Research Article

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Effects of *PON1* QR192 genetic polymorphism and paraoxonase, arylesterase activities on deep vein thrombosis

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Abstract

Objectives: We aimed to evaluate *PON1* QR192 polymorphism's (rs662) effects on levels of triglyceride, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, glucose, and c-reactive protein, and paraoxonase-arylesterase activities among deep vein thrombosis patients and healthy subjects.

Methods: Forty-five deep vein thrombosis patients and 45 healthy subjects participated in the study. Genetic analysis was performed by using polymerase chain reaction and sequencing. Paraoxonase and arylesterase enzyme activities were determined by a spectrophotometer. Serum levels of triglyceride, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, glucose, and c-reactive protein were measured by a similar method.

Results: There were no statistically significant differences between patients and controls regarding the frequency of variant allele for the *PON1* QR192 polymorphism, activities of paraoxonase-arylesterase, and level of high-density lipoprotein-cholesterol. Triglyceride, total cholesterol, low-density lipoprotein-cholesterol, glucose, and c-reactive protein levels were significantly higher in patients compared to controls (p values were 0.005, 0.0002, 0.009, 0.0009, <0.0001, respectively.) Paraoxonase activity was found to be associated with *PON1* QR192 genetic polymorphism (p<0.0001). However, we observed no association of *PON1* QR192 polymorphism with arylesterase activity and, levels of triglyceride, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, glucose, and c-reactive protein.

Conclusions: There was no statistically significant difference between deep vein thrombosis patients and healthy subjects regarding variant allele frequency for the *PON1* QR192 genetic polymorphism. In addition, paraoxonase and arylesterase activities were similar among the groups. These results indicate that *PON1* QR192 genetic polymorphism and activity levels of paraoxonase-arylesterase have no effect on the development of deep vein thrombosis.

Keywords: arylesterase; deep vein thrombosis; genetic polymorphisms; paraoxonase.

Introduction

Despite the current advances in the treatment and prevention of deep vein thrombosis (DVT), it still causes a relatively high rate of morbidity and mortality. DVT increases the risk of pulmonary embolism which can be resulted in death. When DVT keeps relapsing and becomes chronic, it can give rise to post-thrombotic syndrome, deterioration in the quality of life, and chronic pulmonary hypertension [1].

Virchow triad was identified in the early 19th century by Rudolf Ludwig Virchow and it continues to maintain its validity until now. Virchow triad includes; venous stasis (slowing of blood circulation), endothelial dysfunction (injury of the vessel wall), and hypercoagulability. These

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factors disrupt the balance between coagulation and fibrinolysis and, increase the risk for thrombosis. The presence of only one of these factors is sufficient enough to increase the risk of thrombosis [2].

One of the Virchow triad components; endothelial dysfunction is among the first symptoms of vascular diseases and it triggers thrombocyte activation [3]. Factors that may lead to endothelial dysfunction compasses anoxia, mechanical stress, free radicals, immune response, endotoxin, serotonin, histamine, and thrombin. Oxidative stress that is caused by risk factors such as hypertension, hypercholesterolemia, diabetes mellitus, and smoking also gives rise to endothelial dysfunction [4, 5]. Excessive production of free radicals tilts the balance between oxidants and anti-oxidants towards the oxidative side and, this results in the accumulation of reactive oxygen species in the cell cytoplasm. This accumulation causes harm to cells which may lead to endothelial dysfunction [6].

Paraoxonase (PON) is a calcium-dependent esterase that catalyzes the hydrolysis of organophosphates and, it has both antioxidant and antiatherogenic effects. Paraoxonase has three types; PON1, PON2, and PON3. PON1 is a lipolactonase that is associated with high-density lipoprotein-cholesterol (HDL-cholesterol) and, it shows an anti-atherosclerotic effect through its activity on HDL-cholesterol in the circulation. It is indicated that PON1 has a vascular endothelial protective effect because it reduces the oxidation of cell membranes and low-density lipoprotein-cholesterol (LDL-cholesterol) [7]. PON1 also inhibits HDL-cholesterol oxidation and has a role in cholesterol movement [8].

A wide range of differences was found in the level and activity of paraoxonase between individuals because of its polymorphic structure [7, 9]. In addition to paraoxonase's association with cardiovascular diseases, different types of diseases were also found to be associated with it [7].

