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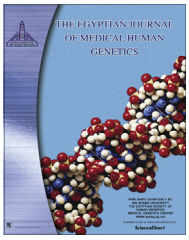

ORIGINAL ARTICLE

Contrasting genetic influence of *PON* 1 coding gene polymorphisms L55M and Q192R on individuals' response to environmental agents

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KEYWORDS

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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 *PON*1 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.

Abbreviations: PON1, paraoxonase 1; OP, organophosphate; AChE, acetylcholinesterase; PO, paraoxon; CPO, chlorpyrifos oxon; DZO, diazoxon.

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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 andDecember, 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 µl, containing 10 mM TRIS pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 1 µl 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 *Hsp*92II (*Hin*1II) (Fermentas, Germany, ER1831) and *Alw*I (*BspP*I) (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 ± 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 ± SD of 10.05 ± 6.35. Demographic characteristics of the cases are given in Table 2.

Genotype frequency distribution of *PON*1 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