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Paraoxonase 1 Phenotype and Paraoxonase Activity in Asthmatic Patients

Nurhan Sarioglu[1], Adnan Adil Hismiogullari[2], Fuat Erel[1], Dudu Demir[3], and Nahit Gencer[4]

[1] Department of Pulmonary Diseases, Faculty of Medicine, Balikesir University Balikesir, Turkey
[2] Department of Biochemistry, Faculty of Medicine, Balikesir University, Balikesir, Turkey
[3] Department of Agricultural Biotechnology, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey
[4] Department of Chemistry, Faculty of Science and Art, Balikesir University, Balikesir, Turkey

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ABSTRACT

Oxidative stress is involved in the pathogenesis of asthma. *Paraoxonase 1* (PON1) and arylesterase are esterase enzymes displaying antioxidant characteristics. PON1 activity varies widely among individuals and ethnic groups, partly related to polymorphisms.

The aim of this study was to determine the activities of PON1 and *arylesterase* including the phenotype distribution of PON1 in asthmatic patients and healthy subjects.

Forty-nine asthmatic patients and 41 healthy people were included in this study. Serum PON1 and *arylesterase* activities were determined by spectrophotometric assays, as well as the lipid profiles. The PON1 ratio (salt stimulated *paraoxonase/arylesterase*) was trimodally distributed and this ratio was used to determine the individual phenotypes of all subjects.

The PON1 activity in the asthmatic patients was significantly lower (p=0.024) when compared to the healthy control group, however no significant difference in the activity of arylesterase was observed between the two groups. The prevalence of the PON1 phenotypes in the asthmatic population were 26.5%, 16.3% and 57.2 % for QQ, QR and RR, respectively.

PON1 activity was significantly lower in asthmatic patients; in addition, the results of this investigation indicated that PON1 RR phenotype may be an important risk factor in asthma disease.

Keywords: Arylesterase; Asthma; *Paraoxonase 1;* Phenotype

INTRODUCTION

Asthma phenotypes and endotypes have been determined recently.[1,2]

Inflammation and airway remodelling are linked to many phenotypes in allergy and asthma. Asthma phenotypes are involved genetic and environmental determinants. Gene and environment interactions are researched in the studies[2,3] however, knowledge is limited on this matter and more works have to be done. Asthma subphenotypes were determined with combination of clinical characteristics but recent studies have evaluated from clinical to molecular approaches.[1] A recent research supports that airway inflammation is associated with increased oxidative stress.[4] Oxidant-antioxidant imbalance is identified as a

Corresponding Author: Nurhan Sarioglu, MD;
Department of Pulmonary Diseases, Faculty of Medicine, Balikesir University Balikesir, Turkey. Tel: (+90 266) 6121 010, Fax: (+90 266) 6121 294, E-mail: nurhangencer@hotmail.com

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major factor concerning chronic inflammation. Oxidative stress suppresses antioxidant mechanisms and consists of reactive oxygen species (ROS) which initiates airway inflammation. It is considered as a significant component in asthma pathogenesis.[5,6] PON1 is known as a high-density lipopprotein HDL-associated antioxidant enzyme and inhibits the oxidative modification of low-density lipoprotein LDL.[7] PON1 activity can vary by more than 10 folds in the population.[8] The hydrolytic efficiencies of PON1 with many substrates are strongly modulated by a single nucleotide polymorphism in the PON1 gene at position 192. PON1 contains two common isoenzymes, one of which is Q isoenzyme which has glutamin at position: 192 or the other is R isoenzyme which has arginine at the same position.[8] PON1 is a critical anti-oxidant enzyme to combat against oxidative stress and it has been involved in the pathogenesis of many diseases including cardiovascular diseases,[9] systemic lupus erythematosus,[10,11] psoriasis[12] and several other diseases. In this study, we investigated PON1 activity and the distribution of PON1 phenotype in asthmatic and healthy subjects. Our aim was to investigate the relationship between PON1 phenotype and asthma.