The QR192 polymorphism for the coding *PON1* gene (rs662) has been shown to alter paraoxonase function. It was reported that some substrates like paraoxon are hydrolyzed faster by the R-isoform of the enzyme than the Q-isoform [10]. Because paraoxonase activity was suggested to contribute to reducing the progression of endothelial dysfunction by its antioxidant activity [7]; there may be an association between *PON1* QR192 polymorphism and diseases that progress with endothelial dysfunction such as DVT.

In this study, we aimed evaluate and compare the effects of genetic polymorphism of *PON1* QR192 on paraoxonase and arylesterase activity among DVT patients and healthy subjects. We also aimed to investigate the association between *PON1* QR192 polymorphism and levels of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and c-reactive protein (CRP) in both DVT patients and healthy subjects, and determine the frequency of *PON1* QR192 polymorphism in the Turkish population.

Materials and methods

The research related to human use has complied with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional Review Board or equivalent committee (Yozgat Bozok University Ethics Committee, 2017-KAEK-189_2018.08.08_01). Informed consent was obtained from all individual participants included in the study.

This study was conducted between January 2018 and January 2019. Forty-five deep vein thrombosis patients who were diagnosed in the Outpatient Clinic for Cardiovascular Surgery, Faculty of Medicine, Yozgat Bozok University, and 45 healthy subjects participated in the study. All participants were aged between 18 and 89. Individuals with severe heart, liver, and kidney diseases, a recent history of surgery or trauma, and pregnancy were excluded from the study.

Venous blood was collected into Vacuette tubes (lot number: A22083DT) with silica particles and a serum separating gel (Greiner Bio-One GmbH, Kremsmünster, Austria). Serum samples were separated from collected venous blood by centrifuging for 10 min at 4,000 rpm, +4 °C, and stored at −20 °C until analysis. Nuve NF 1200 R centrifuge was used for serum separation (Nuve, Ankara, Turkey). Serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, and CRP levels were measured by using Abbott Ci8200 (Abbott Laboratories, Illinois, USA) and Roche Hitachi cobas c501 (Roche, Basel, Switzerland) biochemical analyzers with appropriate commercial kits from the same manufacturers. Direct LDL-cholesterol testing was used to measure LDL-cholesterol.

Paraoxonase and arylesterase activities were determined by using a spectrophotometric method with Roche Hitachi cobas c501 analyzer by using kits according to the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Turkey). Paraoxonase activity was determined as the rate of paraoxon hydrolysis at 37 °C. Two separate sequence reactive solutions; first one containing Tris-HCI, $CaCI_2$ and second one containing paraoxon were used for paraoxonase activity measurement. Meanwhile, three sequence reactive solutions; first one containing Tris-HCI, phenylacetate, second one containing $CaCI_2$, and distilled water as the third solution were used for arylesterase activity measurement. Paraoxonase activity was measured at 412 nm (nm), while arylesterase activity was measured at 548 nm.

For DNA extraction, 200 µl venous blood was collected into Vacusel tubes (lot number: 1,508) with ethylenediaminetetraacetic acid (SEL Medical Materials, Konya, Turkey) and MagPurix 12A Nucleic Acid Extraction System (Zinexts, Taipei, Taiwan) was used. Genotyping was performed by polymerase chain reaction (PCR) and subsequent sequencing by the Miseq platform (Illumina, California, USA). A total volume of 25 mL containing 200 mM of each dATP, dCTP, dGTP, dTTP, 2.5 mM $MgCl_2$, BSA, and 12.5 pmol of each primer, one unit of Taq DNA

Polymerase, and 100 ng of genomic DNA was used as a PCR mixture (Thermo Scientific, Massachusetts, USA). The forward primer for the PCR was; 5′-TAT TGT TGC TGT GGG ACC TGA G-3′, and the reverse primer was 5′-CAC GCT AAA CCC AAA TAC ATC TC-3′ [10]. PCR conditions were; 95 °C for 10 min following 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s, and 72 °C for 10 min. PCR cycles were performed by a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Taipei, Taiwan). After the purification of PCR products, sequencing was performed by using the Miseq platform and a kit according to the manufacturer's instructions (Illumina, California, USA).

