Journal of Diabetes & Metabolic Disorders (2018) 17:1–10
https://doi.org/10.1007/s40200-018-0332-z

RESEARCH ARTICLE

Genotype and phenotype of salt-stimulated paraoxonase 1 (PON1) is associated with atherogenic indices in type 2 diabetes

Durdi Qujeq[1] · Abdolkarim Mahrooz[2,3,4] · Ahad Alizadeh[5] · Parisa Masoumi[4] · Saleh Annemohammadzadeh[4] · Ruzbeh Boorank[4]

Received: 24 October 2017 / Accepted: 8 January 2018 / Published online: 26 March 2018
© Springer International Publishing AG 2018

Abstract

Background Paraoxonase 1 (PON1) and lipid abnormalities contribute to the development of cardiovascular disease, which is the principal cause of mortality in patients with type 2 diabetes (T2D). Data are not available on the potential association between salt-stimulated activity of PON1 (PON1-salt) and the atherogenic indices in T2D, therefore, we focused on these associations and evaluated whether the functional variants PON1-Q192R and PON1-L55M influence the associations.

Methods Paraoxonase activity (PON1-para), arylesterase activity (PON1-aryl) and salt-stimulated activity (PON1-salt) were measured by spectrophotometric assays. The atherogenic index of plasma (AIP) was calculated from the log (TG/HDL-C). The genetic analyses were made by the restricted fragment length polymorphism after PCR amplification.

Results We observed that PON1-salt was negatively correlated with total cholesterol (TC)/HDL-C ($r = -0.441$, $p = 0.006$), LDL-C/HDL-C ($r = -0.415$, $p = 0.011$), and AIP ($r = -0.422$, $p = 0.009$). Correlations between PON1-salt and all three atherogenic indices were significantly affected by PON1-L55M and PON1-Q192R. Linear regression showed that AIP ($p = 0.002$), LDL-C/HDL-C ($p = 0.005$), and TC/HDL-C ($p = 0.002$) were independently associated with PON1-salt. Based on Ridge regression, the standardized coefficients −0.358, −0.297, and − 0.044 were obtained for AIP, LDL-C/HDL-C, and TC/HDL-C, respectively, and this shows that AIP could have more negative effect on PON1-salt than the others.

Conclusions The decreased PON1-salt may be considered as a risk factor for atherosclerosis in T2D, therefore, understanding the associations between PON1-salt as an important although neglected property and atherogenic indices may be valuable in T2D. Accordingly, detection of PON1-salt status (phenotype and genotype) together with the atherogenic indices particularly AIP could be beneficial in identifying the increased atherogenicity in T2D.

Keywords Paraoxonase 1 · PON1 · Salt-stimulated activity · Atherosclerosis · Atherogenic index of plasma · Type 2 diabetes

✉ Abdolkarim Mahrooz
amahrooz@mazums.ac.ir; kmahrooz2@gmail.com

1 Cellular and Molecular Biology Research Center (CMBRC), Health Research Institute, Babol University of Medical Sciences, Babol, Iran

2 Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran

3 Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran

4 Department of Clinical Biochemistry and Genetics, Faculty of Medicine, Mazandaran University of Medical Sciences, Km 17 Khazarabad Road, Sari, Iran

5 Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Background

Paraoxonase 1 (PON1) is a calcium-dependent esterase, expressed in a variety of tissues, but it is mainly produced by the liver and secreted into the blood where it associates predominantly with high density lipoprotein (HDL) particles [1, 2]. This multifunctional enzyme has attracted the attention of researchers, because of its activity to protect low density lipoproteins (LDL) against oxidative stress, reduce macrophage foam cell formation and prevent atherosclerosis [1, 3]. It has also been documented that PON1 plays an important role in hydrolyzing and detoxifying of organophosphate toxins and has interaction with various drugs particularly cardiovascular drugs [3, 4].

Paraoxonases have been introduced as proteins that can be delivered to specific tissues to fight oxidative stress. The enzymes particularly PON1 are associated with various human

diseases [4]. A number of investigations have addressed the role of PON1 in diabetes and its complications [5–7]. Furthermore, the importance of PON1 coding region single nucleotide variants (SNVs), particularly PON1-Q192R (rs662) and PON1-L55M (rs854560), in diabetes and its complications, have been highlighted in several studies [5, 8, 9]. These SNVs are associated with approximately 60% variation in PON1 activity and concentration [10].

Cardiovascular disease (CVD) is the leading cause of death in type 2 diabetic patients and when it has developed, diabetic patients have worse outcomes in comparison with nondiabetic patients [11, 12]. In an eight-year follow-up study [11], it has been shown that individuals who progressed to diabetes during the follow-up had higher baseline values of triglycerides, total and LDL cholesterol and blood pressure, and lower values of HDL than those who remained nondiabetic. Lipid dyslipidemia in patients with type 2 diabetes (T2D) are likely to contribute to the development of atherosclerosis, so the management of dyslipidemia and increased atherosclerotic risk needs a fundamental understanding of diabetic dyslipidemia for finding the efficient preventive and therapeutic strategies [13, 14].

According to studies, the atherogenic ratio total cholesterol (TC)/HDL-C and LDL-C/HDL-C could be better predictors of patients at high risk for atherosclerosis and CVD than single lipid values [15]. Also, investigations have demonstrated that subjects with high atherogenic index of plasma (AIP) have a higher risk for coronary heart disease (CHD) than those with low AIP [16, 17]. The index is positively correlated with the fractional esterification rate of HDL (FER_{HDL}), and it is inversely correlated with LDL particle size [17]. Therefore, AIP has been introduced as the alternative marker of plasma atherogenicity [16, 17].

