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ORIGINAL ARTICLE

Association between activity and genotypes of paraoxonase1 $L_{55}M$ (rs854560) increases the disease activity of rheumatoid arthritis through oxidative stress

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Abstract

Rheumatoid arthritis (RA) is considered as a long-term autoimmune disorder. Gene polymorphism and oxidative stress might be involved in the pathogenesis of the disease. We aimed to determine the association between PON-1L55M polymorphism and its effects on inflammatory markers such as anti-cytroline circulated-peptide (CCP)-antibodies, C-reactive protein (CRP), neopterin serum concentration, arylesterase (ARE) and butyrylcholinesterase (BuChE) activities and total-antioxidant-capacity (TAC) level with the activity of disease in RA patients. This case-control study consisted of 419 RA patients and 397 gender–age-matched unrelated healthy controls from the west of Iran. PON1-L55M polymorphism was detected by real-time-PCR. The TAC level, serum BuChE and ARE activities were determined spectrophotometrically. Anti-CCP-antibody and CRP were measured by ELISA and neopterin level was detected by HPLC. The PON1-M55 allele was associated with increased risk of the RA in cases with moderate or high activity (OR = 1.43, p = 0.023) and also in cases with the presence of anti-CCP antibody (OR = 1.51, p = 0.009). Synergistic effects of PON1 M55 and Q192 alleles resulted in 2.14 times (p = 0.021) increased disease activity among RA patients with moderate or high activity of the disease. RA patients carried both M (PON1 L55M) and Q alleles (PON1Q192R) had higher concentrations of neopterin (p = 0.003), anti-CCP-antibody (p < 0.001) and CRP (p = 0.026) and significantly lower TAC level (p < 0.001) and ARE (p < 0.001) activity compared to controls. The current study suggests there might be a relationship between genetic and activity of PON. Also, the PON1L55M and PON1Q192R could act in synergy to increase the risk of RA and enhance the level of oxidative stress markers.

Keywords Paraoxonase genotypes (PON) · Rheumatoid arthritis (RA) · Anti-CCP-antibody (anti-cytroline circulated peptide (CCP)-antibodies) · CRP (C-reactive protein) · Neopterin · Butyrylcholinesterase activity (BuChE) · Arylesterase activity (ARE)

Abbreviations

RA	Rheumatoid arthritis
MDA	Malondialdehyde
ROS	Reactive oxygen species
CAD	Coronary artery disease
CCP-antibodies	Anti-CCP-antibody anti-cytroline circulated peptide
CRP	C-reactive protein

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BuChE	Butyrylcholinesterase activity
ARE	Arylesterase activity
PON	Paraoxonase

Introduction

Rheumatoid arthritis (RA) is a systemic relapsing autoimmune disease in which inflammatory cells infiltrate into the synovium and subsequently, synovial hyperplasia leads to the damage of bone and articular cartilage [1, 2]. The overall world prevalence of RA (0.18–1.07%) is lower in Asia-Pacific region compared to the United States and Europe [3]. The average age of onset for RA is about 55 years, although it may present at any age and women are more susceptible to it [4]. Although, the exact cause of RA is unknown, genetics, sex, infection, oxidative stress and imbalance in immune system response have important roles in initiation of the disease [5]. Evidences indicate that oxidative stress at sites of chronic inflammation can induce permanent alteration in the DNA sequence and damage of cell membrane [6]. The paraoxonase (PON) gene family; PON1, PON2, and PON3 are located adjacent to each other on the long arm of chromosome 7 between q21.3 and q22.1 [7]. Serum paraoxonase/arylesterase 1 (PON1) is exclusively located on high density lipoprotein (HDL) and coronary artery disease (CAD). The hydrolysis of organophosphate substrates, such as paraoxon attributed to PON1 which explain the ability of HDL to metabolize lipid peroxides and thereby to prevent their accumulation on LDL [8]. Evidence has suggested that gene mutations have different effects on enzymatic activity of PON. One of the polymorphisms of PON1 that results in lower paraoxonase and arylesterase (ARE) activities is $L_{55}M$, where alteration at position 55 leads to Met (M) replacement with the amino acid Leu [9, 10]. We previously reported that decreased ARE activity is associated with Q/Q genotype of $Q_{192}R$ PON1 gene polymorphism in SLE and RA patients, respectively [10, 11]. Butyrylcholinesterase (BuChE) also known as pseudo cholinesterase, or plasma (choline) esterase, that is presented in many organs and choline esters such as the muscle relaxants, succinylcholine, mivacurium, and their related compounds are cleaved by it. The association of BuChE activity with oxidative stress, inflammatory diseases such as lupus, type 2 diabetes mellitus (T2DM), cardiovascular disease, rheumatoid arthritis, preeclampsia, intrauterine insemination and with abnormal lipid profile have been reported [12–15].