MATERIALS AND METHODS

Study Groups

Forty nine asthmatic subjects, (mean age 46.1±11 years, in the age range of 22-65 years) attending the Department of Pulmonology, Faculty of Medicine at Balikesir University for at least one year were included in this study. Physical examination, chest graphs, routine blood tests and respiratory function tests were performed for patients. Diagnosis and classification of asthma were determined using the criteria as expressed in the Global Initiative for asthma guidelines (GINA).[13] The patients with partial asthma or completely controlled asthma were included in the study. According to GINA description, the patients with uncontrolled asthma were excluded from the study. Asthmatic patients were not receiving any controller medication and did not show any symptoms concerning lower or upper respiratory tract infection or asthma exacerbation within the previous 4 weeks prior to study. The control group consisted of 41 healthy individuals (mean age 45.04±5 years, in the age range of 28-58 years) and none of them reported any past clinical problems associated with inflammation such as asthma or had any family history of asthma. Healthy individuals were chosen from those referred to a pulmonology clinic at Balikesir University Hospital, undergoing routine blood tests.

Exclusion criteria included the presence of concomitant inflammatory disease such as autoimmune disorders and infections, neoplastic diseases, liver and kidney diseases, familial hypercholesterolemia and heart disease. Patients who smoked were also excluded. This study was approved by the Institutional Ethics Committee of Celal Bayar University of Medical Sciences and was in accordance with the principles of Declaration of Helsinki and all subjects provided written informed consent.

Blood Samples

Blood samples were drawn after overnight fasting, and the sera following centrifugation (10 min at 3.000 g) were preserved in aliquots at -80˚C until analysed.

Determination of Lipid Parameters

Total cholesterol, HDL, LDL and triglyceride levels were determined by standard biochemical procedures using a COBAS Integra 800 automatic analyser (Roche, Switzerland).

Determination of *Paraoxonase* Activity

Paraoxonase activity was determined by measuring the increase in absorbance at 412 nm (formation of 4-nitrophenol) using paraoxon (O,O diethyl-O-p-nitrophenyl phosphate, Sigma) as a substrate.[14,15] The enzyme activity was calculated by using the molar extinction coefficient of 17,100 $M^{-1}cm^{-1}$ and one unit (U) of *paraoxonase* activity was defined as 1 nmoL of 4-nitrophenol formed per minute.

Determination of *Arylesterase* Activity

Arylesterase activity was determined by measuring the increase in absorbance at 270 by using phenylacetate as a substrate. The reaction was started by adding the serum[15,16] and the enzyme activity was calculated by using a molar extinction coefficient of 1310 M^{-1} cm^{-1} and one unit (U) of *arylesterase* activity was defined as 1 μmoL of phenylacetate hydrolyzed per minute.

PON1 phenotype distribution

The phenotype distribution of PON1 was determined by the dual-substrate method.[17] The

genetic polymorphism at codon 192Q→R is responsible for the presence of two isotypes: Q (low activity) and R (high activity). The ratio of the hydrolysis of paraoxon in the presence of 1 M NaCl (salt-stimulated PON1 activity) to the hydrolysis of phenylacetate was used to assign individuals to one of the three possible (QQ, QR, RR) phenotypes. Cut-off values between phenotypes were as follows: type QQ, ratio <3.0; type QR, ratio 3.0–7.0; and type RR, ratio >7.0. QQ represents low, QR intermediate and RR high enzyme activities.

Statistical Analysis

The results are presented as mean±standard deviation (SD) or median and percentage with 95% CI. The significance of differences between variables of the patient and control groups was determined by the student's t-test and the correlation analyses were performed by using Pearson's correlation test. Kruskall-Wallis Test, a non-parametric test was used to analyse QR subgroups due to abnormal distributed values of groups and an adjusted Mann-Whitney U test with Bonferroni method was performed as a post-hoc test. The statistical significances of differences in the phenotype frequency between the groups were tested using the chi-square (χ^2) test. $P<0.05$ was considered to be statistically significant. SPSS for Windows computing program (version 20.0) was used for all correlation analyses.

RESULTS

Forty-nine patients (mean age 46.1±11 years) and 41 controls were included (mean age 45.04 ±5 years) in the study. The clinical, functional and laboratory features of the subjects are reported in Table 1. The mean age and gender distribution were not significantly different in the patients and the control group. Ten (%20) patients had an accompanying disease (hypertension (n=7) and diabetes mellitus (n=3). There were not meaningful differences in the groups of patients and controls, regarding comorbid diseases. The mean forced expiratory volume in 1 second (FEV1) and FEV1/forced vital capacity (FVC) in the patients were lower than the control subjects ($p<0.001$).

