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# The Impact of Different rs3917538 Genotypes with the PON-1 Activity, Atherosclerosis Severity in Patients of Coronary Atherosclerosis

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## Abstract

**BACKGROUND:** Coronary atherosclerosis is one of the coronary artery diseases (CAD) responsible for a significant percentage of mortality and morbidity in Iraqi subjects. Atherosclerosis compresses about 17 million death cases; 45% are under the age of 70. The improvement in atherosclerosis disease management plays a critical role in preventing this disease; despite that, new risk factors have been studied, including genetic studies. Many polymorphisms have been associated with coronary atherosclerosis in different ethnic populations. Paraoxonase is an enzyme located on HDL in serum. PON acts as a protective factor or agent against oxidative modification of LDL, suggesting that it could play an essential and influential role in atherosclerotic processes prevention.

**AIM:** The aim of the study was to evaluate the rs3917538 genotypes in the PON-1 gene associated with the coronary atherosclerosis risk and the effect of this single-nucleotide polymorphism on the serum activity of PON-1 and the severity of coronary atherosclerosis.

**METHODS:** A case–control study included 60 patients diagnosed with coronary atherosclerosis and 60 healthy volunteers diagnosed with angiography examination. The study was carried out during the period from February 2019 to June 2020. Serum PON-1 was measured by fluorometric technique and the genetic assessment was by RT-PCR technique.

**RESULTS:** The activity for PON-1 in patients was significantly lower than controls; the median activity of PON-1 in controls was 197.32 mU/ml, which was higher than that of patients (median = 163.46 mU/ml). There was an impact of rs3917538 genotypes on the severity of the disease, P = 0.041. The rs3917538 genotypes have no association with the risk of coronary atherosclerosis and the activity of PON-1.

**CONCLUSION:** The measurement of serum PON-1 activity may help in the diagnosis of coronary atherosclerosis patients. The rs3917538 genotypes in the PON-1 gene among the patient's group impact the severity of coronary atherosclerosis.

## Introduction

Coronary atherosclerosis is a chronic inflammatory state and is the primary cause of coronary artery diseases (CAD) [1]. The atherosclerotic process is a series of stages from the intima-media thickening to fatty streak intermediates lesions and fibrous plaque and finally to the complicated lesion [2]. The PON-1 gene regulates the synthesis of the PON enzyme in serum. PON may protect low-density lipoprotein (LDL) from lipid peroxidation and inhibit the formation of foam cells and accumulation of fatty streaks, suggesting that the PON-1 may play a key role in preventing atherosclerotic processes [3], [4]. Several studies and reports have suggested that PON-1 is associated with oxidative stress [5]. Genetic polymorphism is a marker of biological diversity and genotyping variation, which correlate with a specific phenotype and sometimes impact a human disease development among different ethnic groups [4]. PON-1 levels and activities are essential in protecting against cardiovascular diseases (CVD) due to their modulating oxidative stress in the arteries related to coronary atherosclerosis [4]. The low serum levels and activities in the PON-1 enzyme due to some genetic polymorphisms in the gene of PON-1 have been impacted with coronary atherosclerosis [6], [7]. The family gene of paraoxonase includes PON-1, PON-2, and PON-3. In humans, PON-1 and a low level of PON-3 but not PON-2, are found as HDL-associated in serum, PON-2 is found in many tissues and macrophages. However, all forms were shown a protective effect against the development of atherosclerosis [8]. The PON-1 gene is localized on chromosome 7q21.3 with nine exons and a 62,857 bp. The gene ID is 544, coding a glycoprotein associated with HDL and plays a central role in preventing the oxidation of LDL [4]. This might be explained by one mechanism of HDL protecting effect against atherosclerosis [9]. PON-1 decreases the accumulation of lipid peroxide on LDL [10]. This effect refers to the link between PON-1 and atherosclerosis [11]. The present study aimed to

investigate the frequency of rs3917538 SNP genotypes in the PON-1 gene with coronary atherosclerosis and assessed its correlation with the serum PON-1 activity and atherosclerosis severity.