Inflammatory markers, antibodies to cyclic circulated peptide (CCP) are new and highly specific tests that play important roles in early diagnosis and treatment of RA and in achieving better patients health and outcome [16]. C-reactive protein (CRP), an acute-phase protein, and the activation of classical complement cascade, increases cytokine production, and enhances the phagocytosis which are partly of its biological functions [17]. In addition, CRP serum level in RA patients has a direct correlation with subclinical atherosclerosis and increases the cardiovascular (CV) mortality [18]. Another unique marker of innate inflammation, neopterin, a small (250 Da) metabolite of the guanosine triphosphate, is released by activated T cells and its serum concentrations are used to assess the progression of viral infections, renal transplant rejection, severe systemic inflammatory diseases, nephritic syndrome, and several autoimmune diseases [19]. In current study, we evaluated the association between polymorphisms of PON1, $L_{55}M$ (rs854560), $Q_{192}R$ (rs662) with inflammatory markers such as anti-CCP antibodies, CRP, and neopterin and with progression of disease in RA patients.

Materials and methods

This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran and was in accordance with the principles of the Declaration of Helsinki I. Written informed consent was obtained from all the participants.

Study design and patients

The present case-control study included 419 cases with RA patients (377 females and 42 males; aged 20–70 years) with disease duration of 5–13 years. The RA patients were identified according to the 1997 ACR classification criteria for rheumatoid arthritis [20] by a rheumatologist. The ANA profile was used to confirm the absence of other autoimmune diseases such as systemic lupus erythematosus. We used the EULAR activity criteria (clinical remission was considered as values below 2.6, low activity was considered as values between 2.6 and 3.2, moderate activity was the values from 3.2 to 5.1 and high activity was the values over 5.1) to calculate disease activity the score of 28 joints (DAS28-CRP) was used [21]. All of the patients were under treatment with corticosteroids and methotrexate. Patients receiving other drugs including therapeutic monoclonal antibodies (anti-TNF or anti-CD20) and smokers were excluded from the study.

The control group consisted of 397 healthy individuals (362 females and 35 males) with no history of autoimmune diseases. Baseline information including demographic and the age of disease onset were collected by face-to-face interviewing.

Chemical analysis

Serum samples obtained from patient and control groups were aliquoted and stored at − 80 °C until use. One hour

ESR was determined by the Westergren method. IgG anti-CCP antibody and serum CRP (hs-CRP, mg/L) levels were measured by Elisa (Genesis Diagnostics), according to manufacturer's instruction (Monobine Inc., USA and Aesku, Wendelsheim, Germany). Neopterin was measured by HPLC [11].

Measurement of serum arylesterase activity (ARE) of paraoxonase

Serum ARE activity of paraoxonase was measured spectrophotometrically using phenylacetate as substrate according to protocol previously described [15, 21, 22].

Measurement of serum levels of total antioxidant capacity (TAC)

The serum levels of TAC were measured using commercially available kits (Randox Laboratories Ltd., Crumlin, Antrim, N. Ireland, Cat. No. NX2332) as previously described [11].

Determination of serum butyrylcholinesterase activity (BuChE)

BuChE activity was determined spectrophotometrically using benzoylcholine chloride (50 µmol/L) as substrate in the presence or absence of the inhibitors, dibucaine hydrochloride (10 µmol/L) and sodium fluoride (50 µmol/L) as previously described [11, 13].

