Original Article

Paraoxonase-1 gene in patients with chronic obstructive pulmonary disease investigation Q192R and L55M polymorphisms

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BACKGROUND: The effect of increased oxidative stress on the development of chronic obstructive pulmonary disease (COPD) is well known. One of the antioxidative systems against oxidative stress in human body is paraoxonase (PON) enzyme that protects low density lipoproteins (LDL) against oxidation. This study aimed to explore the polymorphisms on PON1, Q192R, L55M genes of patients with COPD.

METHODS: DNAs extraction was obtained from blood samples of 50 patients diagnosed with COPD and 50 patients as a control group who were presented to emergency clinic. Genotypes were obtained with polymerase chain reaction (PCR) and AIw I and Hsp92II restriction enzymes were used for Q192R and L55M polymorphisms, respectively. Analysis of data was done with the Chi-square test and Fisher's exact test.

RESULTS: A statistically significant difference in Q192R polymorphism was found between the COPD patients and the control group (P=0.05). There was no statistically significant difference in L55M polymorphisms between the patient and control groups (P>0.05). Q192R polymorphism was significantly correlated with the PON1 gene and cigarette smoking; however other risk factors did not show any significant correlation with this polymorphism. Though L55M polymorphism was significantly correlated with family history and tuberculosis, there was no significant correlation with other risk factors.

CONCLUSION: We believe that more studies are needed to study the correlation of L55M polymorphism with other factors.

KEY WORDS: Chronic obstructive pulmonary disease; Paraoxonase; Polymorphism; Acute attack

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD), which is a major cause of mortality and morbidity worldwide, is one of the important public health issues. Oxidative stress plays an important role in the pathogenesis and progression of COPD. The shift of susceptible balance between free radicals and antioxidant defense mechanism in favor of oxidant substances causes increased oxidative stress. It is well-known that oxidative stress results in tissue injury. In a large-scale study, it was

shown that oxidative stress markers were increased in the urine, respiratory system, blood and airways of patients with COPD and smokers.[1]

Paraoxanase-1 (PON1) is a lipophylic antioxidant which is found in the liver and serum as bounded to high-density lipoprotein (HDL), and PON1 activity may play a protective role against oxidative stress in the lung.[2] The antioxidant role of PON1 is due to its protective effect on low-density lipoproteins against oxidation. The serum PON1 is found in plasma together with HDL and involved in the prevention of plasma lipoprotein oxidation. As lipids undergoing peroxidation are metabolized by this enzyme, the accumulation of lipid peroxides is prevented in both HDL and LDL. Thus, PON1 accounts from protective effect of HDL against LDL oxidation and it is more effective than vitamin A and E in this context.[3,4]

The PON1 gene is located at the q21-q21 region of chromosome 7. Two common functional polymorphisms are detected for PON1 and these polymorphisms affect serum PON1 activity. These polymorphisms results from amino acid changes at loci 55 and 192. The first polymorphism occurs when glutamine (Q genotype) is substituted by arginine (R genotype) at locus 192, whereas the second polymorphism occurs when leucine (L genotype) is substituted by methionine (M genotype) at locus 55. It has been reported that enzyme activity is altered as a result of these polymorphisms.[2,5,6]

In the present study, we aimed to investigate the association of COPD, in which oxidative stress is involved in pathogenesis, and PON1, which has antioxidant properties, with Q192R and L55M polymorphisms.

METHODS

This study was approved by the Institutional Ethics Committee. All subjects gave informed consent before recruitment. Fifty-five patients were enrolled in the study by taking their symptoms, physical examination results, laboratory findings and pulmonary function test parameters into consideration according to COPD diagnosis. Fifty healthy controls were included in the study. Patient's name, surname, age, gender, risk factors and comorbid diseases were recorded with a data sheet. Age and gender similarities were particularly observed in the selection of the controls.

Blood samples (2 mL) were drawn into tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were stored at –20 °C until DNA isolation. Blood samples were analyzed at Genetic Laboratory of, Medical School, Fırat University.

