLD guidelines.

Subject and controls

Serum PON1 levels were measured spectrophotometrically, modified from Eckerson (5). Initial rates of hydrolysis of paraaxon (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co. London, UK) were determined by measuring liberated-p-nitrophenol at 405 nm at 37°C. Spectrophotometric measurements were carried out by using Shimadzu UU–1201 spectrophotometer (Shimadzu UV 1208, Serial Number: A1038).

Blood samples were collected from 62 inpatients of COPD (40 males and 22 females; mean age of 60.21 years; SD 11.17 years), from 45 non-smokers (control group 1) (32 males and 13 females; mean age of 60.12 years; SD 12.21 years), and 35 smokers without COPD (control group 2) (24 males and 11 females; mean age of 59.16 years; SD 17.10) who were admitted to Dicle University, Faculty of Medicine Department of Chest Diseases and Tuberculosis. Control groups were drawn from the same population as that of the COPD subjects. This study was conducted in accordance with the Helsinki Declaration. As a standard procedure an informed consent was obtained from each participant.

Genotyping

Genomic DNA was extracted from peripheral leucocytes by standard procedures. The PON1 genotypes were determined by PCR amplification of the region containing the polymorphism followed by restriction enzyme digestion (8). Briefly, the DNA fragment that enclosed the codon 55 polymorphism was amplified using forward 5’ TAT TGT TGC TGT GGG ACC TGA G 3’ and reverse 5’ CAC GCT AAA CCC AAA TAC ATC TC 3’ primers. The DNA fragment that included the codon 55 polymorphism, PCR reaction and cycling was carried out by the following steps: one cycle at 95°C for 5 min followed by 95°C for 1 min, 60°C for 1 min and 72°C for 1 min for 35 cycles. Final elongation was one cycle at 72°C for 10 min for the PON1 192 genotype. The 99 bp PCR product was digested with 8U BspI restriction endonuclease (MBI Fermentas, Lithuania) overnight at 55°C and the digested products were separated by electrophoresis on a 3% agarose gel and visualized using ethidium bromide staining. For the PON1 55 polymorphism, PCR reaction and cycling was the same as above. PCR products (170 bp) were digested with NlaIII (MBI Fermentas, Lithuania) in the presence of BSA overnight at 37°C and were separated on 3% agarose gel, and DNA was visualized by ethidium bromide staining.

All genotypes were assigned at least by two researchers independently with no knowledge of the samples. Allele and genotype frequencies of PON1 55 and PON1 192 were obtained by direct counting. Hardy-Weinberg equilibrium was evaluated using chi-square test.

Statistical analysis

The data were analyzed using the Mann-Whitney-U test. Allele and genotype frequencies of PON1 55 and PON1 192 were obtained by direct counting. Hardy-Weinberg equilibrium was evaluated using chi-square test. P<0.05 were considered as significant. Statistical analyses were carried out by using the statistical packages for SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The main characteristics of the study subjects were as shown in Table 1. The values of FEV1 and FEV1/FVC ratio in the COPD group were less than these of the control groups (p<0.001). Male/Female ratio was similar in all groups.

The serum levels of PON1 were significantly low in the COPD patients (p<0.001). The serum levels of PON1 were shown in Table 2.

The genotype and allele frequencies of PON1 192 polymorphism in the COPD patients and in the control groups are shown in Table 3. The frequencies of PON1 192 polymorphism were detected using PCR based RFLP analysis. The frequency of PON1 192 polymorphism was found to be 48.4% for QQ, 37.1% for QR and 14.5% for RR in COPD patients’ group and 60% for QQ, 37.8% for QR and 2.2% for RR in control group1 and 54.3% for QQ, 37.1% for QR and 8.6% for RR in control group2 genotypes, respectively (Table 3). Allele frequencies were found to be 0.67% for Q and 0.33% for R allele in the COPD patients group and 0.79% for Q, 0.21% for R allele in the control group1 and 0.73% for Q allele, 0.27% for R allele in the control group2 (Table 3). The PON1 192 QQ and QR genotypes occurred with similar frequencies in the COPD and control groups with no significant differences while a significant difference was found between the PON1 192 RR allele frequencies (p<0.05) of all groups.

The genotype and allele frequencies of PON1 55 polymorphism in the COPD patients and in the control groups are shown in Table 4. The frequencies of PON1 55 polymorphism were detected using PCR based RFLP analysis. The frequency of PON1 55 polymorphism was found to be 38.7% for LL, 50% for ML and 11.3% for MM in the COPD patients group and 31% for LL, 62% for ML and 7% for MM genotypes in control group1 and 34.3% for LL, 57.1% for ML genotypes in control group2.
TABLE 1

Mean characteristic of the study population

<table>
<thead>
<tr>
<th></th>
<th>COPD patients (n=62)</th>
<th>Non-smoker (control group1) n=45</th>
<th>Smokers without COPD (control group 2) n=35</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.21±11.17</td>
<td>60.1±12.21</td>
<td>59.16±17.10</td>
<td>p&lt;0.001**</td>
</tr>
<tr>
<td>Male/Female</td>
<td>40/22</td>
<td>32/13</td>
<td>24/11</td>
<td></td>
</tr>
<tr>
<td>Pack/years smoking*</td>
<td>54.28±12.24</td>
<td>-</td>
<td>46.60±10.12</td>
<td>p&lt;0.001**</td>
</tr>
<tr>
<td>FEV1 (% predicted)*</td>
<td>35.40±13.02</td>
<td>79.70±10.80</td>
<td>78.24±9.08</td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC (% predicted)*</td>
<td>60.72±6.38</td>
<td>83.42±8.5</td>
<td>82.47±11.01</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SD

