International Immunopharmacology 17 (2013) 974–979

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Effects of paraoxonase 1 activity and gene polymorphisms on long-term pulmonary complications of sulfur mustard-exposed veterans

Ali Taravati [a], Sussan K. Ardestani [a,*], Abed-Ali Ziaee [a], Atefeh Ghorbani [a], Mohammad-Reza Soroush [b], Soghrat Faghihzadeh [c], Hadi Kazemi [d,e], Abbas Rezaei [f], Habibollah Hoseini [g], Tooba Ghazanfari [h,i]

[a] Institute of Biochemistry and Biophysics, Tehran University, Tehran, Islamic Republic of Iran
[b] Janbazan Medical and Engineering Research Center (JMERC), Tehran, Islamic Republic of Iran
[c] Department of Biostatistics and Social Medicine, Zanjan University of Medical Sciences, Zanjan, Islamic Republic of Iran
[d] Shafa Neuroscience Research Center, Tehran, Islamic Republic of Iran
[e] Dep of Pediatrics, Shaheed Mostafa Khomeini Hospital, Shahed University, Tehran, Islamic Republic of Iran
[f] Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran
[g] Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran
[h] Immunoregulation Research Center, Shahed University, Tehran, Islamic Republic of Iran
[i] Department of Immunology, Shahed University, Tehran, Islamic Republic of Iran

ARTICLE INFO

Article history:
Received 2 February 2012
Received in revised form 12 November 2012
Accepted 27 December 2012
Available online 29 January 2013

Keywords:
Sulfur mustard
Paraoxonase 1
Oxidative Stress
Pulmonary problems

ABSTRACT

Sulfur mustard (SM) is an alkylating agent with prolonged adverse effects. The antioxidant paraoxonase 1 (PON1), an endogenous free radical scavenger, plays a protective role against oxidative stress. The possible roles of oxidative stress in the pathogenesis of SM, together with the antioxidant activity of PON1, are enough to warrant the analysis of PON1 polymorphisms and allelic variants in incapacitated veterans. PON1 55 L/M and 192 Q/R polymorphisms were assayed in 289 male veterans with severe pulmonary conditions, who were exposed to SM 20–25 years ago, and 66 gender-, age- and ethnic-matched healthy controls. As we showed previously the PON1 activity decreased significantly in veterans. However, PON1 55 L/M and 192 Q/R genotype distributions were not significantly different between the veterans and the controls. R and L allele carriers have also significantly higher basal and salt-stimulated PON1 activity than Q and M allele carriers. Paraoxonase and arylesterase activities in individuals with the QQ + (MM or LM) genotype were significantly lower than those with the (RR or QR) + LL genotype. Furthermore, basal and salt-stimulated paraoxonase activity in veterans with the (RR or QR) + LL genotype was significantly lower than that in the controls. A positive correlation has been determined between serum PON1 activity and pulmonary function test in QR/LL genotypes. Some of the veterans with RR + QR genotypes have also shown a novel missense change of Asn227Ser in exon 6 of the enzyme. This substitution is close to the binding domain of PON1 and so modifies enzyme activity.

1. Introduction

SM is a powerful vesicant (alkylating agent) with strong cytotoxic properties. It was used extensively during World War I and the Iraq–Iran conflict. Early effects of SM on veterans have been investigated, but there is no comprehensive information concerning the chronic/long-term effects. However, SM-induced toxicity affecting the eyes, skin, and respiratory tract still persist in veterans, many years after exposure to SM. It is proposed that oxidative stress plays an important part in SM intoxication, resulting in the oxidation of the most important macromolecules.

Human serum PON1, located on high density lipoprotein (HDL), prevents the oxidation of LDL and HDL both in vivo and in vitro through the hydrolysis of lipid peroxides [1,2]. The activity of PON1 is subjective to genetic and environmental influences, such as diet, lifestyle, smoking, environmental toxic factors, and physiological and pathological conditions [3]. PON1 seems to contribute to the inhibition of oxidative processes; therefore the study of PON1 activity may prove effective against disease progression in victims of chemical warfare.

