Paraoxonase Activity and Phenotype Distribution in Patients with Chronic Obstructive Pulmonary Disease

Nurhan Sarioglu1, Cigdem Bilen2, Celalettin Cevik3, Nahit Gencer4

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1Department of Pulmonary Diseases, Balikesir University School of Medicine, Balikesir, Turkey
2Department of Chemistry, Yildiz Technical University, Faculty of Arts and Science, Istanbul, Turkey
3Department of Public Health Nursing, Balikesir University Faculty of Health Science, Balikesir, Turkey
4Department of Chemistry and Biochemistry, Balikesir University Faculty Science and Art, Balikesir, Turkey

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Correspondence to: Nurhan Sarioglu
E-mail: nurhangencer@hotmail.com
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ABSTRACT

Objective: Paraoxonase 1 (PON1) and arylesterase (ARE) enzymes have an important role in the prevention of oxidative stress which is related to the pathogenesis of chronic obstructive pulmonary disease (COPD). PON1 levels vary widely among individuals and ethnic groups, which is in part associated with polymorphisms.

Materials and Methods: We investigated PON1 and ARE activity and phenotype distribution in COPD patients and healthy individuals. Sixty six COPD patients and 59 control subjects were involved in the study. Serum PON1 and ARE activities were detected by spectrophotometric method. The ratio of salt-induced PON1 to ARE activity was used to determine phenotypes as QQ, QR, and RR.

Results: COPD patients exhibited higher PON1 activity (199.1 vs 129.2, p=0.002) but lower ARE activity compared to control (21.3 vs 33.5, p=0.021). There was a significant difference between COPD and control group with respect to PON1 phenotype characteristics. RR phenotypic distribution was more common in the COPD group than in control (60.6% [95% CI: 48.8 - 72.3] versus 22.0% [95% CI: 12.0 - 31.9], p=0.001). We also found that smoking (95.0% CI: 0.001-0.036, p<0.001) and RR phenotype (95.0% CI: 0.006 - 0.59, p=0.016) are independent determinants in COPD.

Conclusion: We found that RR phenotype was more common in COPD patients compared to control. Smoking and RR phenotype may be defined as independent factors associated with COPD.

Keywords: COPD, paraoxonase 1, phenotype

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of mortality and morbidity across the globe [1]. It is characterized by enhanced chronic inflammatory response in the airway to toxic particles or gases [1, 2]. Many inflammatory cells and cytokines have a role in the pathogenesis of COPD. Environmental risk factors such as smoking, air pollution, and biomass affect the genome of susceptible individuals and trigger the onset of the disease [3]. The interaction of genetic and environmental factors contributes to the formation of the phenotypes [3]. Several different clinical and pathophysiological phenotypes have been determined in COPD [2-4]. Definition of clinical phenotypes is needed to facilitate the understanding of pathogenesis and management of the disease [4].

It is well known that oxidative stress plays an important role in the pathogenesis of COPD [2]. Paraoxonase 1 (PON1) is an antioxidant enzyme associated with high-density lipoprotein (HDL) and prevents the oxidation of low-density lipoprotein (LDL) [5]. PON1 has been involved in the pathogenesis of many disorders such as asthma, COPD, cardiovascular diseases and sepsis [6-9]. PON1 is expressed in type I cells, endothelial cells, and Clara cells of the alveolar epithelium. Smoking may influence the expression of PON1 by epithelial damage [10]. The human plasma paraoxonase activity in a population displayed a polymorphic distribution, and polymorphisms affect plasma PON1 levels [11, 12]. PON1 polymorphism is an amino acid substitution at the active site of the enzyme. The human PON1 gene is expressed by allelic variants, a glutamine (Q allele) for arginine (R allele) at position 192 polymorphism in relation with substrate dependent [9, 13]. In a few studies, PON1 activity was studied in COPD patients but PON1 phenotypes...
Materials and Methods

Methods

Study design
Sixty-six patients with COPD diagnosed according to GOLD criteria and 59 control subjects were included consecutively between August 2016 and February 2017 [1]. The disease was classified according to the new version of GOLD staging [1]. The mMRC dyspnea scale was fulfilled to assess the dyspnea [15]. The patients with COPD were categorized into A-D subgroups combining exacerbation risk and mMRC dyspnea score. Smokers were defined as current smokers who smoked ≥2 cigarettes daily, non-smokers were defined as subjects who had never smoked, ex-smokers were defined as subjects who had a smoking history but quit smoking more than 6 months ago.