In the genomic DNA samples of the subjects, alleles at locus 192 of the PON1 gene were amplified by PCR. A mixture of 25 μL was prepared for amplification of each sample. This PCR mixture was prepared to contain 100–200 ng DNA, 0.5 μL of each primary, 0.2 mmol dNTP, 1.5 mmol $MgCl_2$, and 1.0 U Tag DNA polymerase. Amplification reactions were performed in a Mini Thermal Cycler.

Deoxyribonucleic acid purification was performed by using Wizard Genomic DNA purification kit purchased from Promega Inc. (Madison, WI, USA).

A PCR program consisted of 35 cycles was used. PCR products were cut by Alw1 restriction endonuclease and subjected to 2% agarose gel electrophoresis. DNA fragments were stained by ethidium bromide and then genotyping was performed by visualizing under UV. Allele A demonstrated band at 99 bp, while Allele B at 69 and 30 bp (Figure 1).

In the genomic DNA samples of the subjects, alleles at locus 55 of the PON1 gene were amplified by PCR; then PCR products were cut by Hsp192II restriction endonuclease and subjected to 2% agarose gel electrophoresis. DNA fragments were stained by ethidium bromide and then genotyping was performed by visualizing under UV. Allele L demonstrated band at 170 bp, while Allele B at 126 and 44 bp (Figure 2).

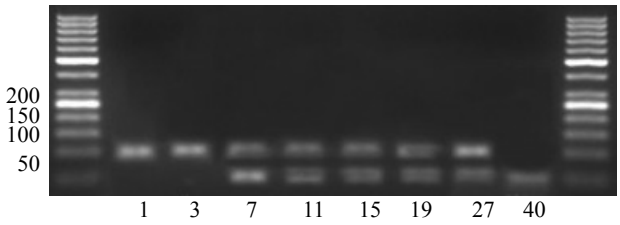

Figure 1. Image of PCR products-related to PON1 192 polymorphism on agarose gel electrophoresis. Alw1 enzyme was used for cutting PCR products. Subjects 1 and 3 have QQ genotype, while subjects 7, 11, 15, 19, 27 have QR and subject 40 has RR genotype. Q allele at 99 bp; R allele at 69 and 30 bp. M: 50–1 000 bp size marker.

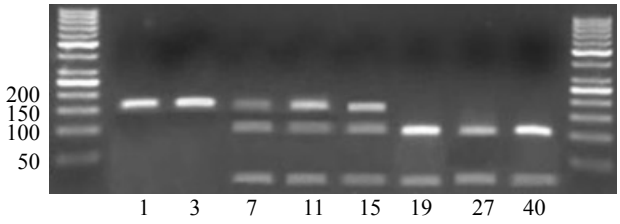

Figure 2. Image of PCR products-related to PON1 55 polymorphism on agarose gel electrophoresis. Hsp91II enzyme was used for cutting PCR products. Subjects 1 and 3 have LL genotype, while subjects 7, 11, 15, have LM and subjects 19, 27, 40 have MM genotype. L allele at 170 bp; M allele at 170 and 126 bp. M: 126 bp size marker.

The primary oligonucleotide used in the present study was synthesized by Biobasic Inc. (Biobasic, Ontario, Canada). The primaries in polymerase chain reaction (PCR) assays were used for amplification of related gene region on human DNA (Table 1).

Statistical analysis

The fitting of genetic distribution to the Hardy-Weinberg equilibrium was analyzed by using the Chi-square test. Difference of genotypic distribution between the patients and controls were analyzed by the Chi-square test. A *P* value less than 0.05 was considered as statistically significant. All statistical analyses were performed by using SPSS for Windows version 15.0.

RESULTS

Fifty-five patients with COPD and 50 healthy controls were included in the study. Table 2 presents the demographic characteristics of the patients and controls. Table 3 presents distribution of risk factors in the patients and controls. Of the patients included, 15 (27.3%) had a positive family history for COPD, while 35 (36.6%) were smokers.

Table 4 presents the distribution of Q192R genotype (as number and percent) in the patients with COPD and controls. In our study, the genotype distribution of PON1 gene Q192R polymorphism was provided. According to our results, QQ was the most common genotype in the patient group (*n*=33, 64.7%), followed by QR genotype (*n*=14, 27.5%). The most seldom genotype was RR, which was seen in 4 (7.8%) patients. In the control group, QQ genotype was seen in 32 (80.0%) patients, whereas QR genotype in 8 (20.0%) patients. No RR genotype was observed.