## Patients and methods

Sixty selected patients with coronary atherosclerosis are all admitted to Al Imamain Al-Kadhimain medical city and Ibn Al-Bitar center for cardiac surgery, Baghdad, Iraq. All patients were investigated by cardiologists and underwent angiography examination to diagnose coronary atherosclerosis and evaluate the arterial stenosis or the number of the affected vessels for assessing the severity of coronary atherosclerosis. The Local Ethics Committee approved the study protocol (Institutional Review Board (IRB) Ethics Committee of College of Medicine, Al-Nahrain University No. 20190991 on August 23, 2021, and written consent were obtained for all patients included in the present study. The study excludes subjects with acute myocardial infarction, history of angina pectoris, congestive heart failure, cardiomyopathy, hypertension, diabetes mellitus, hyperlipidemia, or family history of hyperlipidemia. The collection of samples was from February 2019 till June 2020. The severity of coronary atherosclerosis is classified as the number of diseased vessels as one vessel, two vessels, and three vessels. The patients were (42 males and 18 females) and the controls were (44 males and 16 females). The blood samples were divided into two parts; part one was collected in the plane tube and centrifuged at 1000 X g for 15 min. The serum formed was used to assess paraoxonase-1 by fluorometric assay; part two was collected in EDTA tubes as whole blood and stored at (-20ºC) for the genetic measurements of PON-1 gene polymorphism by (RT-PCR) technique. The principle of the fluorometry measurement of PON-1 activity depends on using a fluorogenic substrate formed by a highly fluorescent product that can be measured on an emission filter/excitation filter (Ex/Em) equal to 368/460 nm. A selective PON-1 inhibitor is provided to verify PON-1 specific activity with a sample volume of 5 μl.

### *Serum PON-1 activity*

The determination of circulating serum paraoxonase-1 activity was according to the use of a commercially available kit produced by MyBioSource, USA. Ten microliters of samples were used and 10 μl of 2-hydroxyquinoline solution (Paraoxonase-1 inhibitor solution) were added to each sample well and allowed the inhibitor to interact with samples for 10 min. Eight microliters of Paraoxonse-1 assay buffer were added to adjust the volume. After incubation, 20 μl of PON-1 substrate solution were added to each reaction well. Without waiting, but immediately within 1 min, the fluorescence was measured at Ex/Em = 380/460 nm, then in kinetic mode for 30 min and 60 min was measured and recorded for calculations.

### *Genetic analysis*

According to the manufactured protocol, ReliaPrep™ Blood gDNA Miniprep System, Promega, USA, genomic DNA was isolated from the blood sample. For each 1.5 ml, microcentrifuge tube 20μl of Proteinase K (PK) solution was dispensed, and then 200μl of blood was added and briefly mixed. For cells lysis, 200 μl of cell lysis buffer (CLB) was added to the tube and mixed by vortex for 10 s. All mixes were incubated in a water bath at 56°C for 30 min; while the blood sample was incubated, a ReliaPrep™ Binding Column was placed into an empty collection tube. After incubation, the tube was removed from the water bath, and 250μl of binding buffer (BBA) was added and mixed by vortex for 10 s. After the washing, the column was placed in a clean 1.5 ml microcentrifuge tube and 100μl of nuclease-free water was added. The genotyping analysis with (RT-PCR) and this reaction done by using sequence-specific primers supplied by Macrogen Company, the primers were as follows:

- PON-1-F-5`-CCAGTCTATCATCCTGCTTTC-3`and
- PON-1-R-5`-GGGTGAAATGTTGATTCCATTAG-3`

The reactions conditions were initial denaturation at 95ºC for 5 min, followed by 30 cycles denaturation for 30 s at 95ºC, annealing at 60ºC for 30 s, the extension at 72ºC for 30 s, with final 7 min at 72ºC. The products of PCR were run on agarose gel electrophoresis to confirm the amplification; ethidium bromide (10 mg/ml) was used. The stained bands in the gel were visualized using a gel image system.