Control subjects were chosen from our hospital after undergoing a routine examination. All subjects signed written informed consent and the Institutional Ethics Committee approved the study. Physical examination, routine blood analysis, chest X-ray, and respiratory function tests were performed on all the patients. Smoking history and the number of exacerbations in the previous year were recorded. Standard blood analysis and respiratory function tests were performed for the healthy control group.

The Population of the Study
Patients over 40 years, current or former smokers of at least 10 pack-years or biomass smokers of at least 10 pack-years or biomass were included. Exclusion criteria included COPD exacerbation within the previous 3 months, having a chronic inflammatory disease or malignancy, and having uncontrolled concomitant disease (arrhythmia, myocardial infarction, etc.).

Statistical Analysis
The results are represented as mean ± standard deviation (SD). Student t-test was used to compare the variances between two groups. The correlation analyses were performed using Pearson’s correlation test. One-way ANOVA was used to analyze QR subgroups. The phenotype distribution between the groups was tested using the chi-square (χ²) test. Logistic regression analysis with the backward elimination method was performed to identify independent determinants associated with COPD. Age, sex, smoking status, phenotype, PON1 and ARE activity were included as independent variables, p<0.05 was considered to be statistically significant. Statistical Package for the Social Sciences for Windows computing program version 22.0 (IBM SPSS Corp., Armonk, NY, USA) was used for all correlation analyses.

Blood Samples
Blood samples were obtained after an overnight fasting, and the serum separated by centrifugation at 10 min at 3,000 g was recovered following protection in aliquots at -80°C until the experiment.

Determination of Lipid Parameters
HDL, LDL, triglyceride, and total cholesterol levels were measured according to standard biochemical procedures using a COBAS Integra 800 automatic analyzer (Roche, Switzerland).

Assay of PON1 and ARE Activity
PON1 activity towards paraoxon was quantified spectrophotometrically at 412 nm [16]. The molar extinction coefficient of 17 100 M⁻¹ cm⁻¹ was used to determine the enzyme activity at 37°C. The micromole of paraoxon hydrolyzed at 25°C. The molar extinction coefficient of 1310 M⁻¹ cm⁻¹ was used to determine the enzyme activity at 25°C. The micromole of phenylacetate formed in 1 min was considered as a unit.

ARE activity towards phenylacetate was quantified spectrophotometrically at 270 nm [17]. The molar extinction coefficient of 1310 M⁻¹ cm⁻¹ was used to determine the enzyme activity at 25°C. The micromole of phenylacetate formed in 1 min was considered as a unit.

PON1 Phenotype Dispersion
PON1 phenotype distribution was determined by the binary substrate process [18]. The genetic polymorphism at codon 192Q→R is liable for the presence of two isotypes: Q and R. The ratio of 1 M NaCl containing buffer paraoxon catalysis to phenylacetate catalysis is applied to find out which of the three (QQ, QR, RR) phenotypes enters. The limit values of the phenotypes are as follows: type RR>7.0; QR: 3.0-7.0; and QQ<3.0 with RR high, QR medium, and QQ low enzyme activities.

Results
The study included 66 patients and 59 controls. Clinical, functional and biochemical parameters of subjects were shown in Table 1. The mean (SD) age of the patients was 63.9 (10) years and that of the controls was 61.0 (6.5) years. Most participants were male, and there was no significant difference between the two groups in terms of age (p=0.077) and sex (p=0.174). The proportion of smokers in the COPD group was higher than that in the control group (p=0.001) (Table 1). Twenty-five (37.9%) patients with COPD had a concomitant disease (hypertension [n=12], diabetes mellitus [n=10], and ischemic heart disease [n=3]). The mean FEV1 was 46.9% of the predicted value and mean FEV/FVC 55.5% in the COPD group. Incidences of comorbidities of two groups were similar (p=0.352).

