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Serum Paraoxonase Activity and Phenotype Distribution in Turkish Covid-19 Patients

Halil İsa Celik[1], Sahabettin Selek[2], Ertan Sonmez[3], Huseyin Metin[3], Bahadir Taslidere[2], Ufuk Sarıkaya[2], İsmail Yurtsever[4], Gülay Okay[5], Mehmet Doymaz[6], Bedia Gulen[1]

[1]Department of Emergency Medicine, Faculty of Medicine, Medipol University, Istanbul, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[3]Department of Emergency Medicine, Faculty of Medicine, Dumlupınar University, Kutahya, Turkey
[4]Department of Radiology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[5]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[6]Department of Medical Microbiology, Faculty of Medicine, Bezmialem Vakif University, 34093 Istanbul, Turkey

Abstract

Background: For the phenotype classification, it is important to determine the relationship between enzyme activity and the severity of the COVID-19 disease. Reaching significant differences between healthy and infected individuals in terms of genotype and allele distributions may be a guide in the fight against the COVID-19 pandemic. This study, it was aimed to investigate the relationship between serum arylesterase PON1 enzyme activity and disease severity in COVID-19 patients.

Materials and Methods: Patients over the age of 18 who applied to the Emergency Service between 01-30 April 2020 and were examined with a preliminary diagnosis of COVID-19 were included in the study. In the study, serum PON1 activity was measured in the venous blood of 56 patients diagnosed with Covid-19 disease by either CT or RT-PCR and who have not received any systemic treatment yet.

Results: The Arylesterase (AREase) and Paraoxonase (POase) activity levels of the study and control groups were 131.49 ± 52.75 kU/L 142.29 ± 38.82 kU/L, 276.48 ± 220.4 U/L 505.30 ± 301.4 U/L, respectively. It was found that 64.3 % of those infected with Covid-19 had the low-activity PON1 phenotype (p= 0.007)

Conclusion: Genetic variability in PON1 may be associated with exposure to or risk of developing the disease. As a result, vaccination of individuals with low activity phenotype can be given priority at the vaccination stage in order to reduce the mortality rate in the fight against the pandemic. Awareness and protection measures of societies with low activity phenotypes can be increased.

Keywords: Phenotype, COVİD-19, Paraoxonase

Introduction

The World Health Organization (WHO) declared on March 10, 2020 that a new coronavirus caused a global epidemic (1). The clearest known clinical information about the COVID-19 disease is that the disease is transmitted by droplets. Although intense information and warning efforts continue by the authorities to prevent the spread through droplets, asymptomatic cases increase the rate of spread. The average incubation period of SARS-CoV-2 is estimated to be 2-14 days, which is quite long (2). In addition, asymptomatic patients can be contagious during the incubation period, which is the cause of supercontamination. All members of the society are susceptible to SARS-CoV-2. However, the data show that the elderly and individuals with comorbidities or those receiving immunosuppressive therapy have the disease more severely (3). There is no gender difference in the incidence of COVID-19, but it is stated that the mortality risk is 2 times higher in men depending on their chromosomal differences and lifestyle (4). Although it has been reported that the causative factors in the course of the disease are age, gender and comorbid diseases, the relationship between phenotypic features and COVID-19 should be investigated. Serum paraoxonase (PON1) is a calcium-dependent antioxidant enzyme associated with high density lipoprotein (HDL), found in the bloodstream, and synthesized in the liver (5). Studies have shown that the lipid peroxidation effect of low density lipoprotein (LDL) is neutralized by the antioxidant activity of HDL-dependent PON1. Therefore, it can be said that PON1 is a protective factor against atherosclerosis (6). It has been shown in different studies that some viruses and bacteria may be effective in the inflammatory mechanism of atherosclerosis (7). Disease courses ranging from ARDS (acute respiratory distress syndrome) to sepsis are observed in the deterioration of the oxidant/antioxidant balance against the host in the pathogenesis of viral, liambacterial, parasitic infective agents. Oxidative stress occurs due to the overproduction of reactive oxygen species (ROS), which causes related cell damage and lipid peroxidation (4-7). Lipid peroxidation is a well-known mechanism of cell membrane in humans, used as an indicator of oxidative stress in cells and tissues. PON1 is known as an antioxidant