PON1 enzyme activity is modulated by a number of polymorphisms in the PON1 gene located on chromosome 7q21.3, which is clustered with at least two other related genes, PON2 and PON3. There is variation in the capacity of PON1 to hydrolyze different substrates. These differences arise from several single nucleotide polymorphisms (SNPs) in coding and non-coding regions. The polymorphism of paraoxonase (A/G) results in glutamine (Q) to arginine (R) substitution at codon

* Corresponding author at: Institute of Biochemistry and Biophysics, Tehran University, P.O. Box 13145-1384, Tehran, Islamic Republic of Iran. Tel.: +98 21 66956976; fax: +98 21 66404680.
E-mail address: ardestani@ibb.ut.ac.ir (S.K. Ardestani).

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http://dx.doi.org/10.1016/j.intimp.2012.12.026

192. PON1Q genotype has lower paraoxonase (paraoxon hydrolyzing), whereas 192 R has a higher hydrolytic activity toward paraoxon. Another coding region polymorphism, amino acid substitution at position 55 Leu(L)/Met(M), has been associated with plasma PON1 protein levels, with PON1M55 having low plasma PON1 levels. The distribution of genotypes in PON1 polymorphisms, Leu55Met (L55M) and Gln192Arg (Q192R), differs greatly among nations. Therefore, it is essential to determine both PON1 activity and PON1 genotype for a more accurate and reliable assessment of veterans' PON1 status. These polymorphisms, together with environmental factors, are responsible for the variation in serum PON1 activity (antioxidant function) among individuals [4].

Based on the importance of PON1's role in risk of disease or exposure, we felt obliged to analyze the polymorphism, allelic variation, and functional activity of PON1 in a population of veterans experiencing the late toxic effects of SM. Also we endeavor to understand the contribution of genetic factors to pulmonary complications in SM-exposed veterans.

2. Materials and methods

The study was performed on 289 SM-exposed male veterans who were exposed to SM 20–25 years later, with clinically significant late pulmonary complications and 66 healthy male members of the victims' families with a similar age distribution as controls. An informed consent was obtained from the subjects prior to admission in the study. A medical history of hypercholesterolemia, hypertension, diabetes, and current smoking habits was obtained from the participants' medical records. Baseline data were collected by trained research assistants during face-to-face interviews. At the time of study (2008), the clinical evaluations and Spirometry was performed for all subjects according to the American Thoracic Society Criteria, and at the same time, the samples were isolated, labeled and aliquots were kept frozen in −70 °C, the experiments were performed after months.

2.1. Blood samples

A fasting venous blood sample was obtained from each participant. The blood samples were collected in K2EDTA-coated tubes. The samples were then centrifuged, aliquot and immediately frozen at −70 °C for later assessment. Serum was used to determine the biochemical parameters [5].

2.2. DNA extraction

A fasting blood sample for DNA isolation was collected in Na-EDTA tubes and DNA was extracted with a salting-out method from peripheral blood leukocytes [6]. The isolated DNA from each subject was quantified by measuring its absorbance at 260 nm.

2.3. Genetic analysis

Standard PCR-RFLP protocols were used to genotype the PON1 192Q/R and 55 L/M polymorphisms.

For the 192 polymorphism, the forward primer was 5′-TATTG TTGCTGTGGGACCTGAG-3′ and the reverse primer was 5′-CCTGAG AATCTGAGTAAATCCACT-3′. For the 55 polymorphism, the forward primer was 5′-GAAGAGTGATGTATAGCCCCAG-3′ and the reverse primer was 5′-TTTAATCCAGAGCTAATGAAAGCC-3′.

Each PCR reaction was performed in a 50 μL final volume containing 1.5 μM MgCl2, 20 pM of each primer, 0.2 μM of the four dNTPs, and 2 units of Taq DNA polymerase (Fermentas, Burlington, Canada). For the Q/R and L/M polymorphisms, 35 and 30 cycles of amplification (95 °C 1 min, 60 °C 1 min, 72 °C 1 min), with a final extension of 7 min at 72 °C, were respectively carried out in a TechNet Touch gene Gradient (USA).