Although PON1 status and lipid abnormalities contribute to the development of atherosclerosis, there have been no previous studies (to the best of our knowledge) that have evaluated the potential association between salt-stimulated activity of PON1 (PON1-salt) as an important although neglected property of the enzyme [10] with the atherogenic indices TC/HDL, LDL/HDL and AIP in T2D. Therefore, in the present study, we focused on these associations and in addition, we evaluated whether the PON1-Q192R and PON1-L55M as the most common variants in the coding region related with variation in PON1 activity and vascular disease [4, 18], influence these associations in patients with T2D.

Methods

Study patients

Forty patients with T2D (age range 52.35 ± 11.86 years) were included in the study. The participants underwent a physical examination and completed a questionnaire of personal information, including medical history, medication, and personal habits. All the patients were taking metformin (1000–1500 mg per day) and none of the patients was taking insulin therapy. The patients were taking lipid-lowering therapy including atorvastatin ($n = 26$) or both atorvastatin and gemfibrozil ($n = 5$). Some of the patients were taking antihypertensive medication including losartan, a beta blocker, or an angiotensin-converting enzyme (ACE) inhibitor. Subjects with previous history of type 1 diabetes, renal failure, chronic hepatic disease, autoimmune diseases, and malignant diseases were not included in this study. The rate of current smoking was 7.5% in the patients. The protocol study was planned in accordance with the ethical criteria detailed in the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was received from all participants before enrollment.

Laboratory assays

Blood samples were obtained from the patients after overnight fasting and were isolated by low-speed centrifugation and their aliquots were stored at −70 °C. Insulin levels were measured using ELISA kit (Demeditec Diagnostics GmbH, Germany). The HbA1c levels were quantified by a commercially available kit (NycoCard, Oslo, Norway). Standard enzymatic methods were used to assay values of fasting plasma glucose (FPG) and total cholesterol (TC) triglycerides (TGs), high-density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT) using an auto-analyzer (Prestige, Japan). AIP was calculated from the logarithmic transformation of TG/HDL-C. The LDL-C levels were calculated according to the Friedewald formula [19].

PON1 activity assays

Hydrolyses rates of the two substrates phenylacetate (arylesterase activity; PON1-aryl) and paraoxon (paraoxonase activity; PON1-para) were determined spectrophotometrically using a double-beam spectrophotometer (UV 1800, Shimadzu, Japan) at 270 nm for PON1-aryl and 412 nm for PON1-para. In the assay cuvette, the levels of paraoxon and phenylacetate were 2 mM and 1 mM, respectively. A Tris/HCL buffer (100 mM, pH 8.0) and 1 mM CaCl2 were used for assaying both the enzyme activities. Blanks were applied to correct for the nonenzymatic hydrolysis of the substrates. The molar extinction coefficients were 17,100 and 1310 M^{-1} cm^{-1}, respectively for PON1-para and PON1-aryl. One unit of PON1-para and PON1-aryl are defined as 1 nmol p-nitrophenol/min and 1 μmol phenol/min formed, respectively. The PON1-aryl and PON1-para assays were each done in duplicate. Salt-stimulated paraoxonase activity (PON1-salt) was measured with 1 M NaCl in the reaction mixture in which paraoxonase activity was assayed.

Genotype determination

PCR-based RFLP (restriction fragment length polymorphism) was used to screen the study PON1 single nucleotide variants. For the PON1-Q192R variant forward primer 5' TATTGTTG CTGTGGGACCTGAG 3′ and reverse primer 5' CCTGAGAATCTGAGTAAATCCACT 3′ were used. Genomic DNA was amplified in 25 μl of reaction mixture. PCR cycling conditions were 94 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 58 °C for 40 s, 72 °C for 35 s, with a final extension step of 5 min at 72 °C. PCR products (238 bp) were quantified by 1.5% gel electrophoresis. Amplification products were digested using the restriction enzyme *AlwI* (Thermoscientific, Lithuania) at 37 °C for 16 h and resulted in 172 and 66 bp fragments, which were subjected to electrophoresis on a 2.5% agarose gel. Allele Q did not contain the *AlwI* site whereas R contained the restriction site giving rise to 172 and 66 bp products. RR genotypes were identified by the presence of 172 and 66 bp fragments. QR genotypes were identified by the presence of 238, 172 and 66 bp fragments, while the presence of an undigested fragment (238 bp) was the basis for identification of QQ genotypes.

For the PON1-L55M variant forward primer 5' GAAGAGTGATGTATAGCCCCAG 3′ and reverse primer 5' TTTAATCCAGAGCTAATGAAAGCC 3′ were used. PCR cycling was the same as above except that the annealing step was performed at 53 °C. The PCR products (170 bp) were digested with the restriction enzyme *NlaIII* and resulted in 126 and 44 bp fragments, which were subjected to electrophoresis on a 3% agarose gel. Allele M contains a unique *NlaIII* site giving rise to 126 and 44 bp products whereas L allele did not contain the restriction site. The restriction digest revealed 126 and 44 bp fragments in the presence of MM genotype, 170, 126 and 44 bp fragments in the presence of LM genotype, and a non-digested 170 bp fragment in the presence of LL genotype.

For the genotyping quality control, approximately 30% of samples were randomly assayed in duplicates and the concordance rate was 100%.

Statistical analyses

Normality of the distributions of data was analyzed with the Lilliefors test. The differences of the parametric variables were analyzed using t-test and the nonparametric variables were analyzed by Mann–Whitney. The chi-square test was used to evaluate the deviation of allelic and genotype frequencies from the Hardy-Weinberg equilibrium expectations. Spearman's correlation coefficient was applied to analyze the association between the study parameters. We used regression analysis to estimate the independent association of PON1-salt with the atherogenic indices. We also applied the predicted residual error sum of squares (PRESS) statistic, which is a form of cross-validation used in regression analysis, and is obtained by removing one observation at a time from the total data set. The smaller the value of PRESS, the better the prediction for a model. According to considerable correlations between independent covariates (multicollinearity), high variance inflation factors (VIF) showed that linear regression was not a suitable method to estimate the effects of covariates on PON1-salt as a response variable, thus Ridge regression was used. To compare effects of the covariates together, standardized coefficients were reported. Two-tailed p less than 0.05 were considered to be significant. Statistical analyses were performed using SPSS (version 16.0) and R (version 3.0.1) software.