The HDL concentrations of the asthmatic patients were lower than the control group whereas LDL, total cholesterol and triglyceride levels were higher in the asthmatic group when compared to the control group ($p<0.05$). The PON1 level in the group with asthma was lower than in the control group (164.1 ± 114 vs 237.4 ± 174 U ml^{-1}) (p=0.024, 95% CI: 9.9 to 136.6). In addition, the *arylesterase* level in the group with asthma was lower than in the control group as well (25.6 ± 18.1 vs 37.02 ± 45), however, this difference was not statistically significant (p= 0.110, 95% CI: -25.5 to 2.6). No significant correlation was detected between PON1 activity and severity of asthma.

Table 1. Clinical, functional and biochemical parameters of subjects

Topics	Control (n = 41)	Asthma (n= 49)	*P value*
Sex (M/F)	34/7	37/12	0.56
Age (yr)	45.04 ±5	46.1±11	0.39
Comorbidity			
Hypertension, n(%)	5 (12.1)	7 (14.2)	0.33
Diabetes mellitus, n(%)	2 (4.8)	3 (6.1)	0.21
FEV_1 (%)	76.9 ± 19	90 ± 8.1	<0.001
FVC (%)	88.6 ± 16.2	92.6 ± 12.6	0.13
FEV_1/FVC	72 ± 11.2	89.3 ± 9.3	<0.001
Triglyceride (mg/dl)	109.8 ± 46.3	167 ± 80.1	<0.001
Cholesterol (mg/dl)	175.7 ± 36	205.8 ± 54.2	0.003
HDL-C (mg/dl)	54.6 ± 12.4	48.6 ± 9.8	0.013
LDL-C (mg/dl)	97.8 ± 30.7	124.6 ± 47.7	0.003
PON1 activity (U ml^{-1})	237.4 ± 174	164.1 ± 114	0.024
ARE activity (U ml^{-1})	37.02 ± 45	25.6 ± 18.1	0.17

NS: Not significant, ARE: Arylesterase, FEV_1: Forced exspiratory volume in 1 second, FVC: Forced vital capacity

Table 2. PON1 phenotypes in asthma and control groups

Phenotypes	Control		Asthma	
	n	% (95% CI)	n	% (95% CI)
QQ	14	34.1 (21.1- 49.4)	13	26.5 (15.8-40.0)
QR	15	36.5 (23.2-51.8)	8	16.3 (8.0-28.5)
RR	12	29.2 (17.1- 44.3)	28	57.1 (43.2-70.3)*

*: RR phenotypes were higher in asthmatic patients when compared to the control group. P= 0.019 (χ^2= 7.9, df=2)

Table 3. Association between lipid profile and PON1 phenotype of the subjects

Variables	QQ	QR	RR	KW	*P* value
Cholesterol (mg/dl)					
asthma	188.2 ± 83.3	195.5 ± 30.3	217 ± 39.7[a]	7.99	0.018
control	173.3 ± 42.2	176 ± 31.1	172.6 ± 31.1	0.31	0.854
HDL-C (mg/dl)					
asthma	50 ± 12.4	46.3 ± 5.3	48.7 ± 9.7	0.54	0.76
control	53.6 ± 10.8	51.9 ± 11.8	59 ± 14.5	2.66	0.265
LDL-C (mg/dl)					
asthma	115 ± 77.4	119.4 ± 32.6	130.6 ± 32	4.34	0.114
control	98.7 ± 33.7	100.1 ± 32.3	94 ±26.9	0.51	0.776
Triglyceride (mg/dl)					
asthma	117.7 ± 83.4	167.7 ± 60.5	189.9 ± 75.3[a]	8.95	0.011
control	97.2 ± 39.8	123.1 ± 52.6	107.8 ± 44.5	3.10	0.212

[A]:Significant differences in PON1 RR as compared to QQ (p<0.05) (Bonferroni adjusted Mann-Whitney Test) KW: Kruskal-Wallis

Table 4. Correlation between PON1 activity and lipid profile

Variables	PON1 activity	
	r	*p*
Cholesterol	-0.137	0.19
HDL	0.136	0.20
LDL	0.143	0.18
Triglyceride	0.213	0.04*

* The PON1 activity had significant negative correlation with triglyceride.

(R) was used to assign a phenotype to each participant: Homozygous QQ (R<3), heterozygous QR, (3<R<7) or
homozygous RR (R>7). There was a significant difference in the phenotype frequency between asthma and control group. In the asthmatic group, the RR phenotype was increased when compared to the control group (57% (95% CI: 43.2-70.3) versus 29% (95% CI: 17.1- 44.3), p=0.019) (Table 2). This might indicate to the increased risk of asthma associated with this phenotype.