For the 192Q/R polymorphism, 238-bp PCR products were digested overnight at 55 °C with 2 units of BspPI. 170-bp PCR product of the L/M polymorphism (10 μL) was digested overnight at 37 °C with 2.5 units of HindIII restriction endonuclease.

Then digested products were separated by electrophoresis on a 3% agarose gel for 90 min at 55 V, and visualized by UV after ethidium bromide staining.

The nucleotide substitution corresponding to position 192 (Glu-Arg) creates an AlwI restriction site [7,8]. The PON1 192 QQ polymorphism resulted in 66- and 172-bp fragments for the 192RR allele, a non-digested 238-bp fragment for the 192QQ allele, and 66-, 172- and 283 bp fragments for the 192QR. The PON1 55 LL polymorphism corresponded to a 170-bp product, the MM polymorphism to 126- and 44-bp fragments, and the LM polymorphism to 170-, 126-, and 44-bp fragments.

2.4. Mutation screening

DNAs from 40 individuals with the lowest paraoxonase activity (QR and RR) were sequenced. For mutation detection, PCR products corresponding to the PON1 coding sequence (GenBank RefSeq NM_000446) were amplified from genomic DNA, and automated sequencing was performed using Sanger sequencing technique by DNA Technology A/S in Aarhus C. Denmark.

2.5. Statistical analysis

Statistical analyses were conducted using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation. Chi-square test was used for genotype distribution of two study groups. Differences of continuous variables were evaluated by the Student's *t* test.

3. Results

As we previously reported no significant differences in blood glucose, cholesterol, triglyceride, LDL and HDL levels were found between the controls and SM exposed veterans. But the mean of basal and salt-stimulated paraoxonase activity was significantly lower in SM-exposed veterans compared with the controls while there was no significant difference in terms of arylestrase activity. Also, Paraoxonase/arylesterase activity, which represents the real catalytic potential of PON1, was significantly lower in SM-exposed veterans compared with the controls (1.77 ± 0.99, 2.19 ± 1.37, P = 0.005) [5].

Genotype distribution and allele frequencies of the two polymorphisms in veterans exposed to SM and the controls are shown in Table 1. As shown in Table 1, QQ, QR and RR genotype frequencies

Table 1
PON1 genotypes and alleles distribution in sulfur mustard exposed veterans and controls.

Genotype/Allele		Control	Exposed	(chi-square test)
		n (%)	n (%)	
Gene polymorphism (Gln-Arg 192)	RR	4 (6.6)	10 (4.2)	0.479
	RQ	36 (59.0)	129 (54.0)	
	QQ	21 (34.4)	100 (41.8)	
	Q	0.64	0.69	
	R	0.36	0.31	
Gene polymorphism (Leu-Met 55)	MM	8 (13.3)	27 (11.3)	0.882
	LM	26 (43.3)	102 (42.7)	
	LL	26 (43.3)	110 (46.0)	
	L	0.65	0.67	
	M	0.35	0.33	

Standard PCR-RFLP protocols were used to genotype the PON1 192Q/R and 55 L/M polymorphisms.
The chi-square test was used for analysis of the data between gene polymorphism groups.

Table 2
Distribution of double combined genotypes in SM-intoxicated veterans and controls.

	N (%)	
	Control	Exposed
QQ/MM	7 (11.7)	21 (8.8)
QQ/LM	9 (15.0)	41 (17.2)
QQ/LL	4 (6.7)	37 (15.5)
QR/MM	1 (1.7)	6 (2.5)
QR/LM	15 (25.0)	57 (23.9)
QR/LL	20 (33.3)	66 (27.7)
RR/MM	0 (0.0)	0 (0.0)
RR/LM	2 (3.3)	4 (1.7)
RR/LL	2 (3.3)	6 (2.5)

Standard PCR-RFLP protocols were used to genotype the PON1 192Q/R and 55 L/M polymorphisms.

in the controls were 34.4%, 59.0% and 6.6% respectively, while those of the SM-exposed veterans were 41.8%, 54.0% and 4.2% respectively. Accordingly, there was no significant difference in both L/M and Q/R genotype distributions between the veterans and controls. Furthermore, Q and L were the most frequent alleles, while RR was the rarest genotype found in both populations. The distribution of double combined genotypes in the SM-exposed veterans and controls is shown in Table 2. According to this table, the most common PON1-192 and -55 genotypes in both groups were QR and LL, respectively.