Availability of data and materials The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Results

Allele frequency and genotype distribution of PON1 variants in the study patients

The genotypes frequencies were 60.0% QQ, 32.5% QR, 7.5% RR for PON1-Q192R variant and 42.5% LL, 47.5% LM, and 10.0% MM for PON1-L55M in the study population. The frequencies of the Q and R allele were found to be 0.76 and 0.24, respectively, and for the L and M allele were 0.66 and 0.34, respectively. There was no deviation from Hardy-Weinberg equilibrium for the variant PON1-Q192R ($p = 0.516$) and PON1-L55M ($p = 0.694$).

Changes in the study parameters according to the genotypes of PON1-L55M and PON1-Q192R

The study parameters were compared according to PON1-L55M and PON1-Q192R genotypes in Tables 1 and 2. There were no significant differences between the genotypes of PON1-Q192R (QQ and QR + RR group) and PON1-L55M (LL and LM + MM) with respect to age, systolic and diastolic blood pressure, BMI, triglyceride (TG), total cholesterol (TC), HDL-C, fasting glucose, HbA1c, and insulin levels (Tables 1 and 2). Our findings showed no significant differences between LL genotypes and LM + MM group with respect to LDL-C and the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP (Table 1). As shown in Table 2, LDL-C and LDL-C/HDL-C levels were significantly higher in QQ homozygotes compared with QR + RR group, however, there were no significant differences between these groups with respect to the atherogenic indices TC/HDL-C and AIP levels.

The activity parameter PON-aryl ($p = 0.001$), PON1-para ($p = 0.009$), and PON1-salt ($p = 0.002$) levels were significantly higher in LL genotypes compared with LM + MM

Table 1 Changes in the study parameters according to the genotypes of PON1-L55M

Parameter	LL ($n = 17$)	LM + MM ($n = 23$)	p value
Age	53.75 ± 9.90	50.77 ± 12.67	0.679
Diastolic blood pressure (mmHg)	80.62 ± 10.63	75.45 ± 7.85	0.058
Systolic blood pressure (mmHg)	131.87 ± 18.79	126.82 ± 16.94	0.206
BMI (kg/m^2)	31.74 ± 6.62	30.05 ± 4.02	0.359
Fasting glucose (mmol/L)	7.11 ± 1.18	7.85 ± 1.93	0.344
HbA_{1c} (%)	6.84 ± 1.05	6.76 ± 1.17	0.866
Alanine aminotransferase (U/L)	18.87 ± 9.19	21.18 ± 10.17	0.574
Insulin (μU/mL)	7.28 ± 4.94	8.31 ± 6.50	0.905
TG (mmol/L)	1.03 ± 0.60	1.62 ± 0.70	0.121
TC (mmol/L)	4.02 ± 0.71	4.18 ± 0.68	0.605
HDL-C (mmol/L)	1.36 ± 0.32	1.25 ± 0.27	0.432
LDL-C (mmol/L)	2.06 ± 0.43	2.17 ± 0.53	0.801
TC/HDL-C	3.05 ± 0.64	3.44 ± 0.75	0.117
LDL-C/HDL-C	1.58 ± 0.45	1.80 ± 0.58	0.315
AIP	0.31 ± 0.23	0.45 ± 0.19	0.063
PON1-aryl (U/mL)	94.03 ± 16.23	75.30 ± 14.73	0.001
PON1-para (U/mL)	112.06 ± 53.43	69.18 ± 36.29	0.009
PON1-salt (U/mL)	234.60 ± 112.68	123.71 ± 77.74	0.002

LL (subjects with LL genotype), LM + MM (M allele-containing subjects), TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C), PON1-para (paraoxonase activity), PON1-aryl (arylesterase activity), PON1-salt (salt-stimulated activity)

group (Table 1). PON1-para ($p < 0.001$) and PON1-salt ($p < 0.001$) levels were higher in patients with the QR + RR genotype than in those with the QQ genotype (Table 2).

There were no statistically significant differences between QQ and QR + RR group, and also LL and LM + MM group with respect to lipid-lowering therapy.

Table 2 Changes in the study parameters according to the genotypes of PON1-Q192R

Parameter	QQ ($n = 24$)	QR + RR ($n = 16$)	p value
Age	51.37 ± 11.50	54.53 ± 12.70	0.285
Diastolic blood pressure (mmHg)	80.62 ± 10.63	75.45 ± 7.85	0.516
Systolic blood pressure (mmHg)	131.87 ± 18.79	126.82 ± 16.94	0.471
BMI (kg/m^2)	31.90 ± 5.93	28.70 ± 3.04	0.094
Fasting glucose (mmol/L)	7.89 ± 1.73	6.98 ± 1.41	0.091
HbA_{1c} (%)	6.92 ± 1.29	6.56 ± 0.61	0.413
Alanine aminotransferase (U/L)	22.83 ± 9.90	16.33 ± 7.86	0.021
Insulin (μU/mL)	8.67 ± 6.40	6.23 ± 4.61	0.117
TG (mmol/L)	1.63 ± 0.72	1.26 ± 0.50	0.078
TC (mmol/L)	4.29 ± 0.74	3.90 ± 0.56	0.097
HDL-C (mmol/L)	1.26 ± 0.25	1.35 ± 0.35	0.347
LDL-C (mmol/L)	2.28 ± 0.53	1.95 ± 0.43	0.033
TC/HDL-C	3.48 ± 0.70	3.02 ± 0.73	0.073
LDL-C/HDL-C	1.87 ± 0.54	1.53 ± 0.52	0.046
AIP	0.44 ± 0.19	0.31 ± 0.23	0.106
PON1-aryl (U/mL)	84.81 ± 19.51	80.64 ± 14.24	0.84
PON1-para (U/mL)	59.41 ± 32.18	128.16 ± 39.74	<0.001
PON1-salt (U/mL)	109.06 ± 76.96	251.02 ± 90.51	<0.001

