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Age and gender related *paraoxonase I* gene polymorphisms rs662 and rs854560 and serum activity in subjects with metabolic syndrome in Fars ethnic group

Abdoljalal Marjani[1*], *Atefe Sajedi*[1]

[1]*Metabolic Disorders Research Center, Department of Biochemistry and Biophysics, Gorgan Faculty of Medicine, Golestan University of Medical Sciences, Golestan Province, Gorgan, Iran*

Abstract

Background: The prevalence of Metabolic Syndrome (MetS) is different among different populations. The human *Paraoxonase (PON)* genes have been less reported in the literature. So it is a need of hour to study *PON*. *Aim and Objectives:* To determine the *Paraoxonase I (PON1) gene* polymorphisms and its paraoxonase activity in subjects with and without Metabolic Syndrome (MetS). *Material and Methods:* Determination of the *PON1* gene polymorphisms were done using Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) analysis. *Results:* The *MM, LM,* and *LL genotype* frequencies of the *PON1- L55M* polymorphism were 2.5, 37.5 and 60%; and 27.6, 34.5 and 37.9% and; 19.4, 50 and 30.6% and 17.2, 58.6 and 34.2% in females and males with and without MetS, respectively. Females with *L allele* of *L55M* polymorphism had a nearly 14.86 -fold risk of developing MetS. *Conclusion:* Our study suggested that the decrease of *PON1* enzymatic activity in both genders is an important finding, but the *L55M genotype* and *L allele* in females with MetS are more important risk factors of MetS than *PON1 Q192R* polymorphism. This difference in *L55M* among females may make them more vulnerable than males to MetS.

Keywords: Age, Gender, Paraoxonase I, Metabolic Syndrome, Fars

Introduction

The prevalence of Metabolic Syndrome (MetS) differs in different populations. Some studies have showed that MetS alterations are ethnic groups and gender dependent [1]. According to studies among males and females in the European and European American population, the prevalence of MetS varies from 20-30% [2]. Today, the knowledge about the genetic role of some enzymes in development, diagnosis and prevention of some diseases are very important. Some genes, such as the human *Paraoxonase (PON)* genes have been shown in the development of some diseases such as cardiovascular diseases, rheumatic diseases and cancer [3]. *PON1, PON2* and *PON3* are the human *PON* genes showing almost 60% and 70% similarity at the level of amino acid and at the level of nucleotide, respectively [4]. *PON1* is the most important member of the *PON* gene family. Studies on different ethnic groups have shown genetic variations for *PON1* polymorphism and its activity in different populations [5]. Some controversial findings show that there are differences between genders in relation to serum *PON1* activity [6] as it has been revealed in a study that serum *PON1* activity in females is higher than in males [7]. The most studied polymorphisms refer to the *PON1* coding region *L55M* or rs854560 (Leucine (L) / Methionine (M)) and *Q192R* or rs662 (Glutamine (Q) / Arginine (R)), which significantly affect activity of *PON1* and functional genotype and phenotypes in the estimation of the association between *PON1* and MetS by genotyping of related

SNPs [8]. Understanding of *PON1* enzyme activities in relationship with genotype, phenotype, ethnicity, gender, and age in different ethnic groups may give an effective interpretation for some of the related diseases with MetS. Hence, the aim of the study was to determine the coding region rs662 and rs854560 gene polymorphisms of *PON1* and its paraoxonase activity in subjects with and without MetS, according to age and gender in the Fars ethnic group.

Material and Methods

The Golestan University of Medical Sciences Ethics Committee (Ethic number: IR.GOUMS.REC.1397.229) approved this study, according to the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all individuals included in this study. The samples were collected from the native Iranian Fars ethnic group who were referred to the health center in Gorgan. The study was performed from December 2021 to June 2022. The related experimental study tests were done at the Metabolic Disorders Research Center, Department of Biochemistry and Biophysics, Faculty of Medicine, Golestan University of Medical Sciences**.**

The sample size was based on the study of Rojekar *et al*. [9] considering a confidence interval of 0.95 and statistical power of 80%, and was calculated as 92 females and 94 males. The study included 47 and 46 females and males subjects with MetS and 45 and 48 female and male subjects without MetS, respectively. The exclusion criteria were subjects with cardiovascular, hepatic, renal, or thyroid diseases. An informed consent was taken from all subjects before start of the study.