PON1 arylestrase and paraoxonase activities in each genotype were evaluated. The relationships between PON1 Q192R genotypes and activity in both groups are summarized in Table 3. Both the veterans and the controls with the QQ192 genotype demonstrated significantly less paraoxonase activity than individuals with either the QR192 or RR192 genotypes. But the basal and salt-stimulated paraoxonase activity in the QR was found to be significantly lower in the veterans compared with the controls. Table 4 also shows PON1 arylestrase and paraoxonase activities in both the veterans and the controls based on PON1 L55M genotypes. As expected, PON1 activity increased in the order MM<LM<LL. But the basal and salt-stimulated paraoxonase activity in the two most active genotypes, LM and LL, were found to be significantly lower in the veterans compared with the controls. However, in terms of arylesterase activity, the PON1 L55M genotypes showed no significant difference between the SM-exposed veterans and controls.

PON1 arylestrase and paraoxonase activities of all double combined genotypes in the SM-exposed veterans and controls are shown in Table 5. In both groups R and L allele carriers have a significantly higher basal and salt-stimulated serum PON1 activity than Q and M allele carriers. Based on high- or low-activity alleles, individuals were split into two groups: individuals with the (RR or QR) + LL genotype belonged to the high-activity group and those with the QQ + (MM or LM) genotype belonged to the low-activity group. Arylesterase, paraoxonase and salt-stimulated paraoxonase activities of each genotype in the two study groups are shown in Table 6. Among the controls, arylesterase activity of the QQ + (MM or LM) genotype (99.17 ± 19.61) was significantly lower than that of the (RR or QR) + LL genotype (126.73 ± 20.14, P<0.001). Also there was a significantly lower arylesterase activity in veterans with the QQ + (MM or LM) genotype compared to those with the (RR or QR) + LL genotype (103.75 ± 28.92 versus 115.82 ± 28.22, P = 0.020). However, there was no significant difference between the controls and veterans in each genotype. Paraoxonase activity in the controls with the QQ + (MM or LM) genotype (75.02 ± 26.77) was significantly lower than those with the (RR or QR) + LL genotype (143.10 ± 38.32, P<0.001). Veterans with the QQ + (MM or LM) genotype have also shown a lower paraoxonase activity compared to those with the (RR or QR) + LL genotype (63.79 ± 31.52 versus 116.95 ± 43.78, P<0.001). Salt-stimulated paraoxonase activity was significantly different between the two genotypes, and the same went for basal paraoxonase activity. But basal and salt-stimulated paraoxonase activity in veterans with the (RR or QR) + LL genotype was significantly lower than those of the controls (P = 0.015). Correlation between spirometric parameter and paraoxonase activity is shown in Table 7. Positive correlation was determined between serum PON1 activity and pulmonary function test in QR/LL genotype. However, in this study as preliminary data genetic variations in exon 6, which is associated with PON1 activity, were examined in 27 SM-exposed veterans with RR or QR genotypes. A novel missense change was identified in this exon. The mutations, 18256 A to T and 18257 A to C, in exon 6 result in a change of codon 227, from AAC to TCC and Asn to Ser substitution. The Asn227Ser change was detected in 40% of veterans with RR + QR genotypes. However, these changes were not observed with the same frequency in normal chromosomes.

Table 3
Paraoxonase and arylesterase activities in Each PON1 Q192R genotype.