We used the Power and Sample Size Program software for statistical power calculation [11]. We applied previously reported frequency of the variant R allele for the *PON1* QR192 polymorphism as 28.4 % [12]. The estimated sample sizes were calculated while the type I error probability and the power were set to 0.05 and 0.8, respectively. With a proposed relative risk of 2 for allelic distributions between DVT patients vs. healthy controls and for a study of independent cases and controls with at least one control per case, calculated number for each group was 46 subjects.

Statistical analyses were performed by using GraphPad Prism version 8 (GraphPad Software, California, USA). Descriptive statistics were shown as mean±standard deviation (95 % confidence intervals) for the results of the parametric tests and, median (interquartile $range_{25}$-interquartile $range_{75}$) for the results of the non-parametric tests. Ages were compared by t-test while genders were compared by Chi-square test. In addition, frequencies of alleles and genotypes for the *PON1* QR192 genetic polymorphism between the groups were analyzed by using Chi-square and Fisher's exact tests where applicable. Shapiro-Wilk test was used to determine whether the numerical data was normally distributed or not. t-test, one way ANOVA and post-hoc Tukey's tests were used for the data that normally distributed while, Mann-Whitney U, Kruskal-Wallis and post-hoc Dunn's tests were used for the data that are not normally distributed. Comparisons of activities of paraoxonase-arylesterase and, levels of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP between DVT patients and controls were performed by using t-test or Mann-Whitney U test. One way ANOVA and post-hoc Tukey's tests or Kruskal-Wallis and post-hoc Dunn's tests were used to compare the same biochemical parameters between *PON1* QR192 genotype groups. $p \leq 0.05$ was accepted as statistically significant.

Results

As mean±standard deviation, ages were 58.6 ± 16.8 and 59.6 ± 15.4 for deep vein thrombosis patients and healthy volunteers, respectively. There was no statistically significant difference between the groups about age (p=0.78). Twenty-four of 45 patients (53.3 %) and 21 of 45 controls (46.7 %) were female. Study groups were similar regarding gender as well (p=0.674).

The distribution of the *PON1* QR192 genetic polymorphism was coherent with the Hardy–Weinberg equilibrium (p>0.05). The frequency of the variant R allele for the *PON1* QR192 genetic polymorphism was determined to be 28.9 % in our cohort of the Turkish population.

Distribution rates of QQ, QR, and RR genotypes for the *PON1* QR192 polymorphism were 26 (57.8 %), 13 (28.9 %), and 6 (13.3 %) in the patient group and, 23 (51.1 %), 17 (37.8 %) and 5 (11.1 %) in controls, respectively. In addition, variant R allele frequencies were 27.8 % (n=25) for the patients and, 30 % (n=27) for the controls. There were no statistically significant differences between the groups regarding the comparisons of both genotypes and alleles (p values were 0.668 and 0.742, respectively.) Results were demonstrated in Table 1.

Evaluation of paraoxonase-arylesterase activities and, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP levels between the groups are shown in Table 2. While the levels of paraoxonase-arylesterase activities and HDL-cholesterol were similar among the groups; triglyceride, total cholesterol, LDL-cholesterol, glucose, and CRP levels were significantly higher in the patient group compared to controls (p values were 0.013, 0.0002, 0.027, 0.019, <0.0001, respectively).

Comparison of enzyme activities of paraoxonase and arylesterase between the genotype groups according to *PON1* QR192 polymorphism revealed a significant association between paraoxonase activity and *PON1* QR192. While the QQ genotype carriers had the lowest paraoxonase activity, QR genotype carriers had intermediate and, RR genotype carriers had the highest level of paraoxonase activity (p<0.0001). Post-hoc Tukey's test revealed that carriers of the QQ genotype had significantly lower paraoxonase activity compared to carriers of both QR and RR genotypes (adjusted p<0.0001 for both comparisons). In addition, paraoxonase activity levels of RR genotype carriers were significantly higher than carriers of QR genotype (adjusted p<0.0001). This finding was valid for in-group analysis for both groups as well as when the analysis was performed as two groups combined. Statistical analysis

Table 1: Frequencies of genotypes and alleles for the *PON1* QR192 genetic polymorphism between deep vein thrombosis (DVT) patients and healthy controls.