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using phenol chloroform extraction method [22]. Genotyping of all individuals was done without knowledge of their groups or disease. Genotyping of PON1, $L_{55}M$ was performed using the TaqMan allelic discrimination assay as previously described [23].

Statistical analysis

The allelic frequencies were calculated by the gene counting method. The χ^2 test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. The genotypes and alleles frequencies of PON1L_{55}M in RA patients were compared to control group and disease activity (remission-to-low and moderate-to-high) using χ^2 test in three different models: the genotype codominant model, the minor genotype dominant/recessive model, and the minor genotype heterozygous model.

Odds ratios (OR) were calculated as estimates of relative risk for the disease and 95% confidence intervals were obtained by SPSS logistic regression.

The correlation of serum BuChE and arylesterase activities, neopterin, TAC, CRP, anti-CCP antibody and CRP with the PON1 $L_{55}M$ polymorphism between RA patients with and without anti-CCP were calculated using linear regression and an unpaired t test. A two-tailed Student's t test, ANOVA, and nonparametric independent sample Mann–Whitney analyses were used to compare quantitative data. Statistical significance was assumed at the $p < 0.05$. The calculations were performed using the SPSS software (SPSS for windows 16; SPSS Inc. Chicago, IL, USA).

Results

The demographic features and the results of laboratory tests for patient and control groups are shown in Table 1. Details of the clinical and laboratory features of patient and control groups have been previously described [11].

PON1 $L_{55}M$ genotype and allele analysis

The frequency of PON1 $L_{55}M$ genotypes and alleles in RA patient and control groups has been demonstrated in Table 2. The overall distribution of PON1 $L_{55}M$ genotypes and alleles in RA patients were not significantly different from that of the control group. RA patients were divided into two subgroups based on their disease activity score i.e. remission-to-low activity (DAS28-CRP values 0 to 3.2) and moderate-to-high activity (DAS28-CRP from 3.2 and more than 3.2) or the presence or lack of anti-CCP in their serum (the anti-CCP level of ≥ 6.25 RU/mL was considered as threshold for a positive result). Comparison of clinical, laboratory features, and the risk factors in subgroups of RA patients are demonstrated in Table 3a and b that have been previously described [11].

The overall distribution of PON1L_{55}M genotypes in RA patients with moderate or high activity was not significantly differences compared to RA patients with remission or low activity ($\chi^2 = 3$, df = 1, $p = 0.08$), while overall distribution of PON1L_{55}M genotypes in RA patients with the presence of anti-CCP was significantly different compared to anti-CCP negative RA patients ($\chi^2 = 6.1$, df = 1, $p = 0.01$).

The PON1L_{55}M polymorphism was significantly associated with increased severity of the disease in patients with moderate or high activity in dominant model [(M/M + L/M vs. L/L: OR = 1.53, 95% confidence interval (CI) = 1.01–2.28, $p = 0.043$ and PON1 $L_{55}M$, M allele: OR = 1.43, 95% CI = 1. 2–1.4, $p = 0.023$] compared to those with remission or low activity and also in cases with the presence of anti-CCP antibody [(codominant M/M vs. L/L

Table 1 The demographic characteristic of patients with rheumatoid arthritis (RA) and control subjects in population of Western Iran

Parameter	RA patients (n = 419)	Control subjects (n = 397)	p values
Age (years)	49.8 ± 12.3	48.6 ± 11.98	0.45
Sex(F/M)	377/42	363/36	0.21
Arylesterase activity (ARE)(U/ml)[a,b]	99 (90–137)	130 (122–187)	< 0.001
Butyrylcholinesterase activity BuChE (U/L)[a]	982 ± 253.5	1037 ± 310	0.006
TAC (mmol/L)	0.967 ± 0.36	1.109 ± 0.555	< 0.001
Neopterin (nmol/L)[b]	4.9 (3.2–9.7)	4 (2.8–5.8)	< 0.001
Anti-CCP Ab (RU/mL)[b]	6.5 (1.4–47.8)	1.6 (0.9–2.6)	< 0.001
CRP (mg/L)[b]	1.6 (0.9–2.6)	3.34 (0.68–8.8)	< 0.001