		Gene polymorphism (Gln-Arg 192)		
		Control mean ± SD (N)	Exposed mean ± SD (N)	P-value (*t* test)
Paraoxonase (U/mL)	QQ	74.75 ± 27.49 (21)	69.52 ± 33.33 (94)	0.505
	QR	128.06 ± 42.85 (36)	101.08 ± 39.18 (122)	**0.001**
	RR	116.92 ± 51.80 (4)	167.44 ± 49.34 (9)	0.121
Salt-stimulated paraoxonase (U/mL)	QQ	149.67 ± 123.07 (21)	128.36 ± 98.07 (94)	0.393
	QR	319.10 ± 170.86 (36)	224.78 ± 120.80 (122)	<**0.001**
	RR	264.89 ± 189.86 (4)	388.17 ± 134.77 (9)	0.204
Arylesterase (kU/L)	QQ	102.25 ± 19.70 (21)	109.45 ± 30.98 (94)	0.311
	QR	117.31 ± 22.40 (36)	107.72 ± 27.69 (122)	0.06
	RR	126.24 ± 20.80 (4)	121.52 ± 36.93 (9)	0.818

Serum levels of paraoxonase and arylesterase activities were measured enzymatically using their substrates, standard PCR-RFLP protocol was used to genotype the PON1 192Q/R, and a comparison was undertaken between the control and exposed veterans in each genotype. Values are expressed as mean ± S.D. P-value: comparison of exposed veterans with control groups (*t*-test). Bold data shows significant differences with p-value<0.05.

Table 4
Paraoxonase and arylesterase activities in Each PON1 L55M genotype.

		Gene polymorphism (Leu-Met 55)		
		Control mean ± SD (N)	Exposed mean ± SD (N)	P-value (*t* test)
Paraoxonase (U/mL)	MM	64.46 ± 24.18 (8)	63.23 ± 18.24 (23)	0.851
	ML	100.56 ± 38.28 (26)	83.17 ± 41.19 (97)	0.055
	LL	130.76 ± 46.72 (26)	104.27 ± 44.73 (105)	**0.008**
Salt-stimulated paraoxonase (U/mL)	MM	108.96 ± 86.78 (8)	110.44 ± 56.64 (23)	0.965
	ML	240.64 ± 183.15 (26)	168.51 ± 110.64 (97)	**0.013**
	LL	317.55 ± 160.31 (26)	231.89 ± 140.38 (105)	**0.008**
Arylesterase (kU/L)	MM	93.23 ± 20.56 (8)	102.69 ± 31.75 (23)	0.440
	ML	106.97 ± 19.36 (26)	102.18 ± 26.92 (97)	0.398
	LL	123.99 ± 20.72 (26)	117.53 ± 30.00 (105)	0.302

Serum levels of paraoxonase and arylesterase activities were measured enzymatically using their substrates, standard PCR-RFLP protocol was used to genotype the PON1 55 L/M, and a comparison was undertaken between the control and exposed veterans in each genotype. Values are expressed as mean ± S.D. P-value: comparison of exposed veterans with control groups (*t*-test). Bold data shows significant differences with p-value<0.05.

4. Discussion

In this study, the PON1 genotype and allele frequencies in SM-exposed veterans 20–25 years later with pulmonary complications were analyzed against healthy controls.

It was observed that patients with asthma, pulmonary tuberculosis, and COPD showed a lower paraoxonase activity [9,10]. The role of PON1 in protecting the airways against tobacco smoke has already been studied [11]. The association between PON1 polymorphisms and the lung function of smokers, in which 192R allele was introduced as a novel risk factor for airway injury, has previously been examined [12]. Previously we showed the low PON1 activities in SM-exposed veterans in comparison to the control group [5]. In this study, we have showed the significant differences in paraoxonase activities between the controls and the veterans with QR, LM and LL genotypes. The insignificance

Table 5
Paraoxonae and arylesterase activities in term of double combined genotypes in SM-intoxicated veterans and controls.