Genotypes	DVT patients n, %	Controls n, %	χ2, df, p-Value
QQ	26 (57.8)	23 (51.1)	0.808, 2, 0.668
QR	13 (28.9)	17 (37.8)	
RR	6 (13.3)	5 (11.1)	
Alleles			
Q	65 (72.2)	63 (70)	0.108, 1, 0.742
R	25 (27.8)	27 (30)	

Table 2: Analysis of paraoxonase-arylesterase activities and, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP levels between deep vein thrombosis (DVT) patients and healthy controls.

	DVT patients (n=45)	Controls (n=45)	p-Value
	Median (IQR_{25}-IQR_{75}) Mean ± SD (95 % CI)	Median (IQR_{25}-IQR_{75}) Mean ± SD (95 % CI)	
Paraoxonase activity, U/L	303 (214–634)	331 (210–623)	0.96
Paraoxonase activity/HDL-cholesterol	7 (4.5–15.1)	7.7 (4.2–14)	0.61
Arylesterase activity, kU/L	841 ± 221 (775–908)	844 ± 219 (778–910)	0.948
Triglyceride, mg/dL	185 (161–219)	171 (148–184)	**0.013**
Total cholesterol, mg/dL	190 ± 37.5 (179–201)	164 ± 25.3 (156–172)	**0.0002**
HDL-cholesterol, mg/dL	43.7 (37.1–52.7)	48.1 (42.4–53.7)	0.062
LDL-cholesterol, mg/dL	109 (82.6–134)	96.3 (72.1–113)	**0.027**
Glucose, mg/dL	97 (89.1–120)	91.8 (88.5–97.6)	**0.019**
CRP, mg/L	3.5 (2–18)	1.5 (1–2.8)	**<0.0001**

IQR, interquartile range; SD, standard deviation; CI, confidence interval. Results are shown as median (interquartile $range_{25}$-interquartile $range_{75}$) for non-parametric tests (Mann-Whitney U test) and mean ± standard deviation (95 % confidence intervals) for parametric tests (t-test). Statistically significant p values are marked as bold.

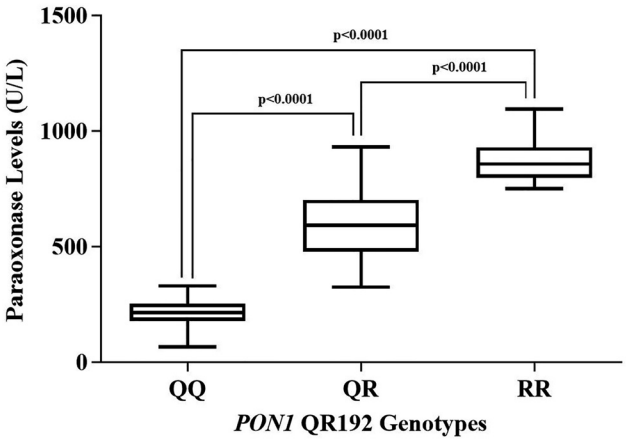


Figure 1: Levels of paraoxonase between the groups according to PON1 QR192 genotypes for all participants. As mean ± standard deviation (95 % confidence intervals), paraoxonase levels were 217 ± 64.6 (199–236), 587 ± 150 (531–643), and 874 ± 100 (806–941) U/L for the QQ, QR, and RR genotype carriers, respectively (p<0.0001). Post-hoc Tukey's test showed that carriers of the QQ genotype had significantly lower paraoxonase levels compared to carriers of both QR and RR genotypes and, RR genotype carriers had significantly higher paraoxonase levels than QR genotype carriers (adjusted p<0.0001 for all comparisons).

yielded similar results for comparison of paraoxonase activity/HDL-cholesterol values between the groups as well. No association of *PON1* QR192 polymorphism with arylesterase activity was determined. Results are shown in Figure 1 and Table 3.

On the other hand, serum levels of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP were not significantly different between the *PON1* QR192 genotype groups in both patients and controls. Results are shown in Table 3.

For the comparison of paraoxonase activity levels between *PON1* QR192 genotype groups, statistical power was calculated as 0.9. Likewise, the estimation of statistical powers for the evaluation of triglyceride, total cholesterol, LDL-cholesterol, glucose, and CRP levels between DVT patients and healthy controls yielded results bigger than 0.8.