The comparison of serum arylesterase activity and butyrylcholinesterase activity, neopterin and TAC between patients and controls were used by non-parametric 2 independent sample test Mann–Whitney and age by the two –tailed Student's t test and sex by the χ^2-test

Results were expressed as mean ± SD for normally distributed data

[a]μmol L^{-1} min^{-1} at 25 °C, substrate benzoylcholine chloride, μmol mL^{-1} min^{-1} at 37 °C, substrate phenylacetate

[b]Median and interquartile range (IQR) for non-normally distributed data

Table 2 Distributions of genotypes and alleles of PON $L_{55}M$ (rs854560) between RA patients and control subjects

	RA patients (n = 419)	Control subjects (n = 397)	
PON 55 rs854560 genotypes			
L/L	210 (50.1%)	210 (52.9%)	$\chi^2 = 1.1$, df = 2, p = 0.56
L/M	172 (41.1%)	148 (37.3%)	
M/M	37 (8.8%)	39 (9.8%)	
PON 55 rs854560 allele			
L	592 (70.6%)	568 (71.4%)	$\chi^2 = 0.16$, df = 1, p = 0.7
M	246 (29.4%)	226 (29.3%)	

Distributions of genotypes and alleles of PON 55 frequency in RA patients compared with control was made using χ^2 test analysis

OR = 2.67, 95% CI = 1.15–5.9, p = 0.016, dominant model M/M + L/M vs. L/L OR = 1.54, 95% confidence interval (CI) = 1.04–2.3, p = 0.033 and PON1 $L_{55}M$, M allele: OR:1.51, 95% confidence interval (CI) = 1.051–1.44, p = 0.009)] compared to RA patients with anti-CCP negative (Table 4a and b).

Synergistic effect of PON 55 and PON 192 polymorphisms in RA patients with anti-CCP positive compared to RA patients with anti-CCP negative

Interaction of PON1 $L_{55}M$ and PON1 $Q_{192}R$ polymorphisms comparing RA patients with anti-CCP positive and those negative for anti-CCP and also comparing patients with remission or low activity and those with moderate or high activity after adjusting sex and age on RA risk are shown in Table 5a and b. As shown in Table 5, the concomitant presence of the Q allele of PON1 $Q_{192}R$ and the M allele of PON1 $L_{55}M$ significantly increased the severity of disease among RA patients with moderate or high activity [OR = 2.14 (CI = 1.05–1.6, p = 0.021)]. In addition, RA patients with anti-CCP positive and the concomitant presence of PON1 Q and PON1 M alleles had a trend toward increased risk of developing the disease [OR = 1.73 (CI = 0.9, 1.6, p = 0.051)].

Comparing inflammatory and oxidative stress parameters between RA patients and controls in the presence of PON-192 Q and PON-55 Met alleles compared to the absence of both alleles

RA patients with both PON1 Met and Q alleles had higher concentration of neopterin [5.6 (3.3–8.7) vs. 3.9 (2.8–5.9) nmol/L, (p = 0.003)], anti-CCP Ab [8 (2–76) vs. 1.4 (0.7–2.7) RU/mL, (p < 0.001)] and CRP [4.4 (1.1–9.2) vs. 1.8 (1.2–2.6) mg/L, (p = 0.026)] and significantly lower TAC level [0.306 (0.23–0.36) vs. 0.33 (0.28–0.42) mmol/L, (p < 0.001)] and ARE activity [101 (91–137) vs. 122 (121–135), U/mL, (p < 0.001)] compared to controls (Table 6). Furthermore, as indicated in Table 6 the RA patients with the lack of PON1 M and Q alleles had significantly higher neopterin (4.5 ± 2.9 vs. 2.9 ± 2.1 nmol/L, p = 0.024), anti-CCP Ab [6 (0.7–42) vs. 1.6 (1.1–2.7) RU/mL, (p = 0.001)], CRP [2.6 (0.3–8.1) vs 1.6 (0.9–2.9) mg/L, (p = 0.039)] and significantly lower TAC level [0.31 (0.25–0.41) vs. 0.37 (0.3–0.43) mmol/L, (p = 0.039)] and ARE activity [95 (91–137) vs. 130 (122–187), U/mL, (p < 0.001)] compared to control subjects. In addition, we compared the results between RA patients M55 and Q192 negative versus M55 and Q192 positive. We found that RA patients with both M55 and Q192 alleles compared to RA M55 and Q192 negative had significantly higher serum