	N		Arylesterase (kU/L)		Paraoxonase (U/mL)		Salt-stimulated paraoxonase (U/mL)	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
QQ/MM	7	17	89.46 ± 19.00	107.82 ± 33.72	56.85 ± 11.90	59.07 ± 17.96	78.55 ± 12.55	97.98 ± 50.04
QQ/LM	9	40	106.71 ± 17.43	102.02 ± 26.91	89.16 ± 26.90	65.80 ± 35.79	203.60 ± 160.45	114.95 ± 94.88
QQ/LL	4	36	108.93 ± 19.56	119.15 ± 32.35	62.90 ± 25.52	78.83 ± 34.87	113.81 ± 40.64	159.05 ± 112.60
QR/MM	1	6	119.59	88.16 ± 21.29	117.73	75.02 ± 14.34	321.79	145.74 ± 63.91
QR/LM	15	54	104.98 ± 21.18	99.86 ± 24.85	104.71 ± 40.02	90.03 ± 33.20	255.28 ± 193.55	195.10 ± 90.01
QR/LL	20	62	126.45 ± 19.64	116.46 ± 27.87	146.10 ± 37.74	113.22 ± 41.79	366.83 ± 143.38	258.27 ± 137.98
RR/MM	0	0	–	–	–	–	–	–
RR/LM	2	3	122.97 ± 9.27	146.10 ± 35.34	120.73 ± 78.67	191.44 ± 48.69	297.56 ± 293.49	404.12 ± 193.94
RR/LL	2	6	129.52 ± 34.19	109.24 ± 33.75	113.11 ± 42.44	155.43 ± 49.28	232.23 ± 133.20	380.19 ± 117.42

Serum levels of paraoxonase and arylesterase activities were measured enzymatically using their substrates, standard PCR-RFLP protocols were used for PON1 genotyping, number of individual with double combined genotypes determined and all PON1 activities expressed as mean ± S.D.

Table 6
Paraoxonae and arylesterase activities in term of QQ + (MM or LM) and (RR or QR) + LL genotypes in SM-intoxicated veterans and controls.

Polymorphism		Control			Exposed			P-value[b]
		N	Mean ± SD	P-value[a]	N	Mean ± SD	P-value [a]	
Arylesterase (kU/L)	QQ + (MM or LM)	16	99.17 ± 19.61	<**0.001**	57	103.75 ± 28.92	**0.020**	0.554
	(RR or QR) + LL	22	126.73 ± 20.14		68	115.82 ± 28.22		0.097
Paraoxonase (U/mL)	QQ + (MM or LM)	16	75.02 ± 26.77	<**0.001**	57	63.79 ± 31.52	<**0.001**	0.198
	(RR or QR) + LL	22	143.10 ± 38.32		68	116.95 ± 43.78		**0.014**
Salt-stimulated paraoxonase (U/mL)	QQ + (MM or LM)	16	148.89 ± 133.79	<**0.001**	57	109.89 ± 83.94	<**0.001**	0.158
	(RR or QR) + LL	22	354.59 ± 144.96		68	269.03 ± 139.92		**0.015**
Percentage activation by 1 M NaCl (%)	QQ + (MM or LM)	16	82.63 ± 114.22	**0.038**	57	64.48 ± 45.25	<**0.001**	0.335
	(RR or QR) + LL	22	147.12 ± 70.25		68	128.58 ± 76.27		0.316
Paraoxonase/arylesterase activity ratio	QQ + (MM or LM)	16	1.50 ± 1.33	**0.002**	57	1.07 ± 0.65	<**0.001**	0.073
	(RR or QR) + LL	22	2.83 ± 1.15		68	2.34 ± 1.09		0.074

Comparison of paraoxonase and arylesterase activities in veterans and controls with QQ + (MM or LM) and (RR or QR) + LL genotypes, comparison between controls and veterans in each polymorphism groups. P-value[a]: Comparison between polymorphism group (*t* test), P-value[b]: Comparison between control and veterans groups (*t* test). Bold data shows significant differences with p-value < 0.05.

of paraoxonase activity in the RR genotype could be attributed to the small sample size of individuals with the RR genotype. Arylesterase activities were also highest in RR and lowest in QQ, however, differences between the genotype groups were insignificant. This finding is consistent with previous studies, which indicate that arylesterase activity is not polymorphic dependent, and that both 192Q/R isoforms hydrolyze phenylacetate at equal rates [13]. Thus, arylesterase activity was accepted to be a measure of the serum PON1 level, irrespective of the 192Q/R genetic polymorphism.