*p value for comparison of FEV1 and FEV1/FVC levels in COPD patients and control groups

TABLE 2

PON1 levels in the COPD patients and in the control groups

<table>
<thead>
<tr>
<th></th>
<th>COPD patients (n=62)</th>
<th>Non-smoker (control group1) n=45</th>
<th>Smokers without COPD (control group 2) n=35</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (U/l)*</td>
<td>43.16±28.64</td>
<td>99.50±44.08</td>
<td>102.40±35.50</td>
<td>p&lt;0.001**</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SD

*p value for comparison of PON1 levels in COPD patients and control groups

TABLE 3

Genotype and allele frequencies of PON1 192 polymorphism

<table>
<thead>
<tr>
<th>Genotype of PON1 192</th>
<th>COPD patients n=62 (no) %</th>
<th>Non-smoker (control group1) n=45 (no) %</th>
<th>Smokers without COPD (control group 2) n=35 (no) %</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>QQ</td>
<td>(30) 48.4</td>
<td>(27) 60</td>
<td>(19) 54.3</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>QR</td>
<td>(23) 37.1</td>
<td>(17) 37.8</td>
<td>(13) 37.1</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>RR</td>
<td>(9) 14.5</td>
<td>(1) 2.2*</td>
<td>(3) 8.6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>(83) 0.67</td>
<td>(71) 0.79</td>
<td>(51) 0.73</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>R</td>
<td>(41) 0.33</td>
<td>(19) 0.21</td>
<td>(19) 0.27</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

*p value of patients and control group 1

TABLE 4

Genotype and allele frequencies of PON1 55 polymorphism

<table>
<thead>
<tr>
<th>Genotype of PON1 55</th>
<th>COPD patients n=62 (no) %</th>
<th>Non-smoker (control group1) n=45 (no) %</th>
<th>Smokers without COPD (control group 2) n=35 (no) %</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>(24) 38.7</td>
<td>(14) 31</td>
<td>(12) 34.3</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>LM</td>
<td>(31) 50</td>
<td>(28) 62</td>
<td>(20) 57.1</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>MM</td>
<td>(7) 11.3</td>
<td>(3) 7</td>
<td>(3) 8.6</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>(79) 0.64</td>
<td>(56) 0.62</td>
<td>(44) 0.63</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>M</td>
<td>(45) 0.36</td>
<td>(34) 0.38</td>
<td>(26) 0.37</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>
and 8.6% for MM in the group 2 (Table 4). Allele frequencies were found to be 0.64% for L and 0.36% for M allele in the COPD patients group and 0.62% for L and 0.38% for M allele in the control group 1 and 0.63% for L and 0.37% for M allele in the control group 2 (Table 4). The PON1 55 LL, LM and MM genotypes occurred with similar frequencies in the COPD and control groups with no significant differences.

In the present study we examined the association between the PON1 55 and PON1 192 polymorphisms and COPD. An association between the PON1 55 and PON1 192 polymorphisms and COPD has not been studied according to our best knowledge. But, there are some studies on PON1 activity in COPD (2, 4, 10, 11, 21).

In this study, we found significant differences in the distribution of PON1 192 genotypes between the COPD and healthy non-smoker individuals. The results of our study support the hypothesis that the PON1 192 gene may be related to genetic susceptibility to COPD. However, we also found no indication of an association between the PON1 55 gene polymorphism and COPD. Our results suggest that the decreasing of the serum level of PON1 may be due to PON1 192 polymorphism in the COPD group in comparison to controls. PON1 192 and PON1 55 polymorphisms and the smoking-induced degradation of PON1 may thus be synergistically involved in the decreasing of PON1 activity in the airways in lungs.

Interestingly, Joppa et al. in their study described the potential relationship between pulmonary hypertension and markers of systemic oxidative stress in well defined patients with COPD (12). We also found relationship between PON1 192 gene polymorphisms and low serum PON1 levels in COPD patients in comparison to healthy non-smokers and smokers without COPD. However, we did not examined pulmonary artery pressure of the patients. We just focus on the relation of COPD and PON1 192 gene polymorphism.

Another result that was obtained showed that the FEV1 value was with low rate in the COPD group in comparison to the control groups. Also FEV1/FVC ratio was found to be low in the COPD group compared with the control groups. These two spirometric parameters are recommended for diagnosis of COPD stage according to GOLD guideline (7). Fletcher and Peto firstly described that the evidence linking the tobacco smoke exposure lead to COPD development and lung function impairment (6). Smoking is predominantly associated with a diminished lung function, more frequent respiratory symptoms, and with increased COPD related deaths (7).

The present study does not deal with all COPD stages. The focus has been set only on the serum levels of PON1 in patients with stage 3 and 4 of COPD, according to GOLD guideline (7). We have not evaluated the relationship between PON1 192 gene polymorphism and COPD in stages 1 and 2 because we evaluated patients who were hospitalized due to COPD exacerbations.

Conclusions

In summary, our findings indicated that in addition to other known risk factors which related to development of COPD, PON1 192 gene polymorphism might cause a reduction in the serum PON1 activity and may contribute to the development of COPD.

Further studies will be necessary to investigate the relationship of the PON-1 192 polymorphism in order that it can be suggested as an independent risk factor for COPD.

REFERENCES