As expected, pulmonary function parameters (FEV1, FVC, FEV1/FVC) of COPD patients were lower than controls (p=0.001). When the patients were subdivided, of the 66 patients, 30.3% were in group A, 39.4% group B, 9.1% group C, and 21.2% group D.

COPD patients exhibited higher PON1 activity (199.1 vs 129.2, p=0.002) but lower ARE activity compared to control (21.3 vs 33.5, p=0.021). Levels of LDL-C, total cholesterol, and triglycerides in the COPD group were found to be higher than those in the control (p<0.05). However, levels of HDL-C in the patient group were found to be lower than those in the control (p<0.001) (Table 1).

We observed that the phenotype distribution of the two groups was different. In the COPD group, the RR phenotype was more common compared to that of the control group (60.6% [95% CI: 48.8-72.3] versus 22.0% [95%CI: 12.0-31.9], p=0.001) (Table 2).

The lipid parameters (HDL, LDL, triglycerides, total cholesterol) did not show any difference between the phenotype subgroups (Table 3).

We compared A, B, C, and D subgroups with respect to their PON1 activity. PON1 activities in B and D groups (179.5 U mL⁻¹, 192.8 U mL⁻¹, respectively) were lower than A and C groups.

Main Points
- Arylesterase (ARE) activity could be a useful marker in COPD.
- PON1 RR phenotype was common in COPD and this result is consistent with PON1 genotype studies in COPD.
- Smoking and RR phenotype can be defined independent determinants relation with COPD.
Table 1. Clinical, functional and biochemical parameters of subjects

|                      | COPD (n=66) | Control (n=59) | p     |
|----------------------|-------------|---------------|-------|
| Sex (M/F)            | 61/5        | 50/9          | 0.174 |
| Age                  | 63.9 (10.7) | 61.0 (6.5)    | 0.077 |
| Smoking status, n (%)|             |               |       |
| Smokers              | 57 (86.4)   | 2 (3.4)       | 0.001 |
| Ex-smokers           | 7 (10.6)    | 7 (11.9)      |       |
| Non-smokers          | 2 (3)       | 50 (84.7)     |       |
| Comorbidity          |             |               |       |
| Any                  | 41 (62.1)   | 45 (76.3)     | 0.352 |
| Hypertension, n (%)  | 12 (18.2)   | 8 (13.6)      |       |
| Diabetes mellitus, n%| 10 (15.2)   | 5 (8.5)       |       |
| Ischemic heart disease,n(%)| 3 (4.5) | 1 (1.7)       |       |
| FEV₁, % predicted    | 46.9 (16.3) | 89.7 (5.5)    | 0.001 |
| FVC, % predicted     | 66.0 (17.8) | 93.2 (5.8)    | 0.001 |
| FEV₁/FVC, %          | 55.5 (13.2) | 89.9 (5.2)    | 0.001 |
| Gold group, n (%)    |             |               |       |
| A                    | 20 (30.3)   |               |       |
| B                    | 26 (39.4)   |               |       |
| C                    | 6 (9.1)     |               |       |
| D                    | 14 (21.2)   |               |       |
| HDL-C (mg/dL)        | 48.8 (5.9)  | 54.9 (10.2)   | 0.001 |
| LDL-C (mg/dL)        | 113.1 (19.5)| 100.0 (26.7)  | 0.003 |
| Total Cholesterol (mg/dL) | 207.2 (23.8) | 180.2 (34.3)  | 0.001 |
| Triglyceride (mg/dL) | 155.6 (30.5)| 125.3 (43.7)  | 0.001 |
| PON1 activity (U mL⁻¹)| 199.1 (34.5)| 129.2 (21.2)  | 0.002 |
| ARE activity (U mL⁻¹) | 21.3 (14.9) | 33.5 (39.5)   | 0.021 |

ARE: Arylesterase; FEV₁: Forced expiratory volume in 1 second; FVC: Forced vital capacity; COPD: chronic obstructive pulmonary disease

Table 2. PON1 phenotype distribution in patient and control subjects

| Phenotypes | COPD n (%) | Control n (%) |
|------------|------------|---------------|
| QQ         | 12 18.2 (8.8-27.1) | 24 40.7 (28.8-52.5) |
| QR         | 14 21.2 (11.3-31.0) | 22 37.3 (25.6-48.9) |
| RR         | 40 60.6 (48.8-72.3)* | 13 22.0 (12.0-31.9) |

*RR phenotypes distribution was more common in COPD group than in control; p=0.001 (χ²=19.20, df=2); COPD: chronic obstructive pulmonary disease

There was also a negative relation between PON1 activity and mMRC dyspnea score (r=-0.25, p=0.044) (Table 4).