After 12 hours of fasting, the blood samples (10 ml) were collected from all participants. The serum was separated to determine Fasting Blood Glucose (FBG), High Density Lipoprotein-Cholesterol (HDL-C) and Triglycerides (TGs) using commercial kits and a spectrophotometer method. Commercial kits (Cat No: ZB-PON-96A, Zellbio, Germany) and the ELISA method were used to measure *PON1* activity. Body Mass Index (BMI), units of kg/m^2 was calculated as weight (kg) divided by square body height (m). Waist Circumference (WC) was measured using a tape in centimeters.

A blood pressure monitor (Omron 70JCP; Omron Maussaka, Mie-Ken, Japan) was used to measure Systolic and Diastolic Blood Pressures (SBP and DBP). According to the NCEP, ATP III [10] general agreement worldwide definition of the MetS, the criteria for subjects with MetS were defined by WC: > 102 cm (male), > 88 cm (female); TG levels: >150 mg/dl; HDL-C levels: < 40 mg/dl (male), <50 mg/dl (female); blood pressure: > 110/85 mmHg; and fasting blood glucose levels: > 110 mg/dl. Subjects who had any three criteria of five MetS components were diagnosed with MetS. The rest of the subjects were included in the without MetS group. For genomic DNA of *PON1* extraction, the peripheral blood sample and salting-out method [11] were utilized. Different SNP genotype determination was performed by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP). Amplification of the *PON1* gene was done to detect the *Q192R* (rs662) and L55M (rs854560) polymorphisms. The following primers were used for *PON1* Genotyping of DNA samples:

Forward: 5′- TATTGTTGCTGTGGGACCTG AG- 3′

Reverse: 5′CCTGAGAATCTGAGTAAATCCA CT-3′ (with PCR product size 238bp) primers for *Q192R* (Initial denaturation at 95° C for 5 min, followed by 35 cycles of denaturation at 95° C for 30 s; annealing at 57° C for 30 s; and then extension at 72° C for 35 s, with a final extension step at 72° C for 7 min.).

Forward: 5′-AGAGGATTCAGTCTTTGAGGA AA-3′

Reverse: 5′-CTGCCAGTCCTAGAAAACGTT-3′ (with PCR product size 386 bp) primers for *L55M* (Initial denaturation at 95° C for 5 min, followed by 35 cycles of denaturation at 95° C for 30 s; annealing at 57° C for 30 s; and then extension at 72° C for 35 s, with a final extension step at 72° C for 7 min.).

Amplification of the *PON1* gene was carried out with 20 µl reaction mixtures. The total volume of the reaction mixture for *Q192R* was 20 µl and it contained 10 µLTaq DNA Polymerase 2 × Master Mix (Ampliqon), forward (0.8 µl) and reverse (0.8 µl) primers, deionized sterile water (6.4 µl) and 20 ng template (2 µl). The same reaction mixture volume was utilized for *L55M*, but the only differences between them in their volume, were forward (1 µl) and reverse (1 µl) primers and deionized sterile water (6 µl). Alw I and H in 1II restriction enzymes were used to digest the PCR product of 238 bp for *Q192R* and 386 bp for *L55M* at 55° C and 37° C overnight, respectively.

Electrophoresis in 1.5% agarose gel was used to segregate the enzyme digested fragments. Electrophoresis of DNA fragments was detected by staining an agarose gel with DNA-safe stain. The bands were detected with a polaroid gel camera.

Statistical analysis

Data were analyzed with SPSS Statistical software (Version 16.0, Chicago for Windows) and were shown as medians with Interquartile Ranges (IQR) and percentages. The Kolmogorov-Smirnov test was used for the definition of normality of data between the subjects with and without MetS. Mann-Whitney U test was used to determine data with non-normal distribution. Comparison of the subjects with and without MetS was done with the Mann-Whitney U test. A *post hoc* test was performed to consider genotype differences in two groups. Pearson's Chi-square and Fisher's exact tests were used to analyze studied variables as a percentage. Values of $p < 0.05$ were considered significant. The association between females with the *L allele* of *L55M* polymorphism with the risk of developing MetS was performed by determent of Odds Ratios (OR) and 95% Confidence Intervals (95% CI) (Logistic regression).

Results

Table 1 shows the clinical and demographic characteristic in females and males with and without MetS. The median age of females and males with and without MetS was 56 and 55 years; and 56 and 54 years, respectively. Females and males with MetS showed significantly higher BMI, WC, TG, SBP, DBP (except males) and FBG levels in comparison with those without MetS. Females and males showed lower HDL-C levels (not significant in females) and *PON1* activity when compared to subjects without MetS (Table1).

