



Ain Shams University
The Egyptian Journal of Medical Human Genetics

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ORIGINAL ARTICLE

Contrasting genetic influence of *PON1* coding gene polymorphisms L55M and Q192R on individuals' response to environmental agents



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Received 5 February 2015; accepted 25 February 2015

Available online 31 March 2015

KEYWORDS

Paraoxonase;
L55M;
Q192R;
Organophosphate;
Poisoning grade

Abstract *Background:* Paraoxonase (PON1) is an A-esterase capable of hydrolyzing the active metabolites (oxons) of many organophosphorus (OP) insecticides. Human PON1 displays two polymorphisms in the coding region (Q192R and L55M) and several polymorphisms in the promoter and the 3'-UTR regions. Animal studies have shown that PON1 is an important determinant of OP toxicity though a direct satisfactory verification in humans is still lacking.

Aim: To investigate the impact of polymorphisms in the PON1 coding region (Q192R and L55M) on individual sensitivity to OP poisoning.

Subjects and methods: This study enrolled 42 subjects (21 females and 21 males, age range 1.5–53 years) diagnosed of acute OP poisoning. They were classified into 4 grades according to manifestations. All subjects were genotyped for the PON1 gene polymorphisms; Q192R and L55M using RFLP-PCR, then genotype frequencies were compared between different OP grades.

Results: Genotype frequency distribution of *PON1* L55M polymorphism among different OP poisoning grades revealed no significant difference ($p > 0.05$) between the four grades. In contrast, frequency distribution of *PON1* Q192R polymorphism showed a highly significant ($p < 0.001$) difference between different grades of OP poisoning, with QQ genotype predominating in grade 4 with a frequency of 66.7%, followed by QR genotype (33.3%), while the RR and QR genotypes were similarly distributed in grade 1 with a frequency of 50% for each.

Conclusion: The current results suggest a possible association between QQ genotype and poor OP poisoning prognosis.

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Abbreviations: PON1, paraoxonase I; OP, organophosphate; AChE, acetylcholinesterase; PO, paraoxon; CPO, chlorpyrifos oxon; DZO, diazoxon.

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Peer review under responsibility of Ain Shams University.

<http://dx.doi.org/10.1016/j.ejmhg.2015.02.007>

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1. Introduction

Pesticides represent a large and important class of environmental chemicals, and are often associated with adverse health effects in non-target species including humans. Among the major classes of pesticides, the insecticides are of most concern with regard to potential adverse effects on the nervous system. A major class of insecticides is represented by the organophosphates (OP), triesters of phosphoric acid that exert their toxicity primarily by inhibiting the enzyme acetylcholinesterase (AChE) [1]. OPs undergo bioactivation and detoxification processes that can be affected by genetic polymorphisms in biotransformation enzymes. As numerous genetic variants exist for all these enzymes, such genetic differences can greatly influence the toxicity and neurotoxicity of OPs. Paraoxonase (PON1), is a polymorphic enzyme that is involved in the detoxification of several important OPs. Paraoxonase takes its name from the ability to hydrolyze paraoxon (PO), the highly toxic metabolite of the insecticide parathion. Other OP substrates of PON1 include chlorpyrifos oxon (CPO) and diazoxon (DZO), the active metabolites of chlorpyrifos and diazinon, respectively, as well as the nerve agents sarin, soman and VX [2].

Early studies examining plasma paraoxonase levels in human populations revealed a polymorphic distribution of activity [3]. A single nucleotide polymorphism in the coding region was found to cause variability in the amino acid present at position 192, where either glutamine (Q) or arginine (R) could be present [4,5].

Another polymorphism was identified in the coding region that resulted in an amino acid substitution at codon 55 [leucine (L)/methionine (M)] [6].

The present study was carried out to investigate the impact of polymorphisms in the *PON1* coding region (Q192R and L55M) on individual sensitivity to OP poisoning.