Table 2. PON1 phenotype distribution in patient and control subjects

Discussion

In this study, we determined PON1 phenotype distribution and PON1 activity in COPD and control subjects.

As is known, reactive oxygen species (ROS) cause the oxidative deformation of basic components of the organism, which also leads to the structural damage of airway cells and connective tissues. Oxidative stress plays a crucial role in COPD and atherosclerotic disorders [2, 19-21]. Furthermore, coronary heart disease, which is related to oxidative stress is also one of the causes of mortality in COPD [20]. PON1 is an antioxidant enzyme and was examined in a few studies in COPD, but PON1 phenotypes have not been determined yet [7, 14].

Rumora et al. [7] have reported that COPD patients had lower PON1 activity compared to the control subjects. They indicate that particularly in GOLD II-stage patients, PON1 and ARE activities significantly decrease compared to controls. The recent study reported no difference between COPD and control subjects with respect to PON1 activity [14]. However, in our study, PON1 activity in patients with COPD was found to be higher than those in the control group. These conflicting results could be explained by the patient groups did not the same with respect to disease severity.

Furthermore, previous studies demonstrated that patients with COPD had a lower ARE activity compared to that of control subjects [7, 22]. Our study also showed similar results. According to these results, we think that ARE activity could be a useful marker in COPD.

The relationship between PON1 activity and the lipid profile has been previously researched, but conflicting results have been reported [23-25]. Some studies revealed an association between PON1 activity and lipid parameters [23] whereas others did not [24, 25]. In the present study, PON1 activity was not associated with lipid parameters, which was consistent with the data from past research on type-2 diabetes mellitus [24, 25]. The inconsistent results can be explained by PON1 activity and concentration being modulated by environmental factors, lifestyle, dietary habits, and genetic properties [26-28].

Comorbid diseases is another factor that can affect PON1 and ARE activities. However, in this study, no difference was observed between patients with COPD and healthy subjects with
respect to comorbidities such as hypertension, diabetes, and heart diseases.

Moreover, in our study, PON1 activity was negatively related to mMRC and FEV\textsubscript{1}/FVC. It can be concluded that PON1 activity decreased as the disease progressed.

In recent years, COPD-linked phenotypes have become the common interest area of researchers [33]. Moreover, it is recommended to choose the treatment according to clinical phenotypes. In a few previous studies, the relationship between PON1 genotype and COPD [34, 35] was determined, but there is no research which examines the relationship between PON1 phenotype and COPD. We determined that RR phenotype was more common in COPD patients compared to those in the control group. Furthermore, we found that smoking and RR phenotype were independent variables of COPD.

In a previous study, a significant difference in Q192R genotype was found between patients with COPD and that of the control group [34]. In another study, Tekes et al. [35] reported that RR genotype was more prevalent in patients with COPD compared to healthy nonsmokers. They have inferred that the PON1192 gene may be related to genetic susceptibility to COPD. Our phenotype results are consistent with previous PON1 genotype studies in COPD.