Table 2 shows clinical and demographic characteristics in females and males with and without MetS according to age groups. At ages < 50, 50-60 and > 60 years, females and males with MetS showed significant differences in FBG and TG levels (except TG at age >60 in females), WC, SBP (except at age < 50 in males) and *PON1* activity

Table 1: Clinical and demographic characteristic in females and males with and without MetS.

Parameters	Females (n=92)			Males (n=94)		
	MetS+	MetS-	*p*	MetS+	MetS-	*p*
Number of subjects n (%)	47(51.08)	45(48.92)	-	46(48.94)	48(51.06)	0.76
Median (IQR)						
Age (years)	56(10)	55(11)	0.43	56.5(12)	54.5(8)	0.2
BMI (kg/m^2)	31.47(5.86)	23.88(3.6)	<0.001	30.67(3.11)	23.12(4.2)	<0.001
FBG (mg/dl)	131(34)	89(10.5)	<0.001	124(39.5)	89.5(9)	<0.001
WC (cm)	111(15)	84(14.5)	<0.001	116.5(12.5)	89(14.75)	<0.001
SBP (mmHg)	13.6(1.2)	11(1.85)	<0.001	13.6(2.5)	11(1.97)	<0.001
DBP (mmHg)	8.6(0.9)	8(2.15)	0.001	8.2(1.7)	8.1(1.65)	0.41
TG (mg/dl)	150(97)	79(29)	<0.001	134.5(106.25)	83.5(27.75)	<0.001
HDL-C (mg/dl)	3(1)	3.3(0.75)	0.09	3.25(0.5)	4(2)	<0.001
PON 1 (U/mL)	13.56(10.6)	38.91(49.53)	<0.001	13.85(8.55)	36.23(58.38)	<0.001

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome. WC: Waist circumference SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood sugar, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol and PON 1: Paraoxinase 1, IQR: Interquartile Range.

when compared to subjects without MetS, respectively. At ages 50-60, there was the only significant difference in DBP and HDL-C levels in females with MetS. At ages < 50 and 50-60 years, males with MetS showed significant differences in HDL-C levels.

The genotypes and allele frequency distribution for the *L55M* and *Q192R* polymorphisms of the *PON1* gene in females and males with and without MetS are shown in table 3. The frequencies of the *MM, LM,* and *LL* genotypes of the *PON1* gene *L55M* polymorphism were 2.5, 37.5 and 60%; and 27.6, 34.5 and 37.9% in females with and without MetS, respectively ($p = 0.007$). The frequencies of these genotypes were 19.4, 50 and 30.6% and 17.2, 58.6 and 34.2% in males with and without MetS, respectively ($p > 0.05$).

The frequencies of the QQ, QR, and RR genotypes of the *PON1* gene *Q192R* polymorphism were 50, 47.6 and 2.4%; and 56.8, 34.1 and 9.1% in females with and without MetS, respectively. Males with and without MetS showed the frequencies of these genotypes 61, 29.2 and 9.8% and 56.2, 35.4 and 8.3%, respectively ($p > 0.05$; Table 3). The frequencies of these genotypes were 61, 29.3 and 9.8% and 56.2, 35.4 and 8.3% in males with and without MetS, respectively ($p > 0.05$; Table 3).