2. Patients

This work was done after taking acceptance of all patients to share in the study as well as acceptance of The Ethics Committee of Ain Shams University. The work has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This retrospective study examined the comprehensive medical, nursing, and intensive care records of 42 patients admitted to the Poisoning Control Center Ain-Shams University Hospitals, for acute OP poisoning between January and December, 2012. Initial diagnoses were established in all cases based on cholinergic clinical features, the odor of OP in the gastric contents, history, and other circumstantial evidence, such as the poison or a label of an OP-containing product found by relatives. The grouping of the OP poisoning cases was done depending upon signs and symptoms as well as their pseudo cholinesterase levels [7] as following (n = number of patients):

Grade 1 – OP poisoned with no signs and symptoms ($n = 2$)

Grade 2 – Diarrhea, vomiting, abdominal pain, giddiness ($n = 9$).

Grade 3 – Pupillary constriction with above symptoms ($n = 16$).

Grade 4 – Pulmonary edema ($n = 15$).

Grade 5 – Unconsciousness ($n = 0$).

3. Methods

DNA was extracted from whole blood using a QIAamp Blood mini-prep Kit (QIAGEN, Germany) according to manufacturer's instructions. Genomic DNA (300 ng) was amplified in a final volume of 50 μ l, containing 10 mM TRIS pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 mM each dNTP, 1 μ l M of each primer and 2 U Taq polymerase (all reagents from MBI Fermentas, St. Leon-Rot, Germany). The thermocycling procedure (9700 apparatus, Applied Biosystem, USA) consisted of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min, repeated for 35 cycles, followed by a final extension at 72 °C for 15 min. PCR was followed by restriction digestion, the nucleotide substitution corresponding to position 55 (Met/Leu) and 192 (Gln/Arg) creates an *Hsp92II* (*Hin1II*) (Fermentas, Germany, ER1831) and *AlwI* (*BspPI*) (Fermentas, Germany, ER1321) restriction site. Individuals homozygous for the L allele showed only the presence of a 384 bp product and those homozygous for the M allele showed 282 and 102 bp products. In Q192R polymorphism, individuals homozygous for Q allele had 150 bp and homozygous for the R allele 89 and 61 bp products. Primer sequences, restriction enzymes and PCR products are listed in Table 1.

3.1. Statistical analysis

Results were analyzed using the Statistical Package of Social Sciences (SPSS) computer software program, version 21.0 (Chicago, IL, USA). Quantitative data were presented as mean \pm SD for normally distributed data. Qualitative data were presented in the form of frequencies and percentages. Differences among groups were tested using Pearson's chi-square test (χ^2). A p value less than 0.05 was considered statistically significant.

4. Results

Among the studied subjects, there were 23.8% smokers and 76.19% were non-smokers. 88% of the cases were poisoned orally, 7.14% were poisoned by inhalation and 4.76% were poisoned dermally. Considering the intention of poisoning; 52.38% were poisoned by accident while 47.61% were poisoned by committing suicide. Delay between poisoning and diagnosis ranged between 2 and 30 h with a mean \pm SD of 10.05 \pm 6.35. Demographic characteristics of the cases are given in Table 2.

Genotype frequency distribution of *PON1* L55M polymorphism among different OP poisoning grades (Table 3) revealed no significant difference between the four grades. However, it was found that grade 4 OP poisoning had a high LM genotype frequency (3.3%), followed by MM genotype with a frequency of 33.3% and LL with a frequency of 13.3%. In contrast, frequency distribution of *PON1* Q192R polymorphism

Table 1 Oligonucleotides used as primers for polymerase chain reaction and restriction endonucleases for restriction analysis [32].

SNP	Primer sequence	PCR product (bp)	Restriction enzyme
L55M	5'-TTGAGGAAAAGCTCTAGTCCA-3' 5'-GAAAGACTTAAACTGCCAGTCC-3'	384	Hsp92II
Q192R	5'-TTGTTGCTGTGGGACCTGAG-3' 5'-AATCCTTCTGCCACCACTCG-3'	150	AlwI

Table 2 Demographic characteristics of the OP poisoned cases.