QQ (subjects with QQ genotype), QR + RR (R allele-containing subjects), TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C), PON1-para (paraoxonase activity), PON1-aryl (arylesterase activity), PON1-salt (salt-stimulated activity)

Correlations between PON1-salt with the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP in total data and according to PON1-L55M and PON1-Q192R genotypes

Correlation analyses were conducted to study the association of PON1-salt with the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP in total data and according to PON1-L55M and PON1-Q192R genotypes. Our results indicated that PON1-salt was negatively correlated with TC/HDL-C ($r = -0.441$, $p = 0.006$), LDL-C/HDL-C ($r = -0.415$, $p = 0.011$), and AIP ($r = -0.422$, $p = 0.009$) in total data (Fig. 1).

As shown in Fig. 2, PON1-salt was found to be negatively correlated with the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP according to the genotypes of PON1-L55M; however, only the correlation in the LL genotypes was statistically significant ($r = -0.529$, $p = 0.043$ for AIP, $r = -0.782$, $p = 0.001$ for LDL-C/HDL-C, and $r = -0.732$, $p = 0.002$ for TC/HDL-C). In the LM + MM group, the correlations were still negative, although not significant ($r = -0.135$, $p = 0.569$ for AIP, $r = -0.117$, $p = 0.622$ for LDL-C/HDL-C, and $r = -0.096$, $p = 0.686$ for TC/HDL-C) [Fig. 2]. Based on the genotypes of PON1-Q192R, there was a negative and significant correlation between PON1-salt and AIP ($r = -0.568$, $p = 0.027$), LDL-C/HDL-C ($r = -0.561$, $p = 0.030$), and TC/HDL-C ($r = -0.618$, $p = 0.014$) in the QR + RR group, however, the correlations were not statistically significant in the QQ genotypes ($r = -0.231$, $p = 0.315$ for AIP, $r = -0.161$, $p = 0.485$ for LDL-C/HDL-C, and $r = -0.271$, $p = 0.235$ for TC/HDL-C) [Fig. 3].

Correlations between the prevalent activities of PON1 (PON1-para and PON1-aryl) with the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP in total data and according to PON1-L55M and PON1-Q192R genotypes

Although the correlations observed between the prevalent activities of PON1, including PON1-para and PON1-aryl with the atherogenic indices TC/HDL-C, LDL-C/HDL-C and AIP were negative, they were not statistically significant in total data. For example, the correlations were: $r = -0.125$, $p = 0.441$ for PON1-aryl and LDL-C/HDL-C; $r = -0.138$, $p = 0.395$ for PON1-aryl and AIP; and $r = -0.273$, $p = 0.070$ for PON1-para and TC/HDL-C. Moreover, these correlations were not statistically significant in either PON1-L55M or in the PON1-Q192R genotypes. For example, the correlations were: $r = -0.168$, $p = 0.535$ in the LL genotypes and $r = -0.112$, $p = 0.619$ in the LM + MM group for PON1-aryl and LDL-C/HDL-C, and $r = -0.117$, $p = 0.596$ in the QQ genotypes and $r = -0.425$, $p = 0.114$ in the QR + RR group for PON1-para and LDL-C/HDL-C.

The association of atherogenic indices with PON1-salt according to regression analysis

As shown in Table 3, linear multiple regression was performed to estimate the independent association of atherogenic indices with PON1-salt. AIP, LDL-C/HDL-C, and TC/HDL-C were included in three separate models [1 to 3] as independent variables. Our results indicated that AIP ($p = 0.002$), LDL-C/HDL-C ($p = 0.005$), and TC/HDL-C ($p = 0.002$) were independently associated with PON1-salt. Further analyses with PRESS statistic showed that among three atherogenic indices, AIP had the smaller value of PRESS; in other words, it could be a better predictor of PON1-salt compared with other indices (Table 3). It should be noted that due to marked correlations between atherogenic indices, statistically it was not possible to enter them in a model simultaneously. So, Ridge regression was used instead of linear regression to estimate the standardized coefficients of atherogenic indices on PON1-salt. Based on the analysis, the standardized coefficients −0.358, −0.297, and − 0.044 were obtained for AIP, LDL-C/HDL-C, and TC/HDL-C, respectively, and this shows that AIP could have more negative effect on PON1-salt than the others.

Discussion

Cardiovascular disease is the principal cause of mortality in T2D patients and several factors such as lipid abnormalities and endothelial dysfunction increase susceptibility to the disease [12, 20]. According to studies, risk factors for CVD such as LDL/HDL and AIP have higher predictive value than individual levels of lipid parameters; therefore, it could be an advantage for the atherogenic indices to be an alert to the increased cardiovascular risk [15, 21]. Furthermore, PON1 as an antioxidant and antiatherogenic enzyme was involved in determining the susceptibility to atherosclerosis and CVD [4, 22]. Serum PON1 activity variations are associated with variations in plasma lipid and lipoprotein [23]. PON1 hydrolyzes lipid peroxides that are bound to the LDL ands HDL, and therefore it is a key contribution to the antioxidant ability of HDL [21, 24]. In the previous studies, less attention has been given to the association between PON1 status, particularly PON1-salt (an important although neglected property), and the atherogenic indices. It should be noted that the salt-stimulated activity is widely used in the determination of phenotype distribution of PON1 by the dual substrate method [25, 26], and on the basis of a study by Eckerson et al. [27], the increased activity by the salt under standard conditions could be related to an increased number of active sites.