Discussion

Our study showed that there were differences between DVT patients and healthy controls regarding levels of triglyceride, total cholesterol, LDL-cholesterol, glucose, and CRP. In addition, we determined an association between *PON1* QR192 polymorphism and the level of paraoxonase activity. In contrast, no associations of *PON1* QR192 polymorphism with DVT, arylesterase activity, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP were found.

It is indicated that dyslipidemias decrease blood viscosity, activate thrombocytes, and increase the expression of tissue factor that triggers the coagulation system. Therefore, high levels of cholesterol and triglyceride lead to a higher risk for not just arterial thrombosis but vein thrombosis as well [13]. A Japanese study conducted by Kawasaki et al. concluded that high levels of total cholesterol and triglyceride increased the risk for DVT [14]. In addition, a meta-analysis study performed by Ageno et al. reported that DVT patients had higher levels of triglyceride and lower levels of HDL-cholesterol compared to healthy controls [15]. Our study found similar results regarding levels of triglyceride and total cholesterol among deep vein thrombosis patients and healthy individuals. Contrary to the findings of the mentioned previous studies, we found no difference between DVT patients and healthy controls regarding the levels of HDL-cholesterol.

Regarding the relationship between LDL-cholesterol levels and risk for DVT, there are conflicting results in the literature. A recent Serbian study conducted by Spasic et al. reported that high serum LDL-cholesterol levels doubles the DVT risk [16]. In contrast, Morelli et al. found no

Table 3: Comparison of paraoxonase-arylesterase activities and, serum levels of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP according to *PON1* QR192 genotype groups.

	DVT patients			p-Values
	***PON1* QR192 genotypes**			
	QQ	**QR**	**RR**	
Paraoxonase activity, U/L	224 ± 65.2 (198–250)	596 ± 163 (498–694)	871 ± 126 (739–1,004)	**<0.0001**
Paraoxonase activity/HDL-cholesterol	5 (3.1–5.6)	13.6 (10.9–21.3)	22.5 (17.3–42.5)	**<0.0001**
Arylesterase activity, kU/L	817 ± 230 (724–910)	899 ± 233 (758–1,040)	822 ± 146 (668–975)	0.542
Triglyceride, mg/dL	118 (85.3–171)	156 (115–359)	151 (94.5–231)	0.189
Total cholesterol, mg/dL	191 ± 39.8 (175–207)	191 ± 38 (168–214)	182 ± 29.8 (151–213)	0.863
HDL-cholesterol, mg/dL	47.3 (38.2–61.5)	43.5 (28.2–54)	39.9 (28.2–44.4)	0.161
LDL-cholesterol, mg/dL	114 ± 37.1 (98.6–129)	101 ± 29.9 (82.6–119)	113 ± 32.7 (78.6–147)	0.535
Glucose, mg/dL	98.5 (88.7–141)	97 (91.4–105)	95.8 (87.3–114)	0.81
CRP, mg/L	3.6 (2–25.7)	3.5 (1.6–13.9)	6.2 (1.8–31.1)	0.707
	Healthy controls			**p-Values**
	***PON1* QR192 genotypes**			
	QQ	**QR**	**RR**	
Paraoxonase activity, U/L	210 ± 64.6 (182–238)	581 ± 145 (506–655)	876 ± 71.3 (788–965)	**<0.0001**
Paraoxonase activity/HDL-cholesterol	4.3 ± 1.4 (3.8–4.9)	12.3 ± 3.4 (10.6–14)	17.6 ± 1.3 (15.9–19.2)	**<0.0001**
Arylesterase activity, kU/L	806 ± 236 (703–908)	885 ± 223 (770–1,000)	883 ± 56.3 (813–953)	0.492
Triglyceride, mg/dL	102 (76.2–143)	107 (76.9–163)	92.5 (85.4–133)	0.892
Total cholesterol, mg/dL	158 ± 28.2 (146–170)	169 ± 23.2 (157–181)	174 ± 8.9 (163–185)	0.273
HDL-cholesterol, mg/dL	48.8 ± 7.3 (45.7–51.9)	47.8 ± 6.8 (44.3–51.3)	50.3 ± 8 (40.4–60.2)	0.774
LDL-cholesterol, mg/dL	87.6 ± 23.5 (77.5–97.8)	98.1 ± 24.4 (85.5–111)	102 ± 5.4 (95.7–109)	0.239
Glucose, mg/dL	94.1 ± 9.2 (90.1–98.1)	91.9 ± 6 (88.8–95)	90.5 ± 2 (88.1–93)	0.519
CRP, mg/L	1.7 (1–3.1)	1.4 (1–2.4)	1.8 (1.3–2.9)	0.438
	All participants (patients and controls combined.)			**p-Values**
	***PON1* QR192 genotypes**			
	QQ	**QR**	**RR**	
Paraoxonase activity, U/L	217 ± 64.6 (199–236)	587 ± 150 (531–643)	874 ± 100 (806–941)	**<0.0001**
Paraoxonase activity/HDL-cholesterol	4.5 (3.4–5.5)	12.4 (9.9–16.3)	17.9 (17.6–23)	**<0.0001**
Arylesterase activity, kU/L	811 ± 231 (745–878)	891 ± 224 (808–975)	849 ± 114 (773–926)	0.292
Triglyceride, mg/dL	116 (83.2–145)	134 (92.8–175)	103 (90–185)	0.38
Total cholesterol, mg/dL	175 (149–194)	179 (156–193)	173 (164–188)	0.91
HDL-cholesterol, mg/dL	48.2 (40.3–54.5)	43.9 (39.6–53.8)	45.8 (39.4–48.6)	0.39
LDL-cholesterol, mg/dL	98.2 (75.3–122)	98.9 (78.1–117)	103 (95.3–111)	0.784
Glucose, mg/dL	96 (88.6–108)	93.6 (89–99.2)	91.8 (89.4–96)	0.464
CRP, mg/L	2.3 (1.5–5.9)	1.8 (1–3.6)	2.1 (1.6–9)	0.23