Table 5 summarizes the distribution of LL, LM and MM genotypes (as number and percent) obtained for PON1 gene L55M polymorphism. In the analysis for PON1 gene L55M polymorphism, LL genotype was seen in 30 (61.2%) patients, whereas LM genotype in 18 (36.7%) and MM genotype in one (2.0%) patient. In the control group, LL genotype was observed in 25 (59.5%) patients, whereas LM genotype in 17 (40.5%) patients. No MM genotype was observed.

PON1 gene polymorphisms Q192R and L55M genotype and allele distributions are shown in Table 6. PON1 gene Q192R polymorphism gave an odds ratio of 0.40 (95%*CI* 0.16–0.96); and PON1 gene L55M polymorphism gave an odds ratio of 0.98 (95%*CI* 0.47–2.04) for developing COPD.

Table 7 summarizes the correlation of COPD risk factors with Q192R and L55M polymorphisms. The result revealed that in the patients with a family history as a risk factor and Q192R polymorphism, QQ genotype was observed in 8 (15.7%) patients, whereas QR genotype in 3 (5.9%) patients and RR genotype in one (2.0%) patient. In the patients with L55M polymorphism, LL genotype was observed in 5 (10.2%) patients, whereas LM genotype in 8 (16.3%) patients, and MM genotype in one (2.0%) patient. In the patients with a smoking history as a risk factor and Q192R polymorphism, QQ genotype was observed in 24 (47.1%) patients, whereas QR genotype in 5 (9.8%) patients and RR genotype in 33 (59.0%) patients. In the patients with a history of previous tuberculosis as a risk factor and L55M polymorphism, LL genotype was

Table 1. Primary sequences used for PON 192 and PON 55 gene regions

Primary sequences used for PON 192 gene region
5'-TAT TGT TGC TGT GGG ACC TGA G-3'
5'-CAC GCT AAA CCC AAA TAC ATC TC-3'
Primary sequences used for PON 55 gene region
5'-GAA GAG TGA TGT ATA GCC CCA G-3'
5'-TTT AAT CCA GAG CTA ATG AAA GCC-3'

Table 2. Demographic characteristics of the patients and controls

Variables	COPD (*n*=55)	Control (*n*=50)
Age (years)	68.92±10.76	50.54±11.80
Sex (Male/Female)	38/17	32/18

Table 3. Distribution of risk factors

Variables	COPD (*n*, %))	Control (*n*, %))
Family history	15 (7.3)	2 (4.0)
Smoking	35 (36.6)	20 (40.0)
Asbestosis	0 (0)	0 (0)
Tbc	4 (7.3)	1 (2.0)
Respiratory tract infections	23 (41.8)	1 (2.0)
Socioeconomic status	9 (16.4)	1 (2.0)
Nutrition	8 (14.5)	0 (0)

Table 4. Genotype distribution of PON1 gene Q192R polymorphism

Groups	*n*	QQ (*n*, %)	QR (*n*, %)	RR (*n*, %)
COPD patients	51	33 (64.7)	14 (27.5)	4 (7.8)
Controls	40	32 (80.0)	8 (20.0)	0 (0)
P value	0.05			

Table 5. Genotype distribution of PON1 gene L55M polymorphism

Groups	*n*	LL (*n*, %)	LM (*n*, %)	MM (*n*, %)
COPD patients	49	30 (61.2)	18 (36.7)	1 (2. 0)
Controls	42	25 (59.5)	17 (40.5)	0 (0)
P value	0.05			

observed in 2 (4.1%) patients, whereas LM genotype in one (2.0%) patient and MM genotype in one (2.0%) patient. A significant association was found between Q192R polymorphism in the PON1 gene (P<0.05), while no significant association was found between other risk factors and Q192R polymorphism. However, L55M polymorphism was significantly associated with a family history and tuberculosis (P<0.05), whereas it was not significantly associated with other risk factors (P>0.05).