In this study, all three PON1 activity parameters (PON1-para, PON1-aryl, and PON1-salt) had a negative correlation with the atherogenic indices. However, one of the issues that

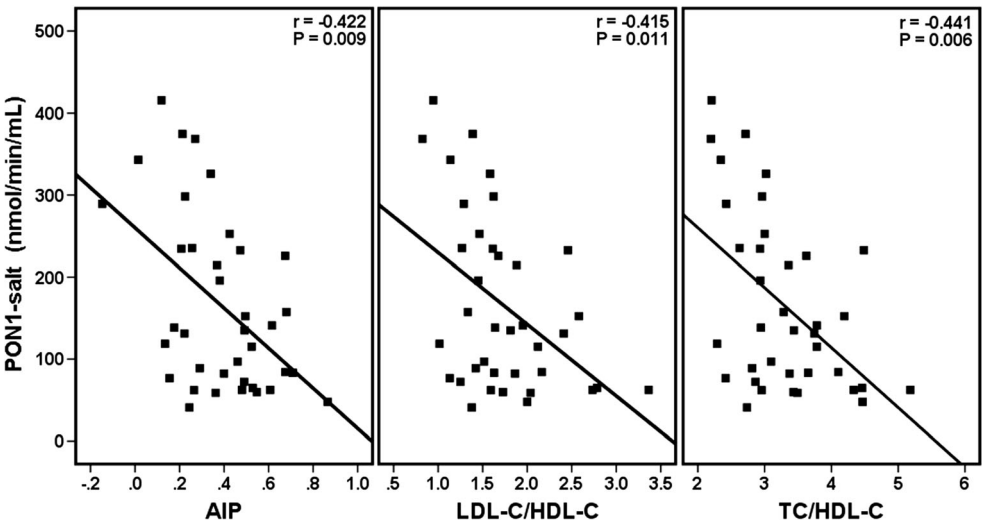

Fig. 1 Associations between salt-stimulated activity of PON1 (PON1-salt) and atherogenic indices in the study patients. TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C)

gained our attention was that among these PON1 activity parameters, PON1-salt was significantly associated with all three atherogenic indices in T2D, and this may highlight the importance of this property of PON1 in atherosclerotic events in patients with T2D. To our knowledge, we did not find the similar investigations in the literature to compare the association of PON1-salt with these indices. However, our results were consistent with those of Patra et al. [28], who found that

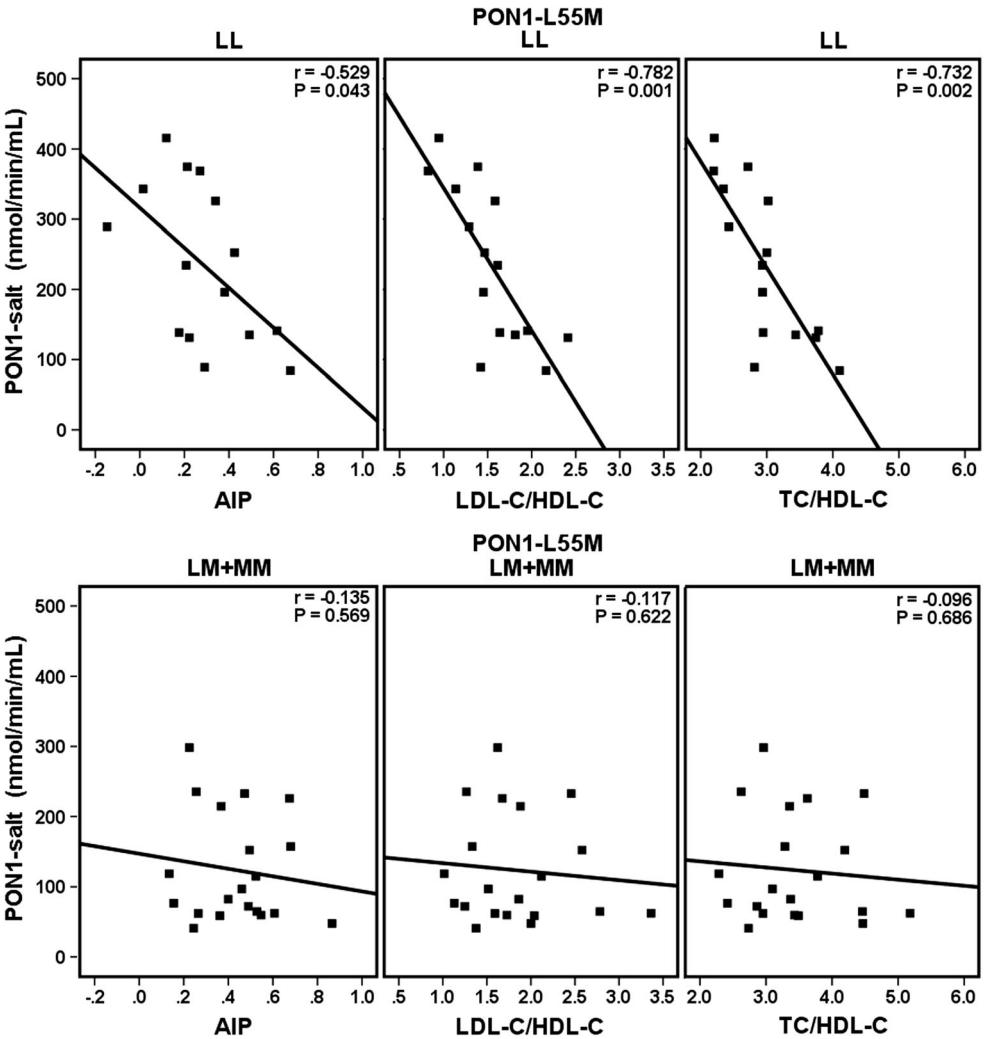

Fig. 2 Associations between salt-stimulated activity of PON1 (PON1-salt) and atherogenic indices according to the genotypes of PON1-L55M variant. TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C)