Results are shown as median (interquartile range$_{25}$-interquartile range$_{75}$) for non-parametric tests (Kruskal-Wallis test) and mean ± standard deviation (95 % confidence intervals) for parametric tests (one way ANOVA). Statistically significant p values are marked as bold.

association of high LDL-cholesterol levels with predisposition to DVT [17]. Similar to the results of the study by Spasic et al., our findings showed an association of high levels of serum LDL-cholesterol with the risk of deep vein thrombosis.

Studies that investigated the paraoxonase activity levels in healthy individuals from various regions of Turkey reported a rate of activity (mean±standard deviation) between 77 ± 36.8 and 429.9 ± 82.7 U/L [18, 19]. We found the rate of paraoxonase activity in healthy controls as 424 ± 259 U/L, which is in the previously reported range. Paraoxonase activity rate was also similar in DVT patients; 418 ± 267 U/L. In addition, the arylesterase activity rate was determined to be 842.5 ± 220 kU/L in all participants at this study.

It has been reported that paraoxonase enzyme activity level differs widely among individuals according to diet, alcohol intake, smoking habits, and genetic polymorphisms

[20]. Further, oxidative stress was indicated as the primary factor that reduces paraoxonase activity because it increases the peroxidation of lipoproteins which includes HDL-cholesterol and, 95 % of paraoxonase in the circulation is attached to HDL-cholesterol. Thus, a reduction in HDL-cholesterol concentration leads to lower expression of paraoxonase [21]. On the other hand, paraoxonase contributes to the antiatherogenic effect of HDL-cholesterol [22]. Also, some studies pointed out that HDL-cholesterol may have an antiplatelet effect [23]. Aykal et al. performed a study that investigated the effects of paraoxonase activity, arylesterase activity, total oxidant status, total antioxidant status, oxidative stress index, total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol on venous thromboembolism. The authors demonstrated that venous thromboembolism patients had lower levels of paraoxonase-arylesterase activities, serum HDL-cholesterol, and higher levels of serum LDL-cholesterol compared to controls [24]. In addition to venous thromboembolism, a reduction in paraoxonase activity was also shown in patients with coronary artery diseases, diabetes mellitus, hypothyroidism, or colorectal cancer [25–28]. One of the mentioned studies indicated that low levels of paraoxonase activity lead to HDL-cholesterol dysfunction which contributes to the development of coronary artery diseases [26] and, the study that was conducted on colorectal cancer patients also found an association between colorectal cancer and reduction in arylesterase activity [28]. The authors concluded that insufficient antioxidant activity, which includes paraoxonase and arylesterase, contributes to the development of colorectal cancer by causing the progression of endothelial dysfunction [28]. In contrast, a thesis study reported no alteration of paraoxonase activity between patients who suffered from ischemic or hemorrhagic stroke and healthy individuals. However, the same study found an association between low levels of arylesterase activity and stroke [19]. As can be seen from the results of the aforementioned studies, paraoxonase and arylesterase affecting thromboembolic disorders were suggested, but not yet clearly defined. Our study demonstrated no significant difference between DVT patients and healthy individuals regarding activity levels of paraoxonase and arylesterase.