Association between PON1 Phenotype and Lipid Levels

The levels of cholesterol and triglyceride were significantly increased in PON1 RR as compared to QQ (p=0.018 vs p=0.011) whilst the levels of HDL and LDL did not show any differences between the groups (Table 3).

Association between PON1 Activity and Lipid Profile

PON1 activity had minimal significant negative correlation with triglyceride levels, however, it had no correlation with cholesterol, HDL and LDL levels (Table 4).

DISCUSSION

The recent studies have shown that production of free radicals in large amounts or the deficiency in antioxidant defence system lead to oxidative stress in asthma.[4-6] ROS has important implication concerning pathophysiological changes such as increased lipid peroxidation, airway responsiveness, production of chemo-attractants and

vascular permeability. PON1, an antioxidant enzyme, is thought to be contributed to pathogenesis of asthma and many diseases. In the previous studies, PON1 activity has been investigated in asthmatic Turkish patients;[18,19] however, PON1 phenotype was not determined. It was shown that PON1 activity reduced in both children and adults with stabile asthma. In a study, it was reported that PON1 activity reduced in asthmatic children during exacerbation.[20] In another study, it was seen that PON1 and arylesterase activity increased which is related to recovering from asthma symptoms.[21] In our study, we only included patients with stable asthma and observed that the PON1 enzyme activity in this group was lower than the control group. This result suggests that respiratory inflammation is persistent even though the disease is in stable state. The mechanism relating the reduction of serum PON1 activity is not clearly known. This reduction can be related to increased lipid peroxidation. It is caused by ROS produced by inflammatory and epithelial cells as oxidized lipids are suggested to block PON1 activity.[22]

There are a few studies concerning relationship between PON1 genotype and asthma whereas there are no studies about PON1 phenotype and asthma. Polonikov et al,[23] have shown that PON1 QR gene polymorphism was significantly associated with risk of asthma. In contrast, Tölgyesi et al [21] found that PON1 polymorphisms did not influence the susceptibility to asthma. We found that RR phenotypes were more common in asthmatic patients when compared to the control group. In order to determine whether RR phenotype poses susceptibility to the disease, more work has to be done in different ethnic groups.

We have also shown that PON1 activity had a significantly negative correlation with triglyceride; however, the correlations were minimal. These results are in agreement with Mohamed et al.[24] The previous studies which were performed to determine the association of PON1 polymorphism with lipid levels, have revealed conflicting results. Few studies have demonstrated that the levels of total cholesterol, triglyceride and LDL were significantly increased in PON1 RR when compared to QQ phenotypes.[25] Similarly, the significant differences were found concerning the cholesterol and triglyceride levels in RR compared to QQ in our study. In contrast, some studies have shown that there is no correlation between PON1 polymorphisms and plasma lipoproteins.[26-28] These contradictory results could partly be explained by intrinsic differences in serum PON1 activities between populations. The factors such as people's life styles, diet, and nutrition could affect blood lipids, and the different results may be associated with these factors. Subject participants in our study were relatively young and had not additional chronic diseases. In our study, some diseases such as cancer, liver dysfunction and kidney failure were excluded. Due to this reason, no differences were observed concerning additional disorders such as chronic diseases concerning PON1 activity and phenotype distribution. However, asthma group indeed had higher cholesterol and LDL. Although the groups had no known chronic diseases they may still have undiagnosed disease.

PON1 polymorphisms have also been investigated in the other diseases such as coronary artery disease[9], systemic lupus erythematosus[10,11] and psoriasis.[12]

There are a few studies in the literature concerning the relationships between PON1 phenotype and genotype. Jarvik et al[29] have shown that PON1 phenotype is a better predictor of vascular disease than PON1192 or PON155 genotypes. Vincent-Viry et al,[30] determined 7.2% phenotype and genotype incompatibility concerning PON192 gene in 918 healthy subjects. Teiber [31] et al, have used the same techniques which we had used in this study. They reported that the phenotypes agreed strongly with the PCR-determined genotypes with disagreement on 2.0%. Another study reported that the measurements of serum hydrolytic activity levels of Q and R isoenzymes more strongly implicated with disorders than the Q192R gene polymorphism alone.[32]

To our knowledge, the current study is the first one to investigate the relationship between asthma and PON1 phenotype. Our observations suggested that the reduced PON1 activity may be involved in the pathogenesis of asthma. The polymorphisms appear to influence the antioxidant capacity of PON1 that may change the susceptibility of this disease. In this field, more studies have to be done in different ethnic groups with more patients.

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