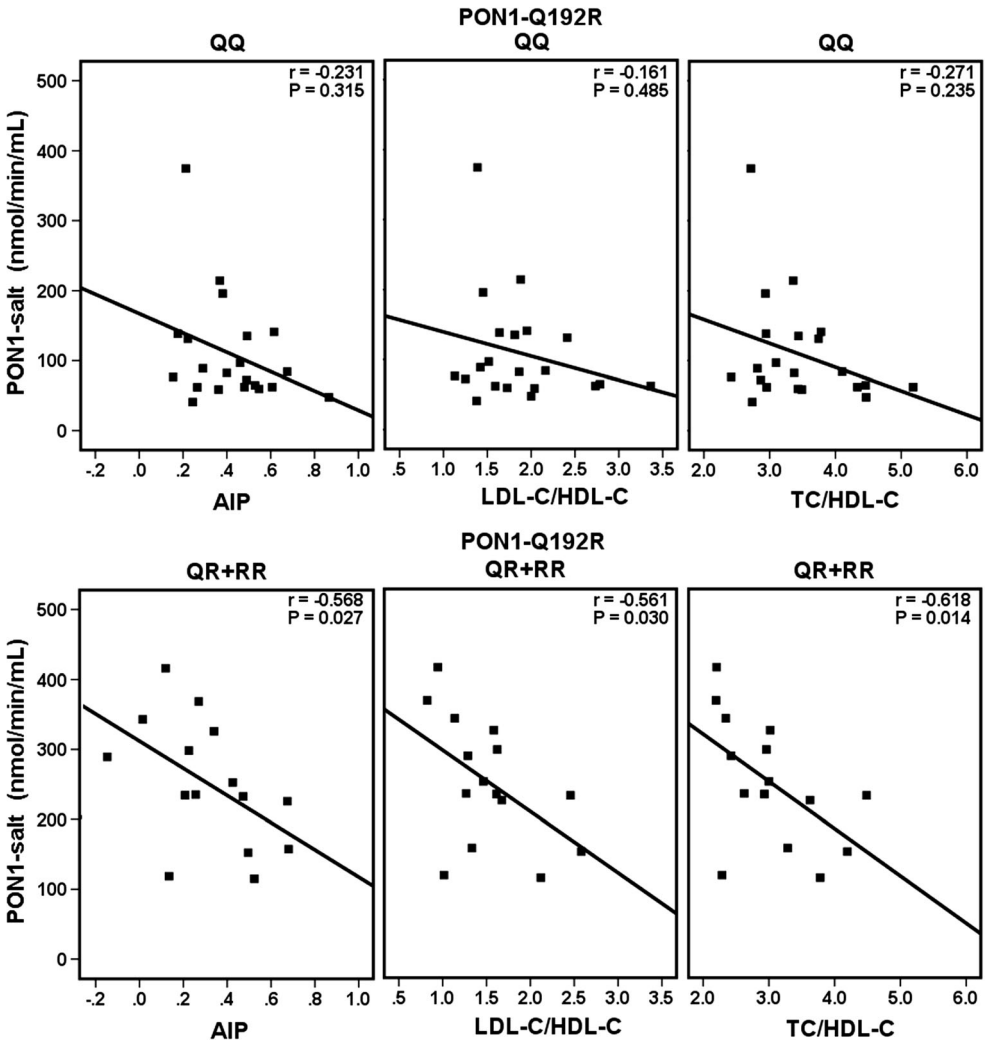

Fig. 3 Associations between salt-stimulated activity of PON1 (PON1-salt) and atherogenic indices according to the genotypes of PON1-Q192R variant. TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C)

PON1 levels were negatively correlated with the atherogenic indices, including AIP, LDL/HDL, and TC-HDL/HDL, and the strength of negative correlation was greater for PON1 than for HDL. Also, in a study, Stefanovic and colleagues [29] reported that lower PON1 activities were independently associated with higher AIP. Our findings were further supported by a recently published investigation [21] in which Viktorinova et al. observed a significant and negative correlation for PON1 activity and non-HDL/HDL ratio. None of these studies evaluated PON1 variants, particularly PON1-Q192R and PON1-L55M, while they are the most common variants in the coding region of PON1 gene, markedly affect the enzyme activity and are associated with vascular diseases [4, 18].

In the present study, when the analyses were performed with regard to PON1-Q192R variant, our findings showed that there were significant correlations between PON1-salt and the atherogenic indices only in the QR + RR group but not in the QQ genotypes. In other words, in the R allele carrying genotypes, the degree of salt stimulation significantly decreased with the increased atherogenicity in patients with T2D. The result may be related to the fact that salt stimulation influences the R allozyme much more than it does the Q allozyme. Oxidative stress could play a role in the association between PON1-salt and the atherogenic indices, because based on previous findings [30, 31], individuals with the R allozyme are more susceptible to oxidative stress than people with the Q allozyme. Our results were further supported by the findings of the previous studies indicating that individuals carrying R allele have less power in preventing the LDL oxidation and production of oxidized-LDL, and thus increased susceptibility for atherosclerosis [30, 32].