Table 2: Characteristic of study subjects according to age groups

Ages (years)	Parameters	Females (n=92)			Males (n=94)		
		MetS+ Median (IQR)	MetS- Median (IQR)	*p*	MetS+ Median (IQR)	MetS- Median (IQR)	*p*
<50	FBG (mg/dl)	126(23.25)	87.5(8.5)	⩽0.001	134(74)	87(11)	⩽0.001
	WC (cm)	109(18)	80.5(16.25)	⩽0.001	112(7)	85(12)	⩽0.001
	SBP (mmHg)	13.2(2.95)	11(2)	0.007	13.4(4)	11(1.7)	0.07
	DBP (mmHg)	8.45(0.8)	8(1.97)	0.22	8.2(0.5)	8.1(1.9)	0.87
	TG (mg/dl)	142(94.25)	73.5(51.5)	0.003	217(210)	91(27)	⩽0.001
	HDL-C (mg/dl)	3(1)	3.1(1.02)	0.55	3.1(0.6)	5(3)	0.001
	PON 1 (U/mL)	18.27(10.88)	30.66(33.92)	0.045	12.97(9.43)	24.76(60.14)	0.003
	FBG (mg/dl)	135(43.5)	89(10.5)	⩽0.001	122(25)	91(9)	⩽0.001
	WC (cm)	111(17)	85(13)	⩽0.001	121(17)	89(17)	⩽0.001
	SBP (mmHg)	13.6(0.85)	11.1(1.9)	⩽0.001	13.6(2)	11(1.2)	⩽0.001
50-60	DBP (mmHg)	8.6(0.9)	6.5(2.6)	0.01	8.3(1.6)	8(2.8)	0.65
	TG (mg/dl)	158(81.5)	74(18.5)	⩽0.001	126(84)	82(21)	⩽0.001
	HDL-C (mg/dl)	3.3(0.8)	4(1.5)	0.03	3.3(0.5)	4(0)	0.001
	PON 1 (U/mL)	12.97(5.6)	38.91(49.83)	⩽0.001	15.3(9.43)	36.5(42.46)	0.001
	FBG (mg/dl)	146(47.75)	94.5(11)	⩽0.001	118(37.25)	89(7.75)	0.001
	WC (cm)	111.5(10.25)	84.5(15)	0.001	116(13.25)	86(19.75)	0.002
	SBP (mmHg)	13.9(2.05)	11(1.85)	⩽0.001	14.8(1.9)	11(1.38)	0.001
>60	DBP (mmHg)	8.6(0.85)	8.1(2.32)	0.16	8.25(0.92)	8.2(1.2)	0.35
	TG (mg/dl)	100.5(111.25)	96(33.25)	0.68	111.5(52.25)	92(48.75)	0.007
	HDL-C (mg/dl)	3(1.25)	3.3(0.4)	0.59	3.25(0.9)	4(1)	0.41
	PON 1 (U/mL)	14.43(20.42)	52.18(68.63)	0.004	12.09(7.36)	72.52(78.28)	0.001

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome, WC: Waist circumference SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol and PON 1: Paraoxinase 1. IQR: Interquartile Range.

The *L* and *M allele* frequencies were 97.5 and 40%; and 72.4 and 62.1% for *PON1-L55M* in females with and without MetS, respectively. The *L allele* frequency in the females with MetS was significantly higher than those without MetS ($p = 0.003$; Table 3). The frequency of the *L allele* was higher than that of the M allele in females with MetS. The *L* and *M* allele frequencies were 80.6 and 69.4%; and 82.8 and 75.9% for *PON1-L55M* in males with and without MetS, respectively. The Q and R allele frequencies for *PON1-Q192R* were 97.6% and 50% and 90.9% and 43.2% % in females and 90.2% and 39%; and 91.7% and 43.8% % in males with and without MetS, respectively ($p > 0.05$; Table 3).

Table 3: Genotype and allele frequency of PON1 (L55M and Q192R polymorphisms) in all subjects

Genotypes	Females (n=92)			Males (n=94)		
	MetS+ n (%)	MetS- n (%)	*p*	MetS+ n (%)	MetS- n (%)	*p*
L55M polymorphism						
MM	1(2.5)	8(27.6)	0.007**	7(19.4)	5(17.2)	0.78*
LM	15(37.5)	10(34.5)		18(50)	17(58.6)	
LL	24(60)	11(37.9)		11(30.6)	7(24.1)	
Allele frequency						
LM	39(97.5)	21(72.4)	0.003	29(80.6)	24(82.8)	0.98**
	1(2.5)	8(27.6)		7(19.4)	5(17.2)	
ML	24(60)	11(37.9)	0.09	11(30.6)	7(24.1)	0.56*
	16(40)	18(62.1)		25(69.4)	22(75.9)	
Q192R polymorphism						
QQ	21(50%)	25(56.8%)	0.24**	25(61)	27(56.2)	0.81**
QR	20(47.6%)	15(34.1%)		12(29.3)	17(35.4)	
RR	1(2.4%)	4(9.1%)		4(9.8)	4(8.3)	
Allele frequency						
QQ	41(97.6%)	40(90.9%)	0.36**	37(90.2%)	44(91.7%)	0.98**
RR	1(2.4%)	4(9.16%)		4(9.8%)	4(8.3%)	
QR	21(50%)	25(56.8%)		25(61%)	27(56.2%)	
RR	21(50%)	19(43.2%)		16(39%)	21(43.8%)	

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome. IQR: Interquartile Range.