Characteristic	Value
Number of patients	42
Females/Males	21 (50%)/21 (50%)
Age mean \pm SD (minimum–maximum)	21.69 \pm 10.89 (1.5–53 years)
Smokers/Non-smokers	10 (23.8%)/32 (76.19%)
<i>Route of OP exposure</i>	
Oral	37 (88%)
Dermal	2 (4.76%)
Inhalation	3 (7.14%)
<i>Mode of exposure</i>	
Accidental	22 (52.38%)
Suicidal	20 (47.61%)
Delayed diagnosis mean \pm SD (minimum–maximum)	10.05 \pm 6.35 (2–30 h)

(Table 4) showed a highly significant ($p < 0.001$) difference between different grades of OP poisoning, with grade 4 having a high QQ genotype frequency, followed by QR, with frequency distribution of 66.7% and 33.3%, respectively, while the RR and QR alleles were similarly distributed in grade 1 with a frequency of 50% for each.

5. Discussion

Serum paraoxonase (PON1) is an esterase that is involved in the detoxification of organophosphate insecticides [8,9]. The *pon1* gene contains functional polymorphisms in both the coding and promoter regions. In the coding region, two common polymorphism are a glutamine (Q) to arginine (R) substitution at codon 192 (Q192R) and a leucine (L) to methionine (M) substitution at position 55 (L55M). In Q192R polymorphism the exchange of codon CAA to CGA in exon 6 of *pon1* gene determines isoforms of the enzyme,

Table 3 PON1 L55M genotype frequency in each OP grade.

		Grade of poisoning				Sig.
		1 OP poisoned with no signs and symptoms	2 Diarrhea, vomiting, abdominal pain	3 Pupillary constriction with above symptoms	4 Pulmonary edema	χ^2
LL	No.	0	2	4	2	NS
	%	0%	22.2%	25.0%	13.3%	
LM	No.	2	2	8	8	
	%	100.0%	22.2%	50.0%	53.3%	
MM	No.	0	5	4	5	
	%	0%	55.6%	25.0%	33.3%	
Total	No.	2	9	16	15	

NS = non-significant difference.

Table 4 PON1 Q192R genotype frequency in each OP grade.

		Grade of poisoning				Sig.
		1 OP poisoned with no signs and symptoms	2 Diarrhea, vomiting, abdominal pain	3 Pupillary constriction with above symptoms	4 Pulmonary edema	χ^2
QQ	No.	0	5	7	10	0.001*
	%	0%	55.6%	43.8%	66.7%	
QR	No.	1	4	9	5	
	%	50.0%	44.4%	56.3%	33.3%	
RR	No.	1	0	0	0	
	%	50.0%	0%	0%	0%	
Total	No.	2	9	16	15	

* Significant difference.

which differ greatly in the rate of hydrolysis of a number of substrates. Paraoxon is hydrolyzed at a far greater rate by the R192 isoform compared to the Q192, but some organophosphates and lactones are hydrolyzed faster by Q192 [10,11]. L55M polymorphism (exchange of codon TTG to ATG in exon 3) is correlated with the blood enzyme level, with isoform L55 associated with higher serum enzymatic activity. It is not clear whether this is because of a decreased stability of the M55 alloenzyme and/or because of the linkage disequilibrium with $-107/-108$ T allele [12,13]. Isoform M55 showed lower stability and loses activity more rapidly and to a greater extent than the L isoform [14]. This is due to the key role of L55 in packing in the propeller's central tunnel, and of its neighboring residues which ligate calcium ions [15]. Emerging lines of evidence have shown that functional polymorphisms in the PON1 gene might play a critical role in increasing susceptibility to organophosphate toxicity, but individually published studies showed inconclusive results [9].

The present results suggest a possible relation between QR genotype and poor OP poisoning prognosis, and also suggest that the RR genotype might be excluded as a predictor of poor prognosis. These results are in accordance with Burton et al. [16], who stated that having one of the two slow metabolism (Gln/Gln; QQ or Gln/Arg; QR) genotypes is one of the independent predictors of chronic OP toxicity. The authors observed that the prevalence of chronic toxicity increased in a stepwise fashion, which differed according to the PON genotype, ranging from 15% (slow metabolism (Gln/Gln; QQ or Gln/Arg; QR) to 75% (fast metabolism Arg/Arg; RR)).