DISCUSSION

Gene polymorphism studies have been performed to investigate predisposition to chronic obstructive pulmonary disease (COPD) in patients with COPD. A study on MEPHX and GSTP1 gene polymorphisms demonstrated that multiple genes play a role in this disease.[7] Another study on CYP2E1 and NAT2 gene polymorphisms in COPD showed a relationship between these detoxification genes and COPD.[8] Charlotte et al[9] studied TNF gene polymorphism in COPD, but no sensitivity was detected.

Paraoxonase-1 activity exhibits individual and community-based variations. The reason for this variation is genetic polymorphism. It has been reported that there is a polymorphism in PON1 genes 148 and 311 in addition to those in PON1 genes 192 and 55. There was a difference in paraoxonase enzyme activities that were associated with polymorphism.[10] Moreover, there were significant differences in paraoxonase activities related to Q192R and L55M polymorphism.[11] And QQ genotype expressed lowest enzyme activity, while QR and RR genotypes expressed moderate and highest enzyme activities, respectively. Similarly, MM homozygote genotype expressed lower enzyme activities compared with LM and LL genotypes. The lowest enzyme activities were observed in QQ and MM genotypes.

In our study, QQ was the most common genotype in the patient group (n=33, 64.7%), followed by QR genotype (n=14, 27.5%). The most seldom genotype was RR, which was noted in 4 (7.8%) patients. In the control group, QQ genotype was seen in 32 (80.0%) patients, whereas QR genotype in 8 (20.0%) patients. No RR genotype was observed. In the analysis of PON1 gene LL55M polymorphism, LL genotype was seen in 30 (61.2%) patients, whereas LM genotype in 18 (36.7%) patients and MM genotype in one (2.0%) patient. In the control group, LL genotype was observed in 25 (59.5%) patients, whereas LM genotype in 17 (40.5%) patients. No MM genotype was observed. A significant relationship was found between the patient and control groups for PON1 gene Q192R polymorphism (P=0.05), while no significant relationship was observed for L55M polymorphism (P>0.05).

In a COPD gene study on 821 patients, Hersh et al[12] evaluated smoking habits, family history and socioeconomic status by using a questionnaire. They found that 85.5% of the patients were smokers and 43.0% had a family history of COPD. They suggested that family history is a strong risk factor for COPD and a family history of smoking is important in COPD. In our study, PON1 gene polymorphism was detected in 12 (23.5%) COPD patients with a family history of COPD, but this finding was found to be insignificant (P>0.05). However, PON1 gene L55M polymorphism was detected in 14 (28.6%) patients, indicating a significant association between a family history and polymorphism (P<0.05).

Orhan et al[13] evaluated the relationship between

Table 6. Genotype and allele distributions of PON1 gene Q192R and L55M polymorphisms

Gene	Genotype Allele	Patients (n=51)	Controls (n=40)	χ^2	P
PON1 gene Q192R	QQ	33 (0.647)	32 (0.800)		
	QR	14 (0.275)	8 (0.200)	4.386	0.111
	RR	4 (0.078)	0 (0.000)		
	Q	80 (0.784)	72 (0.900)	4.358	0.036
	R	22 (0.216)	8 (0.100)		
PON1 gene L55M	LL	30 (0.612)	25 (0.595)		
	LM	18 (0.367)	17 (0.405)	0.950	0.621
	MM	1 (0.020)	0 (0.000)		
	L	78 (0.796)	67 (0.798)	0.0008	0.977
	M	20 (0.204)	17 (0.202)		

Table 7. Association of risk factors with gene polymorphisms

COPD factors	Q192R			L55M		
	QQ (n, %)	QR (n, %)	RR (n, %)	LL (n, %)	LM (n, %)	MM (n, %)
Family history	8 (15.7)	3 (5.9)	1 (2.0)	5 (10.2)	8 (16.3)	1 (2.0)
Smoking	24 (47.1)	5 (9.8)	3 (5.9)	18 (36.7)	12 (24.5)	1 (2.0)
Tbc	2 (3.9)	1 (2.0)	0 (0)	2 (4.1)	1 (2.0)	1 (2.0)
Respiratory tract infections	12 (23.5)	8 (15.7)	2 (3.9)	14 (28.6)	7 (14.3)	0 (0)
Socieconomic status	5 (9.8)	2 (3.9)	1 (2.0)	4 (8.2)	4 (8.2)	0 (0)
Nutrition	5 (9.8)	1 (2.0)	1 (2.0)	4 (8.2)	3 (6.1)	0 (0)