Table 4 shows L allele frequency of *L55M* polymorphism in females with and without MetS. Logistic regression analysis showed that females with L allele of *L55M* polymorphism had a nearly 14.86 -fold risk of developing MetS (ORs 14.86; 95%CI 1.74 to 126.97; $p = 0.01$) (Table 4). Table 5 shows the genotypes frequencies of *PON 1 (L55M* and *Q192R* polymorphisms) in females and males with and without MetS according to age groups. There was no significant difference between the *L55M* and *Q192R* genotypes in females and males with MetS and those without MetS in all age groups (Table 5). There were also no significant differences between age groups with regard to age and gender ($p = 0.55$, not shown).

Table 6 shows age related serum *PON1* activity between age groups and polymorphisms of *L55M* and *Q192R* genotypes in subjects with and without MetS. There were no significant differences between *PON1* activity and age groups and polymorphisms of *L55M* and *Q192R* genotypes in both groups.

Table 4: L Allele frequency of L55M polymorphism in females

Females				*p*	OR	95% CI	
Allele		MetS+	MetS-			Lower	Upper
L	L	39(97.5)	21(72.4)	0.01	14.86	1.74	126.97
	M	8(27.6)	1(2.5)				

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome.
OR: Odd ration, CI: Confidence interval

Table 5: Genotype frequency of PON1 (L55M and Q192R polymorphisms) in all subjects according to age groups

Age (year)	Genotypes	Females Median (IQR)		*p*	Genotypes	Males Median (IQR)		*p*
	L55M polymorphism	MetS+	MetS-		L55M polymorphism	MetS+	MetS-	
<50	LL LM MM	6(54.5) 5(45.5) -	2(33.3) 1(16.7) 3(50)	0.054	LL LM MM	5(38.5) 6(46.2) 2(15.4)	4(33.3) 6(50) 2(16.7)	1
50-60	LL LM MM	13(65) 6(30) 1(5)	7(41.2) 7(41.2) 3(17.6)	0.28	LL LM MM	5(31.2) 7(43.8) 4(25)	3(20) 10(66.7) 2(13.3)	0.43
>60	LL LM MM	5(55.6) 4(44.4) -	2(33.3) 2(33.3) 2(33.3)	0.29	LL LM MM	1(14.3) 5(74.1) 1(14.3)	- 1(50) 1(50)	0.58
Q192R polymorphism								
<50	QQ RQ RR	5(45.5) 6(54.5) -	8(57.1) 5(35.7) 1(7.1)	0.82	QQ RQ RR	10(71.4) 1(7.1) 3(21.4)	7(46.7) 7(46.7) 1(6.7)	0.064
50-60	QQ RQ RR	11(50) 10(45.5) 1(4.5)	11(52.4) 8(38.1) 2(9.5)	0.81	QQ RQ RR	9(50) 8(44.4) 1(5.6)	15(55.6) 9(33.3) 3(11.1)	0.74
>60	QQ RQ RR	5(55.6) 4(44.4) -	6(66.7) 2(22.2) 1(11.1)	0.62	QQ RQ RR	6(66.7) 3(33.3) -	5(83.3) 1(16.7) -	0.60

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome

Table 6: Genotype frequency of *PON1* (*L55M* and *Q192R* polymorphisms) in all subjects according to age groups

Ages/ Genotypes	< 50 (years) Median (IQR)		*p*	50-60 (years) Median (IQR)		*p*	>60 (years) Median(IQR)		*p*
	MetS+	MetS		MetS+	MetS		MetS+	MetS	
L55M									
LL	19.45 (10.83)	47.76 (76.65)	0.68	12.38 (15.33)	42.15 (65.01)	0.43	9.13 (7.53)	10.67 (27.12)	0.76
LM	12.38 (8.25)	29.48 (50.70)		15.91 (6.48)	34.20 (33.90)		17.09 (12.92)	58.37 (81.96)	
MM	12.08 (2.96)	27.71 (22.13)		14.15 (5.30)	66.04 (115.86)		15.32 (6.30)	40.09 (34.79)	
Q192R									
QQ	12.97 (8.29)	29.48 (30.06)	0.28	12.09 (10.17)	31.25 (35.66)	0.65	11.70 (13.56)	74.18 (68.99)	0.58
QR	17.09 (50.70)	20.34 (65.89)		15.91 (6.19)	61.40 (106.43)		15.91 (8.34)	38.90 (0)	
RR	23.80 (0)	24.17 (0)		20.90 (0)	64.27 (48.05)		20.12 (11.28)	48 (72.26)	

MetS+: With metabolic syndrome, MetS-: Without metabolic syndrome. IQR: Interquartile Range.