Our analyses, according to PON1-L55M variant, revealed that significant correlations between PON1-salt and the atherogenic indices exist only in the LL genotypes but not in the LM + MM group. According to the literature, the antioxidant property of PON1 is lower in LL homozygotes than in those containing the M allele and more importantly, an individual with LL genotype is more exposed to the development of atherosclerosis [33]. It seems that the reason for the role of the study variants in the associations between PON1-salt and the

Table 3 Regression analysis for the association of atherogenic indices with salt-stimulated activity (PON1-salt)

Parameter	Coefficient (95% CI)	Standardized coefficient (95% CI)	P value	R^2 (%)	Adjusted R^2 (%)	PRESS statistic
Model 1			<0.001	23.8	21.6	349,765
Intercept	259.6 (193.9, 325.3)	−0.038 (−0.33, 0.26)				
AIP	−243.39 (−392.81, −93.97)	−0.499 (−0.80, −0.19)	0.002			
Model 2			<0.001	20.5	18.3	367,817
Intercept	317.6 (210.2, 424.99)	−0.003 (−0.30, 0.30)				
LDL-C/HDL-C	−87.6 (−146.8, −28.5)	−0.444 (−0.74, −0.1)				
Model 3			<0.001	24.8	22.7	351,696
Intercept	406.2 (259.4, 553.1)	−0.017 (−0.31, 0.28)				
TC/HDL-C	−73.08 (−116.69, −29.47)	−0.494 (−0.79, −0.20)	0.002			

AIP, LDL-C/HDL-C, and TC/HDL-C were included in three separate models (1 to 3) as independent variables. TC (total cholesterol), AIP (atherogenic index of plasma; the logarithmic transformation of TG/HDL-C), PRESS (predicted residual error sum of squares)

atherogenic indices lies in the fact that oxidative stress influences the antioxidant property of PON1. It should be noted that racial differences may influence these associations [34].

In linear regression analysis, we found that all three atherogenic indices were independently associated with PON1-salt. In order to find which of all those indices could be a better predictor of PON1-salt, we performed PRESS statistic. According to this analysis, AIP was found to be a better predictor of PON1-salt in comparison with other indices. Further analyses using Ridge regression confirmed that AIP had more negative effect on PON1-salt than the others. Since AIP has been introduced as a suitable marker for plasma atherogenicity in patients with T2D [16, 17], it may be better to pay more attention to this marker. On the basis of studies, AIP is not only positively correlated with the FER_{HDL}, but is also inversely correlated with LDL particle size, and therefore AIP directly would be related to the risk of atherosclerosis [16, 17, 29]. The importance of the issue becomes more apparent when you realize that the atherogenic indices including AIP could be a warning for the increased cardiovascular risk in people who do not yet have extremely altered lipid levels [21].

In the present study, the small sample size should be considered as a limitation and thus studies with larger sample sizes are warranted to clarify and confirm these findings.

Conclusion

The diminished PON1-salt may be considered as a risk factor for atherosclerosis in T2D, thus, understanding the associations between PON1-salt as an important although neglected property of PON1 and atherogenic indices may be valuable in T2D. Accordingly, detection of PON1-salt status (phenotype and genotype) together with the atherogenic indices may be beneficial in identifying the increased atherogenicity in T2D.

Author's contributions A.M. obtained the funding, contributed to the design and conduct of the study, interpretation of data and writing of the manuscript. D.Q. contributed to study design, clinical interpretation, reviewed the manuscript, and contributed to the discussion. A.A. contributed to the statistical analysis, and reviewed and edited the manuscript. P.M., S.A., and R.B. contributed to study design, researched and analyzed data, and performed the experiments. All authors drafted the manuscript and gave final approval.

Funding This study was funded by Mazandaran University of Medical Sciences (Grant number: 1394–3).

Compliance with ethical standards

Ethics approval The study protocol was approved by the review committee and the Ethical committee at Mazandaran University of Medical Sciences.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

Abbreviations ACE, Angiotensin-converting enzyme; AIP, Atherogenic index of plasma; ALT, Alanine aminotransferase; CHD, Coronary heart disease; CVD, Cardiovascular disease; FER_{HDL}, Fractional esterification rate of HDL; FPG, Fasting plasma glucose; HDL, High density lipoprotein; LDL, Low density lipoproteins; PON1, Paraoxonase 1; PON1-aryl, Arylesterase activity of PON1; PON1-para, Paraoxonase activity of PON1; PON1-salt, Salt-stimulated activity of PON1; PRESS, Predicted residual error sum of squares; RFLP, Restriction fragment length polymorphism; SNV, Single nucleotide variant; TC, Total cholesterol; T2D, Type 2 diabetes; TG, Triglyceride; VIF, Variance inflation factors