| Table 3. Association between lipid profile and PON1 phenotype of the subjects |
|-----------------------------|-----|-----|-----|-----|
| Variables                  | QQ  | QR  | RR  | p   |
| HDL-C (mg/dL)              |     |     |     |     |
| Patient                    | 46.9 (3.9) | 52.2 (7.4) | 48.0 (5.2) | 0.093 |
| Control                    | 53.8 (8.6) | 53.2 (10.1) | 59.7 (12.3) | 0.135 |
| LDL-C (mg/dL)              |     |     |     |     |
| Patient                    | 113.9 (17.49) | 110.7 (21.5) | 113.9 (19.7) | 0.892 |
| Control                    | 102.3 (24.2) | 100.1 (32.3) | 94.0 (26.9) | 0.585 |
| Total Cholesterol (mg/dL)  |     |     |     |     |
| Patient                    | 208.6 (24.9) | 210.2 (30.3) | 205.6 (21.0) | 0.852 |
| Control                    | 183.0 (35.6) | 180.5 (35.4) | 172.6 (31.0) | 0.411 |
| Triglyceride (mg/dL)       |     |     |     |     |
| Patient                    | 164.6 (36.1) | 158.5 (26.1) | 151.7 (30.4) | 0.468 |
| Control                    | 131.1 (41.0) | 127.0 (48.0) | 107.8 (44.5) | 0.177 |

HDL: high-density lipoprotein; LDL: low-density lipoprotein

| Table 4. Relation between PON1 activity and other parameters |
|-----------------------------|-----|-----|
| Variables                  | r   | p   |
| FEV\textsubscript{1}        | -0.17 | 0.052 |
| FVC                        | -0.09 | 0.270 |
| FEV\textsubscript{1}/FVC    | -0.21 | 0.019 |
| HDL-C (mg/dL)              | -0.06 | 0.496 |
| LDL-C (mg/dL)              | 0.13  | 0.153 |
| Total Cholesterol (mg/dL)  | 0.11  | 0.216 |
| Triglyceride (mg/dL)       | -0.03 | 0.735 |
| mMRC                       | -0.25 | 0.044 |
| Smoking                    | 0.10  | 0.226 |

mMRC: modified Medical Research Council dyspnea scale; FEV\textsubscript{1}, FVC: pulmonary function parameters; HDL: high-density lipoprotein; LDL: low-density lipoprotein

| Table 5. Logistic regression analysis of variables in COPD |
|-----------------------------|-----|-----|-----|-----|
| Variable                  | β   | SE  | p   | Exp (β) |
| Age                       | 0.044 | 0.004 | 0.311 | 1.045 |
| Female (ref)              |     |     |     |     |
| Male                      | 1.250 | 0.979 | 0.202 | 3.490 |
| Smoking                   |     |     |     |     |
| No                        | -5.290 | 1.001 | 0.001 | 0.005 |
| Yes (ref)                 |     |     |     |     |
| PON                        | 0.004 | 0.004 | 0.349 | 1.004 |
| ARE                       | -0.024 | 0.023 | 0.311 | 0.977 |
| Phenotype                 |     |     |     |     |
| QQ (ref)                  |     |     |     |     |
| QR                        | -1.190 | 1.317 | 0.366 | 0.304 |
| RR                        | -2.814 | 1.167 | 0.016 | 0.060 |

95% CI

| β   | SE  | p   | Exp (β) |
|-----|-----|-----|---------|
|     |     |     | 0.933   |
|     |     |     | 1.022   |
|     |     |     | 0.512   |
|     |     |     | 23.78   |
|     |     |     | 0.001   |
|     |     |     | 0.036   |
|     |     |     | 0.996   |
|     |     |     | 1.012   |
|     |     |     | 0.933   |
|     |     |     | 1.022   |
|     |     |     | 0.023   |
|     |     |     | 4.016   |
|     |     |     | 0.006   |
|     |     |     | 0.590   |

-2 log-likelihood: 69.585; Chi-square: 101.799; (p<0.001) Nagelkerke R²: 0.74
One of the strengths of our study was that it is the first study investigating PON1 phenotypes in COPD. The present study has some limitations such as, a single center study and small sample size.

The relationship between smoking and COPD is well known. In addition to other known risk factors, our results suggested that RR phenotype may be an independent risk factor associated with COPD. Further studies are needed to determine whether RR phenotype constitutes a risk in COPD progression.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Balkesir University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.S., C.B., N.G.; Design - N.S., N.G.; Supervision - N.S., C.C., N.G.; Resources - N.S., C.B., N.G., C.C.; Materials - N.S., C.B.; Data Collection and/or Processing - N.S., C.B., N.G., C.C.; Analysis and/or Interpretation - N.S., C.B., C.C.; Writing Manuscript - N.S., C.B., N.G.; Critical Review - C.C., N.G.

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