Discussion

Results of our study showed that at ages < 50, 50-60 and > 60 years, females and males with MetS showed significant differences in FBG and TG levels (except TG at age >60 in females), WC, SBP (except at age < 50 in males) and PON1 activity when compared to subjects without MetS, respectively. At ages 50-60, there was a significant difference in DBP and HDL-C levels in females with MetS, while males showed significant differences only in HDL-C levels in the age group <50. The frequencies of the *MM, LM,* and *LL* genotypes of the *PON1* gene *L55M* polymorphism showed significant differences in females with and without MetS (p = 0.007). The L allele frequency in the females with MetS was significantly higher than those without MetS (p = 0.003). Studies on the genetic variation in the activity of *PON1* along with SNPs in different ethnic populations are important for showing the possible role of genetic variants in vulnerability to some diseases.

Some results revealed that there was no significant difference when comparing *PON* activity of females and males. They also found that *PON* activity decreases in females, but increases in

males with age [12]. These studies were not in accordance with findings of our study. It has been reported that *PON1* activity stays stable during adulthood or reduces in elderly subjects [7]. It seems that *PON1* activity is new and independent marker for MetS. Sepahvand *et al*. [13] showed that age and gender did not affect *PON1* activity, while another study revealed that *PON1* activity was affected by gender [14]. Some studies reported that according to genetic factors, age had no or negative significant effect on enzyme activity. Then, changes in *PON1* activity seem to be as a result of time progression [7]. This may show a possible effect of age on the levels of *PON1*.

Our study also found lower *PON1* activity in subjects with MetS in both genders and all studied age groups. Studies have shown that subjects with MetS demonstrated no changes in *PON1* activity compared to subjects without MetS in a young population in Turkey with MetS [15]. These may show that age and gender do not affect *PON1* activity. It is also reported that *PON1* activity indicated no significant age-related changes [7]. These studies were in agreement with findings of our study that *PON1* activity is reduced in all subjects of both genders [7]. Gender might have no important effect on *PON1* activities, but it may depend probably on genetic heterogeneity [16]. Subjects with different genotypes may show *PON1* activities changing at different ages, but the findings are not exactly clear. Some studies have demonstrated that there was no significant difference between subjects with and without MetS in the *PON1* polymorphism frequency (*L55M* and *Q192R*) [17]. It seems that the *PON1 L55M* polymorphism genotype may be a risk factor for females in the progression of MetS (nearly 14.86-fold risk of developing MetS) as our study showed.

It has been reported that for the *L55M* SNP, the frequency of 72% of females had the L allele, while the frequency of this allele in the American and European populations were 79% and 64%, respectively [18]. These studies were not in agreement with findings of our study (The female's *L allele* frequency was 97.5%). The findings of some studies have indicated that *allele L* was associated with higher levels and lower activity of the enzyme [19], which is in agreement with our study while, it was also reported that there was no significant difference between the genders in *PON* allele frequency [8], which is in contrast with our study findings.

It may be interpreted that subjects with high *allele L* may have high or low enzyme activity. Some studies have revealed that reduced level and activity of *PON1* has been linked with impaired glucose tolerance in healthy subjects [20]. The reduced *PON1* level and activity may show an important role in the pathogenesis of MetS. Decreased glucose uptake from blood by muscle cells may cause insulin resistance. This may happen with decreased level and activity of *PON1* [20].

In our study, we found polymorphic differences in *PON1 L55M* and not *PON1 Q192R* genotype polymorphism in females with MetS, but gender indicated no significant effect on any of *PON1* activity, as it is also shown in some other study [17]. The study by Himbergen *et al*. on middle-aged females revealed that there is no relationship between *PON1* genetic variants (*L55M* and *R192Q*) and *PON1* activity [21].

Our study indicated that the genotype frequencies of the *MM* were lower and; *LM* and *LL* were higher for the *PON1* gene *L55M* polymorphism in females with MetS compared to those without

MetS. The study by Rios *et al*. has shown that there is an association between gender and *PON1 L55M* polymorphism alteration. They showed that *PON1 LL* genotype was higher among female Caucasian-Brazilians, similar to our study [22].

PON1 Q192R genes *Q* and *R* allele frequencies widely change in different populations. A study showed that *PON1 Q192R* polymorphism is associated with a low risk of heart diseases in Asian and African populations, but not among Europeans and Americans, implicating that ethnicity differences play an important role in the polymorphism effects [23]. *PON1 Q192R* allelic frequencies in our study were different when compared to American, English, Finnish, German and French population [23]. Studies on Chilean and Mexican populations indicated that *Q* allele frequencies were 56% and 51%, respectively [24-25]. *Q* allele frequencies were 54% in a healthy young Turkish population, and also it was 67% in different case control studies [26]. Another study on the Turkish population revealed no significant difference in the distribution of *PON1 Q192R* polymorphism [27].