Corresponding Author: Bedia Gulen **e-mail:** drbediagulen@yahoo.com
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enzyme because it hydrolyzes lipid peroxides in oxidized lipoproteins. Because of the genetic polymorphism PON1 activity varies between individuals and populations (8). As a result of paraoxone hydrolysis, individuals are included in one of three possible phenotypes. First, QQ (homozygous low activity) low activity phenotype, second genotype QR (heterozygous medium activity) and third type RR (homozygous high activity) showed the highest level of enzyme activity. For the phenotype classification, it is important to determine the relationship between enzyme activity and the severity of the COVID-19 disease. So far, no specific research has been conducted on the relationship between the novel coronavirus and the different phenotype activities of PON1. Reaching significant differences between healthy and infected individuals in terms of genotype and allele distributions may be a guide in the fight against the COVID-19 pandemic. Identifying people at risk, treatment plans, vaccination studies can provide clues about the course of the disease. In this study, it was aimed to investigate the relationship between serum arylesterase PON1 enzyme activity and disease severity in COVID-19 patients.

Materials and Methods

***Study design*:** This prospective cross-sectional study was performed in the Medical Biochemistry Laboratory of Bezmialem Foundation University Hospital between April and September 2020. The study was carried out with the permission no 54022451-050.05.04- obtained from Bezmialem Vakif University Non-Interventional Research Ethics Committee. Inform consent was obtained from each study patient.

***Selection of participant and data collection process*:** Patients over the age of 18 who applied to the Emergency Service of Bezmialem Vakıf University Medical Faculty Hospital between 01-30 April 2020 and were examined with a preliminary diagnosis of COVID-19 were included in the study. As part of the COVID-19 pandemic management, preliminary triage was created in front of the hospital emergency department. In the preliminary triage, possible COVID-19 patients were differentiated from other emergency patients and taken to the isolation area. Potential COVID-19 patients evaluated in an isolated area in the emergency department according to the guide published by WHO and the Scientific Committee of the Ministry of Health of the Republic of Turkey, possible COVID-19 patients were identified by a senior emergency medicine assistant/ emergency medicine specialist. As part of the fight against the pandemic, the patients were recorded in the patient notification data system with no exception. Informed consent was obtained from each of the patients, and laboratory blood tests (hemogram, kidney function tests, liver function tests, troponin), blood gas and computerized thorax tomography (CT) if necessary, were performed. In order to confirm the presence of COVID-19 nasal and throat swab were taken from all patients for the Real Time Polymerase Chain Reaction (RT-PCR) test. According to the isolation rules, patients were promptly referred to the appropriate services. Before administering any medication, at least 2 cc of study blood was taken into a gel-free biochemistry tube from patients with informed consent. The tubes were centrifuged at 3000/min for 10 minutes and the serums were separated and stored in eppendorf tubes at -80 degrees Celsius until the operating time. Clinical data (age, gender, symptoms, comorbidities, laboratory findings, treatments, and outcomes) were collected by two researchers and recorded for statistical analysis in the electronic data system. Clinical results were updated after patient follow-up. During the study period, 56 patients with positive CT findings and RT-PCR test were recruited from COVID-19 outpatient and emergency department admissions (Table 1). The data of the COVID-19 patients included in the study were compared with the control group. The control group consisted of 60 healthy individuals without any known chronic or recurrent disease. Each of them was selected from volunteers who did not show COVID-19 symptoms for at least 14 days during the pandemic process and did not have a history of contact with COVID-19 patient.

Sample collection and measurement of the rate of paraoxonase and arylesterase activities: Paraoxonase and arylesterase activities were measured with the commercially available Rel Assay Diagnostics kits[9] that are equipped with an auto analyzer (AerosetR, AbbottR, Illinois, USA). Measurements to determine the rate of paraoxonase activity were performed in the alternating absence (basal activity) by using the paraoxon substrate. The rate of the paraoxon

Table 1: Demographic, clinical and laboratory findings

Parameters	COVID 19 + (N=56)	CONTROL (N=60)	*p*
Age	49,53 ± 10,93	44,21 ± 14,34	ns
BMI (kg/m^2)	22,3 ± 1,1	21,5 ± 0,9	ns
Glucose (mg/dL)	109,74 ± 9,06 [a]	90,91 ± 4,46 [b]	< 0.01
HbA1c (%)	5,46 ± 0,28 [a]	5,23 ± 0,24 [b]	< 0.05
Insulin (µIU/mL)	5,82 ± 2,47 [b]	6,28 ± 3,04 [b]	< 0.05
LDL (mg/dL)	133,82 ± 39,46 [a]	105,61 ± 36,84 [b]	< 0.05
Triglyceride (mg/dL)	128,71 ± 48,64 [b]	120,92 ± 66,62 [b]	< 0.05
HDL (mg/dL)	51,17 ± 11,3 [a]	56,68 ±16,61 [b]	< 0.05
Total Cholesterol (mg/dL)	205,15 ± 53,83 [a]	169,22 ± 42,99 [b]	< 0.05
Paraoxonase activity (U/L)	276,48 ± 220,4 [a]	505,30 ± 301,4 [c]	< 0.01
Arylesterase activity (kU/L)	131,49 ± 52,75 [a]	142,29 ± 38,82 [b]	< 0.05