Table 3 Comparison of risk factors with activity of rheumatoid arthritis disease in (RA) patients in a population from west of Iran

(a) Parameter	RA patients with remission and low activity (n = 300)	RA patients with moderate and high activity (n = 119)
Age (years)	49.2 ± 12.1 p = 0.08	51.5 ± 12.7
Sex (F/M)	268/31 p = 0.73	108/12
Joint tenderness	0.947 ± 1.73 p < 0.001	4.69 ± 3.4
Joint swelled	0.258 ± 0.834 p < 0.001	1.966 ± 2.167
1 h Erythrocyte sedimentation rate (mm/h)	16.24 ± 13.6 p < 0.001	24.18 ± 19.5
CRP (mg/L)	4.2 ± 5.5 p < 0.001	10.29 ± 8.9
Anti-CCP (U/mL)	40.2 ± 73.5 p = 0.6	43.6 ± 66
Duration RA (Years)	9.8 ± 6.8 p = 0.39	10.5 ± 6
RA activity (DAS28-CRP)[a]	2.12 ± 0.75 p < 0.001	3.83 ± 0.98
(b) Parameter	RA patients with anti-CCP positive(n = 193)[b]	RA patients with anti-CCP negative(n = 199)
Age (years)	49.2 ± 12.1 p = 0.08	49.8 ± 12.8
Sex (F/M)	193/27 p = 0.11	183/16
Joint tenderness	2.26 ± 3.26 p = 0.062	1.73 ± 2.37
Joint swelled	00.95 ± 1.87 p = 0.002	0.52 ± 1.11
1 h Erythrocyte sedimentation rate (mm/h)	19.53 ± 16.9 p = 0.16	17.35 ± 14.69
CRP (mg/L)	6.75 ± 8.15 p = 0.027	5.1 ± 5.9
Anti-CCP(U/mL)	76.9 ± 83.9 p < 0.001	1.75 ± 1.77
Duration RA (years)	10.2 ± 6.6 p = 0.37	9.8 ± 6.5
RA activity (DAS28-CRP)[a]	2.68 ± 122 p = 0.17	2.52 ± 1

[a]For calculation of disease activity score of 28 joints (DAS28-CRP), we used the EULAR activity criteria (clinical values 0 to 3.2., remission and low activity, clinical values moderate and high activity from 3.2 and more than 3.2 values)

[b]The anti-CCP level ≥ 6.25 RU/ml was considered as threshold for a positive result

neopterin and CRP concentrations and significantly lower ARE activity.

Also, we compared the same parameters (inflammatory and stress parameters) between controls with protective haplotype versus controls with risk haplotype (Table 6). Results of the analysis demonstrated that healthy controls with the protective haplotype (the absence of M55 and Q192 alleles) versus controls with the risk haplotype (the presence of both M55 and Q192 alleles) had significantly higher ARE activity [130 (122–187) vs. 122 (121–135), U/mL, (p = 0.001)], and TAC level [0.37 (0.3–0.43) vs. 0.33 (0.28–0.42), mmol/L, (p = 0.043)] and lower level of neopterin [2.9 ± 2.1 vs. 3.9 ± 2.8 nmol/L, (p = 0.038)].