These studies were almost in accordance with our study in subjects with and without MetS in both genders ($p > 0.05$). Study on *PON1 192 RR* polymorphism also showed *PON1* activity was higher compared with *QR* and *QQ* carriers in both young males and females [28]. They reported that serum *PON1* activity is possibly a suitable marker than the *PON1* genotype in the estimation of the acuteness of some diseases such as atherosclerosis [28]. The relationship between *PON1* polymorphism and some diseases may change by genetic differences of the populations. Findings of the study by Altuner *et al*. showed that *RR* genotype associated with higher *PON* activity than *QQ* or *QR* genotypes and *LL* genotype associated with higher *PON* activity than *MM* genotype in Turkish population [29]. A study has shown that children with the *PON1 192R allele* show increase in *PON* activity with age, showing an important modification by the *PON1* 192 genotype [30]. Along with gender, *PON1* genotype could be influenced by some non-genetic factors like the dietary-related variables, environmental conditions and some lifestyle factors and; additional genetic polymorphisms may cause these differences.

Limitations

Small sample size and age distribution of subjects may cause bias in our study. The estimation of the role of *PON* gene polymorphism in the general population requires large prospective studies with appropriate sample size. Our study did not include information about the dietary-related variables, the influence of environmental factors and some lifestyle factors that may affect MetS. Our study assessed age and gender related gene polymorphisms and serum activity in subjects with MetS in our small selected area, and hence results cannot be generalized.

Conclusion

Our study suggested that the decrease of *PON1* enzymatic activity in both genders is an important finding, but the *L55M* genotype and L allele in females with MetS is more important in risk of MetS than *PON1 Q192R* polymorphism, and suggest that the *PON1* activity and the role of its genetic differences may play an important role in the pathogenesis of the MetS. This genotyping difference for *L55M* in females may be important to make the females more vulnerable than males to MetS (nearly 14.86 -fold risk of developing MetS),

which may mean that the *L55M* genotype in females should be considered an important factor in the interpretation of MetS associated with *PON* polymorphism.

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References

1. Marjani A, Shahini N, Atabay OA, Tabari RG. Prevalence of metabolic syndrome among sistanee ethnic women. *Adv Stud Biol* 2012; 4(8): 363-372.
2. For ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: Findings from the Third national health and nutrition examination survey. *J Am Med Assoc* 2002; 287(3): 356-359.
3. Mackness M, Mackness B. Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. *Gene* 2015; 567(1):12-21.
4. Mohammed CJ, Lamichhane S, Connolly JA, Soehnlen SM, Khalaf FK, Malhotra D, *et al.* A PON for All Seasons: Comparing Paraoxonase Enzyme Substrates, Activity and Action including the Role of PON3 in Health and Disease. *Antioxidants (Basel)* 2022; 11(3):590.
5. Shin BS. Paraoxonase gene polymorphism in south-western Korean population. *J Korean Med Sci* 2009; 24(4):561-566.
6. Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Fontana L, Castaldo M, *et al.* Association between serum paraoxonase (PON1) gene promoter T(-107)C polymorphism, PON1 activity and HDL levels in healthy Sicilian octogenarians. *Exp Gerontol* 2004; 39(7):1089-94.
7. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol* 2005; 69(4):541-550.
8. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 2001; 68(6):1428-1436.
9. Rojekar M, Mogarekar M, Adhe-Rojekar A. Study of Oxidative stress marker serum paraoxonase in metabolic syndrome. Turkish *J Endocrinol Metabol* 2016; 20(4):83-87.
10. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001; 285(19):2486-97.
11. Chang M, Dahl ML, Tybring G, Götharson E, Bertilsson L. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics* 1995; 5(6):358-63.
12. Davis KA, Crow JA, Chambers HW, Meek EC, Chambers JE. Racial differences in paraoxonase-1 (PON1): a factor in the health of southerners? *Environ Health Perspect* 2009; 117(8):1226-1231.
13. Sepahvand F, Shafiei M, Ghaffari SM, Rahimi-Moghaddam P, Mahmoudian M. Paraoxonase phenotype distribution in a healthy Iranian population. *Basic Clin Pharmacol Toxicol* 2007; 101(2):104-107.
14. Sumegová K, Blažíček P, Fuhrman B, *et al.* Paraoxonase 1 (PON1) and its relationship to lipid variables, age and gender in healthy volunteers. *Biologia* 2006; 61:699-704.
15. Tabur S, Torun AN, Sabuncu T, Turan MN, Celik H, Ocak AR, *et al.* Non-diabetic metabolic syndrome and obesity do not affect serum paraoxonase and arylesterase activities but do affect oxidative stress and inflammation. *Eur J Endocrinol* 2010;162(3):535-541.
16. Eckerson HW, Romson J, Wyte C, *et al.* The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. *Am J Hum Genet* 1983; 35(2):214-227.