a, b, c: Within rows, means followed by the same letter are not significantly different according to ($p<0.05$)
$p > 0.05$ shown as ns (not significant).

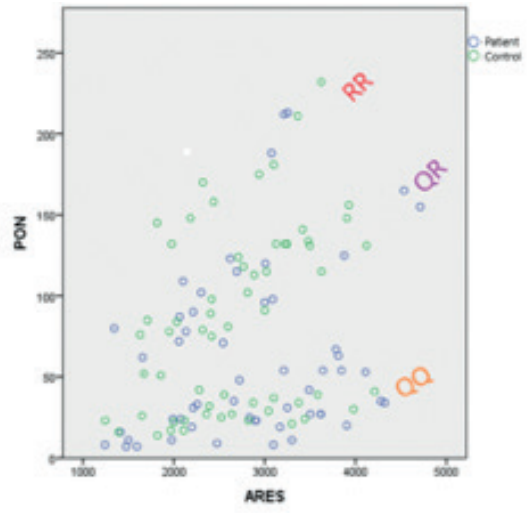

Figure 1: Paroxanase and arylesterase activities of groups. QQ (homozygous low activity), QR (heterozygous medium activity), RR (homozygous high activity).

hydrolysis (diethyl-p-nitrophenyl phosphate) was measured by monitoring the increase in the rate of absorbance at 412 nm. The amount of generated p-nitrophenyl was calculated from the molar absorptivity coeffi cient at a pH value of 8, which was 17.000 M−1 cm−1. The rate of activity was measured briefly at 37 °C by adding 20 µL of the stored serum to 200 µL of the Tris buffer (0.1 M, pH: 8.0), which contained 2 mM of CaCl2 and 7 mM of paraoxon. The rate of paraoxonase activity was expressed as the unit U/L serum. [9-12] Next, in order to measure the rate of the arylesterase activity, phenylacetate was employed as a substrate. The required reaction was initiated by adding the stored serum to the substrate and then reading the increase in the degree of absorbance at 270 nm. The blank readings were included in the evaluations to correct the value obtained for the spontaneous process of phenylacetate hydrolysis. The rate of enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M−1 cm−1. For the evaluations conducted here one unit of the degree of arylesterase activity was defined as 1 µmol of phenol generated/min under the above conditions and expressed by the unit U/L serum. The phenotype distribution of PON1 was determined by calculating ratio of the paraoxonase activity to the degree of arylesterase activity [Figure 1]. The ratio was used to allocate the subjects to one of 3 possible phenotypes (9-12). The subjects were assigned to 1 of the following 3 phenotypes: QQ (homozygous low activity), QR (heterozygous medium activity), or RR (homozygous high activity).

Real-Time Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR): The diagnosis of COVID-19 was made by reverse transcriptase coupled with quantitative polymerase chain reaction assay (RT-qPCR) as described earlier (13). Briefly, the primer probe sets for the RNA-dependent RNA polymerase gene of Wuhan strain of SARS-CoV-2 was utilized in the assay as published by World Health Organization (World Health Organization 2020, Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases: Interim Guidance. World Health Organization, Geneva, Switzerland). A commercial test kit (Bio-Speedy SARS-COV2-2019-nCoV-qPCR Detection Kit; Bioeksen R&D Technologies, Istanbul, Turkey) for RT-qPCR was used for the testing and the tests were performed at Clinical Microbiology Laboratories of Bezmialem Vakif University. The assay was originally validated by the Public Health Central Laboratories of Turkish Health Ministry.