Discussion

RA is a chronic disease with unknown etiology that typically follows an unpredictable course with progressive joint destruction and persistent pain [5, 8, 24]. One of the most

Table 4 Odd ratio of genotypes and alleles of PON 55 rs854560 in patients with remission or low activity and moderate or high activity and comparing RA patients with anti-CCP positive and anti-CCP negative subjects after adjusting sex and age

(a)	RA patients with moderate or high activity (n = 146)	RA patients with remission or low activity (n = 273)
PON1 55 genotypes		
Codominant M/M versus L/L	n = 17 (20.2%) versus n = 66 (79.8%) 1.52 (1.01–2.3, p = 0.043) $\chi^2 = 3$, df = 1, p = 0.086	n = 20 (12.5%) versus n = 106 (87.5%)
Dominant M/M + M/L versus L/L	n = 83 (43.2%) versus n = 63 (53%) 1.53 (1.01–2.28, p = 0.043) $\chi^2 = 4.1$, df = 1, p = 0.043	n = 126 (50%) versus n = 147 (50%)
PON1 55 alleles		
L	Reference group n = 192 (65.8%)	Reference group n = 400 (73.3%)
M	n = 100 (34.2%) 1.43 (1. 2–1.4, p = 0.023) ($\chi^2 = 5.2$, df = 1, p = 0.023)	n = 146 (26.7%)
(b)	RA patients with anti-CCP positive (n = 247)	RA patients with anti-CCP negative(n = 172)
PON1 55 genotypes		
Codominant M/M versus L/L	n = 28 (19.9%) versus n = 113 (n = 80.1%) 2.67 (1.15–5.9, p = 0.016) $\chi^2 = 6.1$, df = 1, p = 0.013	n = 9 (8.5%) versus n = 97 (91.5%)
Dominant M/M + M/L versus L/L	n = 134 (54.2%) versus n = 113 (45.8%) 1.54 (1.04–2.3, p = 0.032) $\chi^2 = 4.5$, df = 1, p = 0.033	n = 75 (43.6%) versus n = 97 (56.4%)
PON1 55 alleles		
L	Reference group n = 332 (67.2%)	Reference group n = 260 (75.6%)
M	n = 162 (32.8%) 1.51 (1.05.1–1.44, p = 0.009) ($\chi^2 = 8.9$, df = 1, p = 0.009)	n = 84 (24.4%)

important signs of the disease is infiltration of synovium by inflammatory cells that cause the synovial inflammation [25]. Increased oxidative stress, decreased antioxidant levels, and impaired antioxidant defenses exacerbate the disease condition [9, 26].

In the present study we observed that healthy controls with protective haplotype (the absence of M55 and Q192 alleles) compared to controls with the risk haplotype (the presence of both M55 and Q192 alleles) had significantly higher ARE activity, and serum level of TAC and lower neopterin level. These findings could indicate a possible baseline effect of PON1 variants on inflammatory parameters. Also, the presence of PON1M allele significantly increased the severity of the disease both in RA patients with moderate or high activity and in RA patients with anti-CCP positive.

Tanhapoor et al. have previously implied that inflammation, oxidative stress, inflammatory markers, neopterin, and MDA had major roles in the progression of autoimmune diseases such as systemic lupus erythematosus (SLE) [26, 27].

Charles-Schoeman et al. demonstrated that there was a relationship between paraoxonase 1 gene polymorphism and enzyme activity with carotid plaque in rheumatoid arthritis [8]. In addition, they reported oxidative stress from active RA increased oxidized fatty acids in HDL, enhanced HDL dysfunction, and thereby increased the atherosclerotic risk [9].