### *Statistical analysis*

The study data were stored in Microsoft Spread Sheet and analyzed on the computer using the SPSS software version 25 (SPSS, Chicago). Continuous data were subjected to a normality test (Shapiro–Wilk test). Data with normal distribution were presented as mean and standard deviation and analyzed with analysis of variance (ANOVA). Data with non-normal distribution were presented as median and range and analyzed with Mann–Whitney U test (for two groups comparison) or Kruskal–Wallis (for three groups comparison). Categorical variables were expressed as numbers and percentages and analyzed with the Chi-square test. The receiver operating characteristic curve (ROC) was used to evaluate the diagnostic value of PON-1 activity. The risk association between PON-1 gene SNP and atherosclerosis was estimated by calculating the odds ratio (OR) and corresponding 95% confidence intervals (CI) using binary

logistic regression. For this analysis, subjects who were homozygous for the wild-type genotype were considered dependent variables and different variants entered the model as independent variables. Chi-square was used to test Hardy–Weinberg equilibrium (HWE) [12].

## Results

The basic anthropometric and clinical parameters of the subjects studied are presented in Table 1. In the present study, the mean age of the patients was 54.53±8.48 years which did not differ significantly from that of controls (52.9±8.86 years). Furthermore, the two groups were comparable in gender distribution with no significant difference. However, the control group had a higher BMI than patients (23.07±2.06 kg/m$^2$ vs 21.48±3.46 kg/m$^2$) with a significant difference. Among the patient group, there were 16 patients (26.67%) who had only one vessel involved, 18 patients (30%) had two vessels, and 26 patients (43.33%) had three vessels involved (Table 1).

**Table 1: Demographic characteristics of the study population**

| Variables | Patients (n=60) | Controls (n=60) | p-value |
|---|---|---|---|
| Age, years | | | |
| Mean ± SD | 54.53 ± 8.48 | 52.9 ± 8.86 | 0.576 |
| Range | 42–65 | 40–65 | |
| Gender | | | |
| Male | 42 (70%) | 44 (73.33%) | 0.685 |
| Female | 18 (30%) | 16 (26.67%) | |
| Severity of atherosclerosis | | | |
| One vessel | 16 (26.67%) | ---------- | -------- |
| Two vessels | 18 (30%) | | |
| Three vessels | 26 (43.33%) | | |

### *Serum levels of PON-1 activity*

PON-1 activities were non-normally distributed and the Mann–Whitney U test was used to compare the medians between groups. The median activity of PON-1 in controls was 197.32 mU/ml (range 122.96–249.75 mU/ml), which was higher than that of patients (median =163.46 mU/ml, range 46.64–228.33 mU/ml) with a significant difference (Figure 1).

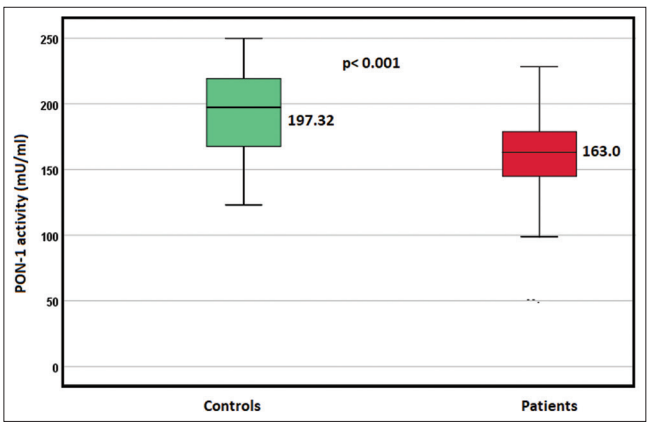


*Figure 1: The medians of PON-1 activities in patients and controls*

### *Rs.3917538*

The frequency of these genotypes in patients and controls was comparable with no significant difference. Similarly, there was no significant difference in different models or allele distribution between the two groups (Tables 1 and 2).

**Table 2: The frequency of different genotypes and alleles of the polymorphism rs3917538 in patients and controls**

| rs3917538 | Patients (60) | Controls (54) | p-value | OR (95%CI) |
|---|---|---|---|---|
| Genotypes | | | | |
| CC | 32 (53.33%) | 34 (62.96%) | 0.534 | 1.0 |
| CT | 24 (40%) | 18 (33.33%) | 0.402 | 2.15 (0.36–12.41) |
| TT | 4 (6.67%) | 2 (3.70%) | 0.660 | 1.5 (0.25–9.11) |
| HWE | 0.860 | 0.840 | | |
| Dominant model | | | | |
| CC+CT | 56 (93.33%) | 52 (96.3%) | 0.485 | 1.0 |
| TT | 4 (6.67%) | 2 (3.70%) | 0.299 | 1.85 (0.33–10.57) |
| Recessive model | 32 (53.33%) | 34 (62.96%) | | 1.0 |
| CC | 28 (46.67%) | 20 (37.04%) | | 1.49 (0.7–3.15) |
| CT+TT | | | | |
| Alleles | | | | |
| C | 88 (73.33%) | 86 (79.63%) | 0.265 | 1.0 |
| T | 32 (26.67%) | 22 (20.37%) | | 1.42 (0.77–2.64) |