With respect to 192 Q/R and 55 L/M polymorphisms of PON1, serum PON1 activity increased significantly in the order QQ<QR<RR and MM<LM<LL in all three groups, in agreement with previous studies [14,15]. In our samples, as expected, individuals with genotypes 192 RR and PON1 55 LL had the greatest paraoxon hydrolytic activity, whereas individuals with PON1 192 QQ and PON1 55 MM genotypes had the least activity. Heterozygote combinations of these genotypes had intermediate levels of activity. Our previous results indicated that PON1 activity is lower in SM-exposed veterans with pulmonary complications, and showed a significant relationship between PON1 activity and the severity of complications [5].

Oxidative stress status is known to have a major impact on PON1 activity. Enzyme inactivation could be the consequence of increased oxidative stress [5,16]. It has been established that the PON1R genotype is more sensitive to inactivation by oxidized lipoproteins than the PON1Q genotype [17]. In vitro study has shown that PON1 192Q reduces the lipid peroxide content more than PON1 192R. Also oxidant incubation led to further inactivation of PON1 192*R* than PON1 192Q [18]. This increased oxidative stress presumably constitutes a problem in individuals carrying the 192RR genotype, given the lower efficiency of the 192R isoform in preventing the oxidation of LDL, and its lower resistance compared to 192Q [19]. Because of its lower potential to decrease the lipid peroxides content, the 192R allele or RR genotype is reasonably

Table 7
Correlation of spirometeric parameter and paraoxonase activity in term of Q/R 192 and L/M 55 genotypes in veterans.

		Paraoxonase (U/mL)								
		QQ/MM	QQ/LM	QQ/LL	QR/MM	QR/LM	QR/LL	RR/MM	RR/LM	RR/LL
FVC (%)	r	0.143	0.228	0.127	0.500	−0.200	0.318			−0.447
	P-value	0.625	0.225	0.511	0.667	0.216	**0.034**			0.450
	N	14	30	29	3	40	45	0	3	5
FEV1(%)	r	−0.069	0.131	0.182	0.500	−0.339	0.304			−0.200
	P-value	0.815	0.490	0.345	0.667	**0.032**	**0.043**			0.747
	N	14	30	29	3	40	45	0	3	5
FEV1/FVC (%)	r	−0.057	−0.023	0.089	0.500	−0.186	0.156			0.700
	P-value	0.846	0.903	0.646	0.667	0.252	0.305			0.188
	N	14	30	29	3	40	45	0	3	5

The paraoxonase activity and the pulmonary function parameters (FVC, FEV1, and FEV1/FVC) were assessed in veterans with Q/R 192 and L/M55 genotypes.
r: Spearman's correlation coefficient, FVC: forced vital capacity, FEV1: forced expiratory volume in 1 s. Bold data shows significant differences with p-value < 0.05.

identified as a risk factor for ischemic stroke or carotid atherosclerosis compared with the 192Q allele [20]. Also contradictory result, based on fatty acid oxidation products, has been reported, which asserts that individuals with the 192Q variant have higher levels of PON1 activity and lower levels of systemic oxidative stress [21].

The 55 L/M polymorphism is associated with the enzyme concentration, with no effect on the interaction of PON1 with its substrates. In particular, PON1 55 L allele has a higher concentration in serum, and plays a key role in the correct packing of proteins [22,23]. Also correlation of paraoxonase activity with spirometeric test was determined in order to investigate the importance of Q/R 192 and L/M genotypes on lung function of veterans. Among all genotypes only the QR/LL genotype showed a positive significant correlation with FVC and FEV1. This may be indicative of effective role of paraoxonase with QR genotype on lung function.

A significant increase was found in the PON1 192 RR allele frequencies of the COPD group [10]. However, the PON1 192 QQ and QR genotype frequencies of COPD patients and control subjects were similar. In veteranse Q/R allele frequencies were not significantly different from those of the controls. In addition, no differences in genotype distribution or allele frequency were found between both groups and the general Iranian population [8].