One of the reasons that paraoxonase enzyme rate differs among individuals and ethnic groups is genetic polymorphisms. There is a glutamine amino acid at the 192nd position of the paraoxonase enzyme in the Q allele for the *PON1* QR192 genetic polymorphism while, arginine amino acid exists in the R allele at the same position. It was shown that carriers of the QQ genotype have the lowest, carriers of the QR genotype have intermediate and, RR genotype carriers have the highest paraoxonase activity levels [10]. A study by Fridman et al. indicated that *PON1* QR192 variants and subsequent changes in paraoxonase activity level are associated with higher risk for cardiovascular diseases [29]. Furthermore, a thesis study that investigated the blood flow in coronary arteries by Akgun et al. demonstrated that coronary artery disease patients with reduced coronary blood flow had a higher distribution rate of the Q allele compared to controls [30]. On the contrary, *PON1* QR192 polymorphism was not found to be associated with another type of thromboembolic disorder; pulmonary embolism [31]. On the other hand, a study by Ergun et al. identified the QQ genotype for the *PON1* QR192 polymorphism as a risk factor for type 2 diabetes mellitus [32]. However, a thesis study found no significant differences between metabolic syndrome patients and healthy individuals regarding frequencies of *PON1* QR192 genotypes and alleles despite demonstrating different rates of paraoxonase activity between groups [33]. Another thesis study by Onderci et al. investigated the association between *PON1* QR192 polymorphism and type two diabetes. The investigators also examined the effects of *PON1* Q1R92 on serum triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels among type two diabetes patients and healthy controls in the same study. They demonstrated that frequencies of *PON1* QR192 genotypes and alleles were similar between type two diabetes patients and healthy individuals. In addition, levels of triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol were found not to be related to *PON1* QR192 polymorphism. However, statistically significant differences in levels of triglyceride, total cholesterol, and LDL-cholesterol among type two diabetes mellitus patients and controls were discovered [34]. Coherent with the results of the previous studies, our study demonstrated alteration in paraoxonase activity according to *PON1* QR192 genotypes. Because, the Q allele for the *PON1* QR192 polymorphism results in lower activity of paraoxonase and, paraoxonase has an antioxidant effect that was suggested to contribute to reduce the progress of endothelial dysfunction; we hypothesize that *PON1* QR192 polymorphism may be associated with DVT, which endothelial dysfunction plays a role in its development. But, our finding of no association between *PON1* QR192 polymorphism and DVT did not support our hypothesis. In addition, arylesterase activity was not determined to be associated with *PON1* QR192 polymorphism. This result was expected because it was indicated that *PON1* QR192 polymorphism has minimal effect on arylesterase activity rate [35].

There were some limitations in our study. Sample size of the study population can be considered as relatively insufficient to reach adequate statistical power level for the analysis of *PON1* QR192 genetic polymorphism.

In conclusion, we found associations of DVT with high serum levels of triglyceride, total cholesterol, LDL-cholesterol, glucose, and CRP. Further, we observed significantly different paraoxonase activity levels between the groups according to *PON1* QR192 polymorphism regardless of having DVT. In contrast, our study showed no association of *PON1* QR192 polymorphism with DVT and, levels of arylesterase activity, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, and CRP. Considering these results, *PON1* QR192 genetic polymorphism and activity levels of paraoxonase-arylesterase may not affect the development of DVT. To our knowledge, the present study is one of the first studies that investigated the relationship between DVT and *PON1* Q192R genetic polymorphism in the literature.

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