### *The impact of different rs3917538 genotypes with the PON-1 activity, atherosclerosis severity in patients and controls*

To evaluate the influence of rs3917538 SNP genotypes on serum PON-1 activity in both patients and controls was inspected, and a comparison between their medians of PON-1 was made. There was no significant impact of rs3917538 SNP genotypes on serum PON-1 activity among patients and controls; there was a significant correlation with the severity of atherosclerosis p = 0.041, as shown in Table 3.

## Discussion

Atherosclerosis is one of the coronary artery diseases (CAD), which compresses about 17 million death cases; 45% are under the age of 70 [13]. The median activity for PON-1 in serum of patients was significantly lower than controls; this result agrees with the study done by Wysocka *et al*., 2019 [14]. Who found lower PON-1 activity in patients? The low activity in patients with coronary atherosclerosis is related to the increased oxidative stress, which might result in a decrement in the activity of PON-1. The reduction in the activity of PON-1 in patients with coronary atherosclerosis is inversely correlated with the OX-LDL levels. An effective nutritional or therapeutic antioxidants usage is necessary to improve PON-1 activity in those patients with high oxidative stress [8]. The frequency of the rs3917538 genotypes in patients and controls was comparable with no significant difference. Similarly, there was no significant difference in different models or alleles distribution between the two groups. Most studies clinically support it, but other studies exclude

**Table 3: The impact rs3917538 genotypes on serum PON-1activity and atherosclerosis severity**

| Variables | Patients | | | p-value | Controls | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | CC (n=32) | CT (n=24) | TT (n=4) | | CC | CT | TT | |
| PON-1 activity | 195.6 (122.9–241.7) | 198.17 (140.9–249.7) | 204.22 (159.3–249.1) | 0.746 | 155.11 (46.6–209.9) | 169.6 (88–228.3) | 174.25 (174.2–174.2) | 0.180 |
| Severity | | | | | | | | |
| 1 vessel | 4 (12.5) | 10 (41.67%) | 2 (50%) | 0.041 | ------- | ------- | -------- | ---- |
| 2 vessels | 14 (43.75%) | 4 (16.67%) | 0 (0%) | | | | | |
| 3 vessels | 14 (43.75%) | 10 (41.67%) | 2 (50%) | | | | | |

a relationship between PON-1 genetic polymorphisms and atherosclerosis development. However, the PON-1 concentration and activity in serum is a more effective and better predictor of the risk of coronary atherosclerosis than the PON-1 genotypes [8]. The association of different rs3917538 genotypes with the PON-1 activity was investigated; there was no impact of these SNP genotypes with the PON-1 activity. Similarly, there was no significant difference in different models or alleles distribution between the two groups. It must be noted that the polymorphisms affect only a part of PON-1 enzymatic activity variations. Besides the genetic factors, enzyme activity is affected by other environmental factors such as nutrition, lifestyle including smoking, alcoholic subjects, chemical materials, sex, age, and other factors [15]. The associations of rs3917538 genotypes with the number of diseased vessels of patients of coronary atherosclerosis were that the CC genotypes carriers were significantly higher than other genotypes with two and three diseased vessels. In contrast, the same carriers (CC) were significantly lower than other genotypes with one disease vessel. It seems to be the CC genotype associated with the severity of coronary atherosclerosis in this study. The study of (Fallah *et al*., 2010) [5] found that the diseased vessels number did not impact the gene variants of PON-1.

## Conclusion

Measurement of serum PON-1 activity helps exclude normal subjects from coronary atherosclerosis states. The rs3917538 genotypes in the PON-1 gene among the patient's group impact the severity of coronary atherosclerosis.

### *Study limitation*

The study was a relatively small sample size which hampered the ability to detect some significant associations.

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