17. Paragh G, Seres I, Harangi M, Pocsai Z, Asztalos L, Locsey L, *et al.* Discordance in human paraoxonase-1 gene between phenotypes and genotypes in chronic kidney disease. *Nephron Clin Pract* 2009;113(1):c46-c53.
18. Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, *et al.* The international Genome sample resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. *Nucleic Acids Res* 2017; 45(D1):D854-D859.
19. 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(1):62-66.
20. Deakin S, Leviev I, Nicaud V, Brulhart Meynet MC, Tiret L, James RW, *et al.* Paraoxonase-1 L55M polymorphism is associated with an abnormal oral glucose tolerance test and differentiates high risk coronary disease families. *J Clin Endocrinol Metab* 2002; 87(3):1268-1273.
21. van Himbergen TM, van der Schouw YT, Voorbij HA, van Tits LJ, Stalenhoef AF, Peeters PH, *et al.* Paraoxonase (PON1) and the risk for coronary heart disease and myocardial infarction in a general population of Dutch women. *Atherosclerosis* 2008; 199(2):408-414.
22. Rios DL, D'Onofrio LO, Cerqueira CC, 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(7): 874-878.
23. Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop IE, González-Castro TB, Rodríguez-Pérez C, López-Narváez ML, *et al.* Effects of paraoxonase 1 gene polymorphisms on heart diseases: Systematic review and meta-analysis of 64 case-control studies. *Medicine (Baltimore)* 2016; 95(44):e5298.
24. Acuna M, Eaton L, Cifuentes L. Genetic variants of the paraoxonases (PON1 and PON2) in the Chilean population. *Hum Biol* 2004; 76(2):299-305.
25. Gamboa R, Zamora J, Rodríguez-Pérez JM, Fragoso JM, Cardoso G, Posadas-Romero C, Vargas-Alarcón G. Distribution of paraoxonase PON1 gene polymorphisms in Mexican populations. Its role in the lipid profile. *Exp Mol Pathol* 2006; 80(1):85-90.
26. Demirdöğen BC, Demirkaya S, Türkanoğlu A, Bek S, Arinç E, Adali O. Analysis of paraoxonase 1 (PON1) genetic polymorphisms and activities as risk factors for ischemic stroke in Turkish population. *Cell Biochem Funct* 2009; 27(8): 558-567.
27. Bayrak A, Bayrak T, Tokgözoglu SL, Volkan-Salanci B, Deniz A, Yavuz B, *et al.* Serum PON-1 activity but not Q192R polymorphism is related to the extent of atherosclerosis. *J Atheroscler Thromb* 2012; 19(4): 376-384.
28. Bounafaa A, Berrougui H, Ghalim N, Nasser B, Bagri A, Moujahid A, *et al*. Association between paraoxonase 1 (PON1) polymorphisms and the risk of acute coronary syndrome in a North African population. *PLoS One* 2015; 10(8):e0133719.
29. Altuner D, Ates I, Suzen SH, Koc GV, Aral Y, Karakaya A. The relationship of PON1 QR 192 and LM 55 polymorphisms with serum paraoxonase activities of Turkish diabetic patients. *Toxicol Ind Health* 2011; 27(10):873-878.
30. Huen K, Harley K, Brooks J, Hubbard A, Bradman A, Eskenazi B, Holland N. Developmental changes in PON1 enzyme activity in young children and effects of PON1 polymorphisms. *Environ Health Perspect* 2009; 117(10):1632-1638.

Author for Correspondence:
Dr. Abdoljalal Marjani, Metabolic Disorders Research Center, Department of Biochemistry and Biophysics, Faculty of Medicine, Golestan University of Medical Sciences, Golestan province, Gorgan, Iran. E-mail: abdoljalal@yahoo.com and drmarjani@goums.ac.ir Tel & Fax: +98(173)4421651

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