Chen et al. reported that decreased plasma HDL-cholesterol concentration had a straight association with coronary artery disease (CAD). Antioxidant activity of HDL has been found to be influenced by the activity of paraoxonase (PON) [28]. In addition, in current study, according to dominant model (M/M + L/M vs. L/L) genotypes of PON1 $L_{55}M$ polymorphism had a positive association with the severity of the disease in RA patients with moderate or high activity of the disease in the presence of anti-CCP antibody. Also, the presence of PON1M allele significantly increased the severity of the disease both in RA patients with moderate or high activity and in RA patients with anti-CCP positive. To the best of our knowledge, there is no study examining association between PON1 $L_{55}M$ variants and their effects on inflammatory markers such as anti-CCP antibodies, CRP, and neopterin with susceptibility to RA in different populations. Consistent with our results Hashemi et al. reported that 'the PON1 MM genotype is a risk factor for rheumatoid arthritis' in a population from East of Iran [29]. Asefi et al. reported that not only PON1 M allele is a risk factor for the autoimmune disease of psoriasis but also carriers of this allele had high levels of MDA, APOB and LP (a), high APOB/APOA1 ratio and low ARE activity. The importance

Table 5 Interaction of PON1 55 and PON 192 polymorphisms in RA patients with anti-CCP positive and RA patients anti-CCP negative and in patients with remission or low activity and moderate or high activity after adjusting sex and age on RA risk

(a) PON1-192 Q allele	PON1-55 Met allele	RA patients with anti-CCP positive (n = 247) ORs (95% CI)[a]	RA patients anti-CCP negative subjects n = 172
−	−	Reference group (n = 33, 13.4%)	Reference group (n = 32, 18.6%)
−	+	(n = 4, 1.6%)	(n = 0.0%)
+	−	(n = 78, 31.6%) 1.28 (0.8–1.4, p = 0.64) $\chi^2 = 0.21$[b], df = 1, p = 0.65	(n = 66, 38.4%)
+	+	(n = 132,.53.4%) 1.73 (0.9–1.6, p = 0.051) $\chi^2 = 3.7$[b], df = 1, p = 0.052 $\chi^2 = 8.18$, df = 3, p = 0.042	(n = 74, 43%)
(b) PON1-192 Q allele	PON1-55 Met allele	RA patients moderate or high activity (n = 146) ORs (95% CI)[a]	RA patients with remission or low activity n = 273
−	−	Reference group (n = 15, 10.3%)	Reference group (n = 50, 18.3%)
−	+	(n = 4, 2.7%)	(n = 1, 0.4%)
+	−	(n = 47, 32.2%) 1.6 (0.91–1.8, p = 0.16) $\chi^2 = 2$[b], df = 1, p = 0.16	(n = 97, 35.5%)
+	+	(n = 80, 54.8%) 2.14 (1.05–1.6, p = 0.021) $\chi^2 = 6.1$[b], df = 1, p = 0.014 $\chi^2 = 10.3$, df = 3, p = 0.016	(n = 125, 45.8%)

[a]ORs were calculated using standard logistic regression after adjusting age and sex

[b]Distribution has been compared between corresponding alleles in control group with those in RA patients

of oxidative stress and inflammation in the progression of autoimmunity have been confirmed in a western population of Iran [15]. The SLE patients with one or two copies of the PON1 (L/M + M/M) allele or with BuChE non-UU phenotype had considerable lower serum ARE and BuChE activities compared to carriers of PON1 $L_{55}M$, L/L or BuChE-UU phenotypes, respectively. Moreover, SLE patients had significantly high serum concentrations of MDA, neopterin and LDL-C that suggested these patients could be more susceptible to cardiovascular disease (CVD) development in a population from Western Iran with Kurdish background [12].

Previously, we and Charles-Schoeman et al. reported that PON1 $Q_{192}R$ polymorphism was associated with increased activity of RA [8, 11]. In present study a synergism was observed between the presence of both PON1 Met and PON1 Q alleles with increased severity of the disease in RA patients with anti-CCP positive by 1.73 times (p = 0.051) and in RA patients with moderate or high activity of the disease by 2.14 times (p = 0.021). The RA patients with both PON1 M and PON1Q alleles had significantly lower TAC level and ARE activity and higher serum concentrations of neopterin and anti-CCP Ab. For the first time, our results suggest that M allele of PON1 $L_{55}M$ affects the antioxidant capacity and oxidative stress biomarkers in RA patients from Western Iran and poor antioxidant defense significantly exacerbates the disease severity. In addition, there are numerous studies in the literature confirming the association between the biomarkers of oxidative stress and increased risk or progression of different types of autoimmune diseases [30, 31]. Not only oxidative stress markers of biomolecules such as lipid, protein and DNA, but also antioxidant enzymes are used to assess the antioxidant defense system in the human body against ROS damage [32]. Our results indicated that without considering the effect of PON1 gene polymorphism, RA patients had significantly lower serum ARE and BuChE activities, and TAC level compared to controls. This association was consistent with Gullo et al. study indicated impairment of the antioxidant system leading to arterial stiffness and atherosclerosis in RA subjects [33]. Tak et al. reported that markers of oxidative stress were considerably elevated in synovial fluid in RA patients compared to patients suffering from other forms of arthritis and its genotoxic effects in peripheral blood lymphocyte DNA was observed [6]. This