Computed Tomography Protocol: Depending on the severity of the disease, SARS-COV-2 causes interstitial damage in the lung and then pathologies at different levels in the parenchyma. The literature also strongly supports the use of CT in the initial diagnosis and follow-up of the disease [7]. CT with 98% accuracy was also used in COVID-19 patients with false negative RT-PCR results (14). All CT examinations were performed using a multi-detector CT scanner with 64 channels (Somatom Definition, Siemens Healthineers, Erlangen, Germany). The detailed parameters for CT acquisition were as follows: tube voltage, 120 kVp; tube current, standard (reference mAs, 60–300) with automatic exposure control; slice thickness, 1.0 mm; reconstruction interval, 1.0–3.0 mm; and a sharp reconstruction kernel. CT images were obtained with the patient in the supine position at the end of inhalation and without contrast medium.The CT images were evaluated with both lung (width, 1500 HU; level, −600 HU) and mediastinal (width, 400 HU; level, 40 HU) window settings.

Statistical Analysis: The collected data was expressed as the mean ± standard deviation (X ± SD). The student t test was employed to compare the parameters of both these groups. The chi-square test was used to test the distribution of the phenotype. In addition, the correlations existing between the parameters in both groups were determined through Pearson's correlation analysis. A linear regression analysis was used to determine the exact relationships that existed between the parameters of age and gender among the evaluated subjects; the serum TG, TC, HDL-C, and LDL-C levels; and the degree of PON1 activities. P-values < 0.05 were accepted as significant. The data was analyzed by using a computer program.

Results

Demographic data of the Covid-19 patients and control group participating in our study are given as in (Table 1). The Arylesterase (AREase) and Paraoxonase (POase) activity levels of the study and control groups were 131.49 ± 52.75 kU/L 142.29 ± 38.82 kU/L, 276.48 ± 220.4 U/L 505.30 ±

Table 2: Phenotypes Group Crosstabulation

		Group		
		Patient	Control	Total
UcPhenotype	QQ	36	24	60
	QR	17	27	44
	RR	3	9	12
Total		56	60	116

301.4 U/L, respectively. PON1 phenotype distribution was analyzed in two groups. The ratio obtained was used for the phenotype distributions of those who were infected and those who did not. Accordingly, 64.3 % (n=36) of those with COVID-19 disease had the QQ homozygous low activity phenotype, while this rate was 40% (n= 24) in the control group (Table 2). It was determined that individuals with low activity of the PON1 enzyme were more likely to get Covid-19 disease (Figure 1). By calculating Cramer's Value, a statistically significant difference was found between the groups in terms of all three phenotypes (p=0.023, Table 3-4).

Discussion

Until the day this article was written, approximately 4.5 million patient deaths have been reported worldwide due to Covid-19. The disease progresses at different levels from asymptomatic to fatal. ARDS, which develops with hyperinflammation due to cytokine storm, is the most important cause of mortality. The causative pathogen SARS-CoV-2 causes high oxidative stress, lipid peroxidation, protein oxidation and ultimately cell death (15). As stated in the literature, PON1 activity is a determinant of the oxidative stress and inflammation response of the disease. PON1 phenotype status as determined by activity analysis of two substrates reveals the functional Q192R genotype and activity levels of an individual (16). Serum PON1 activity varies depending on lipids and lipoproteins in plasma (17). PON1 activity has been widely used to determine phenotype distributions in previous studies (18-19). It has been suggested that genetic variability in PON1 may be associated with exposure or risk of developing the disease (20). In this study, serum PON1 activity and phenotype distribution in Covid-19 patients were investigated. We think that the difference in PON1 activity between individuals and societies may also be a factor in the transmission and course of Covid-19 disease. In the study, serum PON1 activity was measured in the venous blood of 56 patients who were diagnosed with Covid-19 disease by either CT or RT-PCR and have not received any systemic treatment yet. Accordingly, it was found that 64.3 % of those infected with Covid-19 had the low-activity PON1 phenotype (p= 0.007). It has been emphasized in the literature that PON1 activity differs according to the individual and race in direct proportion to the antioxidant capacity (21). Advanced age and concomitant diseases are not effective factors on phenotype difference. As a result, vaccination of individuals with low activity phenotype can be given priority at the vaccination stage in order to reduce the mortality rate in the fight against the pandemic. Awareness and protection measures of societies with low activity phenotype can be increased.

Table 3: Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	,255	,023	,024
	Cramer's V	,255	,023	,024
	Contingency Coefficient	,247	,023	,024
Number of Valid Cases		116		

Table 4: Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	7,544[a]	2	,023	,024		
Likelihood Ratio	7,711	2	,021	,024		
Fisher-Freeman-Halton Exact Test	7,409			,024		
Linear-by-Linear Association	7,355[b]	1	,007	,008	,005	,003
N of Valid Cases	116					

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