Similar to a previous study [8], the most frequent alleles in this study were found to be Q and L in both the veterans and the controls. The RR genotype was the rarest genotype found in both populations. The QR/ML and QR/LL haplotypes were the most common haplotypes in both the controls and the veterans. However, consistent with a previous study, the RR genotype did not occur in the MM homozygotes, suggesting that this haplotye may be rare in the Iranian population [8]. Other studies have shown that PON1-192RR homozygosity occurs in only about 10% of Caucasians, but in about 40% of East Asians [24]. The ethnic distribution of human PON1 polymorphisms shows great variability around the world, even within the same countries. We found the prevalence of the PON1-192R allele to be 0.36 and 0.31 in the controls and the veterans, respectively. This is similar to that found among Iranians [8], Caucasians of Northern Europe [25], and populations from Turkey and India [8], but lower than other Asian populations from Japan and China [26,27].

The prevalence of the PON1-55 L allele was found to be 0.65, which is similar to that found in the Caucasian population [25], and lower than that found in Japanese and Chinese populations [27]. It is obvious that the PON1 genotype distribution in SM-exposed veterans is not different from that of the controls, therefore, PON1 55 and 192 genetic polymorphisms or combined haplotypes cannot induce the difference in PON1 activity between the two groups. Clearly, other modifications as to the enzyme activity must exist within each genotype group. These modifications may result from intrinsic factors, such as physiological and pathological conditions, as well as polymorphisms that affect the expression and activity of PON1. SM is considered to be genotoxic. However, only limited data are available on the specific mutations caused by SM. We tried to identify possible SM-induced mutations in the exons 6 of the PON1 gene, which contributes to PON1 activity, and to determine whether these mutations are associated with decreased paraoxonase activity in the veterans.

Paraoxonase activity significantly decreased in veterans with either the RR192 or QR192 genotype, R carrier, compared with healthy controls. In agreement with our study, results from recent studies on multiple sclerosis patients were indicative of low PON1 activity in the RR genotype compared to healthy subjects [28,29].

Individuals, who had a lower PON1 activity relative to others with the same genotype, were predicted to have genetic alterations. Marja Marchesani et al. found a new I102V mutation in the coding region of the PON1 gene, reported to be associated with an increased risk of prostate cancer in Finnish men [30].

In the present study, in addition to the evaluation of PON1 activities and 192 and 55 polymorphisms, we decided to undertake a sequence analysis of the R allele carriers with low paraoxonase activity by sequencing exon 6. Comparison of the exon 6 mRNA sequence with other known mammalian species showed 98% nucleotide identity with the corresponding genes from Homo sapiens (GenBank NM_000446).

A novel mutation of PON1 exon 6 has been identified in SM-exposed veterans with severe pulmonary complications. The described mutations in exon 6 result in a change of codon 227, from AAC to TCC and Asn to Ser substitution close to the binding domain of PON1. These changes were not observed with the same frequency in normal chromosomes. This suggests that the N227S is a deleterious mutation, supporting its causative role in pulmonary complication development, and not a genotype distribution. Accordingly this missense change could possibly damage the enzyme activity. Mutations in the regions of the gene that constitutes important sites in the enzyme activity usually lead to a loss of catalytic activity. Although the exact mechanism of how the mutation alters enzyme activity is not known, it seems that the N227S mutation may affect protein function. The glycation of PON1 at Lys70 and Lys75 also showed some conformational changes in the residues D269, N168, and N224, which might contribute towards the lowering of PON activity [31]. Another study on PON1 postulated that residue F222, which is nearby N227, is important for substrate specificity and would influence substrate binding [32]. However, further investigations of the transfected cells with the novel mutation are needed to reveal its structural effect on PON1.

In conclusion the PON1 genotype distribution in SM-exposed veterans was not different from that of controls. Also positive correlation determined between serum PON1 activity and pulmonary function test in QR/LL genotypes and reduced PON1 type R activity among SM-exposed veterans, as opposed to healthy controls, supports the theory that some SM-exposed veterans with this allele may have sustained pulmonary damage from exposure to the chemical.

Acknowledgment

This study was carried out by Institute of Biochemistry and Biophysics of Tehran University, Immunoregulation Research Center of Shahed University, Janbazan Medical and Engineering Research Center (JMERC) and financially supported by the Iranian Foundation of Martyr and Veterans Affairs and the Research Council of University of Tehran. We would like to kindly thank all the participants who took part in this study.

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