Table 6 Comparing inflammatory and oxidative stress parameters between RA patients and controls carrying both PON-199 Q and PON-55 Met alleles with those negative for both PON-199 Q and PON-55 Met alleles

	PON-192 Q allele and PON-55 Met allele both negative		PON-192 Q allele and PON-55 Met allele both positive	
	RA patients n = 66	Control subjects n = 52	RA patients n = 203	Control subjects n = 184
ARE (U/mL)	95 (91–137) p < 0.001	130 (122–187) p = 0.012#	101 (91–137) p < 0.001 p = 0.043*	122 (121–135)
Neopterin (nmol/L)	4.5 ± 2.9 p = 0.024	2.9 ± 2.1 p = 0.0122#	5.6 (3.3–8.7) p = 0.003 p = 0.8*	3.9 (2.8–5.9)
Anti-CCP Ab (RU/mL)	6 (0.7–42) p < 0.001	1.6 (1.1–2.7) p = 0.085#	8 (2–76) p < 0.001 p = 0.038*	1.4 (0.7–2.7)
TAC (mmol/L)	0.31 (0.25–0.41) p = 0.039	0.37 (0.3–0.43) p = 0.046#	0.306 (0.23–0.36) p < 0.001 p = 0.25*	0.33 (0.28–0.42)
CRP (mg/L)	2.6 (0.3–8.1) p = 0.039	1.6 (0.9–2.9) p = 0.036#	4.4 (1.1–9.2) p = 0.026 p = 0.038*	1.8 (1.2–2.6)
BuChE (U/L)	1008 ± 224 p = 0. 32	1038 ± 311 p = 0.74#	998 ± 279 p = 0.17 p = 0.9*	1014 ± 311

Results were expressed as mean ± SD for normally distributed data, a median and interquartile range (IQR) for non-normally distributed data

*p = compare the results between RA patients Q192 and M55 negative versus Q192 and M55 positive

#p = compare the results between control groups Q192 and M55 negative versus Q192 and M55 positive

study suggests that negative reciprocal effects of oxidative stress and the PON1 gene polymorphism resulted in the increased levels of lipid peroxidation products and impaired antioxidant systems that are very closely factors affecting the progression and activity of RA disease. New methods of detecting approaches to decrease these biomarkers may assist in the effectiveness of the therapy and improvement of patient's conditions.

Conclusion

For the first time we indicated that in RA patients with anti-CCP positive and one or two copies of PON1 M allele the activity disease of the RA significantly increased in a population from Western Iran. In addition, we found a positive association between the presence of both PON1 L_{55}M, M and PON1 Q_{192}R, Q alleles with significantly lower TAC level and ARE activity and higher serum concentrations of neopterin and anti-CCP Ab in patients. The positive association between PON1 L_{55}M, M and PON1 Q_{192}R, Q alleles and significantly increased the activity of the disease was observed in RA patients with anti-CCP positive and in RA patients with moderate or high activity of the disease. Our findings could be useful in management of the disease. Our results need to be confirmed in various populations with larger sample size.

Limitations of the study

The limitation of the present study is the absence of large sample that could affect the level of obtained p-values for studied parameters especially considering interaction between two polymorphisms. So, the findings of present study need to be confirmed with larger sample size.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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