COPD and smoking, pulmonary infection or inflammation. They found that functional regression of pulmonary functions in patients with COPD after cessation of smoking. In their patients the levels of malonaldehyde which has antioxidant features in the lungs increased after treatment of infection and inflammation. Christopher et al[14] evaluated microsomal epoxide hydrolase gene polymorphism in patients with emphysema. They proposed that epoxide derivates in smokers led to emphysema. In our study, we found PON1 gene polymorphism in 32 (62.7%) patients with COPD who were smokers and also found a significant association between smoking and PON1 gene Q192R polymorphism in these patients with COPD. Although PON1 gene L55M polymorphism was detected in 31 (63.3%) patients with COPD who were smokers, the association was found to be insignificant (P>0.05).

The fact that occupational exposure caused by long-term inhalational exposure to several gases and smoking that can be harmful for the lungs doesn't result in COPD suggests the possibilities including presence of identified or unidentified risk factors and genetic abnormalities leading predisposition to COPD in these individuals. Although it is primarily seen in developing or underdeveloped countries, NHANES III study showed that 19.2% of COPD patients in the USA were due to occupational environment. In the same study, occupational exposure accounted for 31.1% of the COPD patients seen in lifetime non-smokers.[15] No history of asbestosis was seen in COPD patients in our study. Thus, no conclusion can be made about the gene polymorphism in such patients.

The Platino study on patients with tuberculosis suggested that there is evidence indicating a 2–4 fold increase in the incidence of COPD.[16] Ben-Selam et al[17] reported a significant association between active tuberculosis and RANTES gene 28C/G and 403G/A polymorphisms in a Tunisian population. Han et al[18] also found that IL-18 gene polymorphism was more frequent in patients with tuberculosis in the Chinese population. Hahn[19] found a significant relationship between COPD and *C. pneuominia* infections. Studies[20,21] showed that antibiotics were used to treat acute exacerbations and decreased oxidative stress by treating inflammation caused by infection in COPD. In our study, PON1gene Q192R polymorphism was observed in 3 (5.9%) COPD patients with a history of tuberculosis, but this finding didn't reach a statistical significance (P<0.05). However, PON1 gene L55M polymorphism was detected in 4 (8.2%) patients (P<0.05). In the COPD patients with a history of respiratory tract infection, 22 (43.1%) had PON1 gene Q192R polymorphism (P>0.05), whereas 21 (43.3%) had L55M polymorphism (P>0.05).

Although there is an association between COPD development and malnutrition or lower socioeconomic status, this association either represents a true risk factor or result of exposure to several risk factors in the context of poor socioeconomic conditions.[22,23] In our study, no significant association was found between PON1 gene Q192R and L55M polymorphisms in COPD patients with poor socioeconomic status or malnutrition (P>0.05).

Tekes et al[2] found that PON-1 Q192R gene polymorphism can cause a decrease in serum PON1 activity and contribute to COPD development. Another study showed that Q192R and –108>Ct polymorphisms could be associated with serum PON1 and aril esterase levels in patients with COPD in a Croatian population.[24]

In conclusion, we found a difference in PON1 gene Q192R polymorphism between the patient and control groups, whereas there was no significant difference in PON1 gene L55M polymorphism in the two groups. A significant association was detected between PON1 gene Q192R polymorphism and smoking in patients with COPD, but no significant association was found between other risk factors and the polymorphisms. Moreover, L55M polymorphism was significantly related to family history of smoking or tuberculosis. We think that supporting these findings by extending paraoxonase enzyme activities in a large cohort will contribute to understanding of the association between PON1 and COPD.

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Ethical approval: This study was approved by the Institutional Ethics Committee.

Conflicts of interest: There was no conflict of interest related to this report.

Contributors: Gürbüz S proposed the study and wrote the first draft. All authors read and approved the final manuscript.

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