Meanwhile, the present results revealed that the distribution of the L55M polymorphism did not differ among the OP poisoning grades. Comparable observation was previously reported [9,17,18], elucidating that L/M polymorphism at position 55 does not affect catalytic activity, but is associated with plasma PON1 protein levels, with PON1M55 being linked with low plasma PON1 [17,18]. This may result primarily from linkage disequilibrium with the low efficiency -108 T allele of the -108 promoter region polymorphism [19]. In contrast, the Q192R polymorphism significantly affects the catalytic efficiency of PON1. This polymorphism is substrate-dependent, as for example, the PON1R192 alloform hydrolyzes chlorpyrifos oxon and paraoxon more rapidly than PON1Q192 in vitro, while both PON1 alloforms hydrolyze diazoxon with the same efficiency [20].

While PON1 has been known for decades to hydrolyze a number of OP substrates in vitro, evidence of the role played by this enzyme in modulating the toxicity of OPs in vivo has emerged only in the past 20 years [9]. Initially, exogenous PON1 was directly injected into rats or mice to determine whether increasing plasma PON1 levels would protect against OP toxicity. Some studies confirmed that administration of PON1, via different routes, protected animals against the toxicity of OPs through decreasing the rate of acetylcholinesterase (AChE) inhibition [21–23], suggesting a potential therapeutic use in OP poisoning, possibly in combination with other conventional treatments [24].

In accordance with our results about PON1Q/R192 are various human studies on OPs toxicities, in several countries under different circumstances, reported that human toxicity with OPs differed according to their genotypes and PON1 polymorphism. In the Gulf area in 1990–1991, studies investigated PON1 polymorphisms in military personnel, who were

exposed to low levels of sarin, OP insecticides, in addition to several other biological and chemical agents [25,26]. They found that PON1R192 homozygotes or PON1Q/R192 heterozygotes were more likely to have neurologic symptoms than individuals homozygous for PON1Q192 [26]. Low activity of the plasma PON1Q192 isoform also appeared to better correlate with illness than the PON1 genotype or the activity levels of the PON1R192 genotype [27]. Another study on South African workers, reported that symptoms of chronic OP toxicity were significantly more likely among subjects with the QQ or QR genotypes than those with the RR genotype [28]. A different supporting study is an occupational one on Brazilian agricultural workers, which conveyed that workers with homozygotes for PON1Q192 presented with higher genotoxic effects (as determined by a higher lymphocyte micronucleus frequency) than other workers [29]. However, this population of workers was exposed to a large number of pesticides; among the few OPs, none was a known PON1 substrate. Thus, the significance of this finding is obscure, at best. In a similar study in India, agricultural workers exposed to unknown OP insecticides were reported to have higher DNA damage in lymphocytes if they had low PON1 status (only identified as QQ/MM genotypes) [30]. Another study in Spain investigated the association between PON1 statuses, OP exposure and thyroid function [31]. Results indicated that impaired thyroid function in OP-exposed agricultural workers was greater in individuals with lower PON1 activity.

6. Conclusion

Overall, the human studies available so far provide initial evidence that low PON1 status may increase susceptibility to adverse effects of certain OP insecticides. Nevertheless, while animal studies indicate that PON1 status is an important determinant of sensitivity to acute toxicity of certain OPs, it is still uncertain whether it may play a significant role at lower dose levels of exposure.

7. Recommendations

Further studies, with larger sample size, under chronic (instead of acute) OP exposure would be needed to ascertain individual's susceptibility to OPs' adverse effects upon chronic low level exposure. Without such confirmatory studies, genetic testing in the work place to screen for individuals at high risk for OP-induced adverse health effects would be inconclusive.

Conflict of interest

The authors declare no conflict of interest. There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

Acknowledgments

This work has been developed at the Toxicology Department, Poison Control Center, Faculty of Medicine, Ain Shams University. We would like to thank the Poison Control Center at the Faculty of Medicine, Ain Shams University for providing us the patients' samples and data.

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