References

1. Précourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis. 2011;214:20–36.
2. Mackness M, Mackness B. Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. Gene. 2015;567:12–21.
3. Mahrooz A. Pharmacological interactions of paraoxonase 1 (PON1): A HDL-bound antiatherogenic enzyme. Curr Clin Pharmacol. 2016;11:259–64.
4. Kim DS, Marsillach J, Furlong CE, Jarvik GP. Pharmacogenetics of paraoxonase activity: elucidating the role of high-density lipoprotein in disease. Pharmacogenomics. 2013;14:1495–515.
5. Hegele RA, Connelly PW, Scherer SW, Hanley AJG, Harris SB, Tsui LC, et al. Paraoxonase-2 G148 variant in an aboriginal Canadian girl with non-insulin-dependent diabetes. Lancet. 1997;350:785.
6. Koren-Gluzer M, Aviram M, Meilin E, Hayek T. The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates β-cell insulin release. Atherosclerosis. 2011;219:510–8.
7. Hofer SE, Bennetts B, Chan AK, Holloway B, Karschimkus C, Jenkins AJ, et al. Association between PON1 polymorphisms, PON activity and diabetes complications. J Diabetes Complicat. 2006;20:322–8.
8. Aubo C, Senti M, Marrugat J, et al. Risk of myocardial infarction associated with Gln/Arg 192 polymorphism in the human paraoxonase gene and diabetes mellitus. Eur Heart J. 2000;21:33–8.
9. Li J, Wang X, Huo Y, Niu T, Chen C, Zhu G, et al. PON1 polymorphism, diabetes mellitus, obesity, and risk of myocardial infarction: Modifying effect of diabetes mellitus and obesity on the association between PON1 polymorphism and myocardial infarction. Genet Med. 2005;7:58–63.
10. Mahrooz A, Alizadeh A, Gohari G. The salt stimulation property of serum paraoxonase (PON1) could be a valuable factor in evaluating the enzyme status in ischemic stroke: The role of activity-determined PON1 192Q/R phenotypes. J Neurol Sci. 2014;338:197–202.
11. Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, Manson JE. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. Diabetes Care. 2002;25:1129–34.
12. Irie Y, Katakami N, Kaneto H, Nishio M, Kasami R, Sakamoto K, et al. The utility of carotid ultrasonography in identifying severe coronary artery disease in asymptomatic type 2 diabetic patients without history of coronary artery disease. Diabetes Care. 2013;36:1327–34.
13. Verges B. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. Diabetes Metab. 2005;31:429–39.
14. Brown WV, Clark L, Falko JM, Guyton JR, Rees TJ, Schonfeld G, et al. Optimal management of lipids in diabetes and metabolic syndrome. J Clin Lipidol. 2008;2:335–42.
15. Brehm A, Pfeiler G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotein ratios and insulin resistance in obesity. Clin Chem. 2004;50:2316–22.
16. Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate inapoB-lipoprotein-depleted plasma (FER_{HDL}). Clin Biochem. 2001;34:583–8.
17. Tan MH, Johns D, Glazer NB. Pioglitazone reduces atherogenic index of plasma in patients with type 2 diabetes. Clin Chem. 2004;50:1184–8.
18. Mahrooz A, Gohari G, Hashemi MB, Zargari M, Musavi H, Abedini M, et al. R-carrying genotypes of serum paraoxonase (PON1) 192 polymorphism and higher activity ratio are related to susceptibility against ischemic stroke. Mol Biol Rep. 2012;39:11177–85.
19. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
20. Garin MC, James RW, Dussoix P, Blanché H, Passa P, Froguel P, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Invest. 1997;99:62–6.
21. Viktorinova A, Svitekova K, Stecova A, Krizko M. Relationship between selected oxidative stress markers and lipid risk factors for cardiovascular disease in middle-aged adults and its possible clinical relevance. Clin Biochem. 2016;49:868–72.
22. Rizzi F, Conti C, Dogliotti E, Terranegra A, Salvi E, Braga D, et al. Interaction between polyphenols intake and PON1 gene variants on markers of cardiovascular disease: a nutrigenetic observational study. J Transl Med. 2016;14:186.
23. Sarkar PD, Shivaprakash TM, Madhusudhan B. Association between paraoxonase activity and lipid levels in patients with premature coronary artery disease. Clin Chim Acta. 2006;373:77–81.
24. Zargari M, Sharafeddin F, Mahrooz A, Alizadeh A, Masoumi P. The common variant Q192R at the paraoxonase 1 (PON1) gene and its activity are responsible for a portion of the altered antioxidant status in type 2 diabetes. Exp Biol Med. 2016;241:1489–96.
25. Van den Berg SW, Jansen EH, Kruijshoop M, et al. Paraoxonase 1 phenotype distribution and activity differs in subjects with newly diagnosed Type 2 diabetes (the CODAM Study). Diabet Med. 2008;25:186–93.
26. Mirdamadi HZ, Sztanek F, Derdak Z, Seres I, Harangi M, Paragh G. The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. Brit J Clin Pharmacol. 2008;66:366–74.
27. Eckerson HW, Romson J, Wyte CM, la du BN. The human serum paraoxonase polymorphism: Identification of phenotypes by their response to salts. Am J Hum Genet. 1983;35:214–27.
28. Patra SK, Singh K, Singh R. Paraoxonase 1: a better atherosclerotic risk predictor than HDL in type 2 diabetes mellitus. Diabetes Metab Syndr. 2013;7:108–11.
29. Stefanović A, Kotur-Stevuljević J, Vujović A, Spasić S, Spasojević-Kalimanovska V, Jelic-Ivanović Z, et al. Association of the atherogenic index of plasma and oxidative stress status with weight gain during non-complicated pregnancy. Clin Chem Lab Med. 2012;50:2019–25.
30. Aydin M, Gencer M, Cetinkaya Y, Ozkok E, Ozbek Z, Kilic G, et al. PON1 55/192 polymorphism, oxidative stress, type, prognosis and severity of stroke. IUBMB Life. 2006;58:165–72.

31. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA. 2008;299:1265–76.
32. Aviram M, Billeke S, Sorenson R, et al. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. Arterioscler Thromb Vasc Biol. 1998;18:1617–24.
33. Barbieri M, Bonafè M, Marfella R, Ragno E, Giugliano D, Franceschi C, et al. LL-paraoxonase genotype is associated with a more severe degree of homeostasis model assessment IR in healthy subjects. J Clin Endocrinol Metab. 2002;87:222–5.
34. Rios DL, D'Onofrio LO, Cerqueira C, Bonfim-Silva R, Carvalho HG, Santos-Filho A, et al. Paraoxonase 1 gene polymorphisms in angiographically assessed coronary artery disease: evidence for gender interaction among Brazilians. Clin Chem Lab Med. 2007;45:874–8.