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Serum paraoxonase and arylesterase activities in patients with lacunar infarction: A case control study

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ABSTRACT

Objectives: The aim of this study was to assess paraoxonase (PON1) and arylesterase (ARE) activities and polymorphism in patients with lacunar infarctions.

Design and methods: The PON1 activity, ARE and lipid profile were determined in 37 patients and 53 healthy individuals. Descriptive and inferential statistics were used to analyze the data.

Results: A total of 37 patients (20 males and 17 females) were studied. The levels of PON1 activity in patients and healthy individuals were 63.5 ± 46.7 IU/L and 95.5 ± 75.5 IU/L, respectively ($p = 0.024$). The same values for ARE were 54.3 ± 19.3 KU/L for patients and 69.1 ± 29.3 KU/L for controls ($p = 0.008$). Polymorphism in Q192R location shows statistically different presentation in patient group compared to healthy controls with an odds ratio of 3.42 (CI 95%: 1.24–9.44, $p = 0.017$).

Conclusions: According to this study, we suggest that PON1 192R polymorphism may play a minor role as a risk marker for developing lacunar infarctions in a group of Iranian population.

Introduction

Human serum paraoxonase (PON 1) is a polymorphic enzyme protein with 354 amino acids. This gene family consists of three related genes located on the long arm of chromosome 7, proximal to the cystic fibrosis gene [1]. The proteins synthesized mainly in the liver; have 60 to 65% similarity at the amino acid level [2] and bind exclusively to HDL in plasma [3]. The produced enzyme can hydrolyze several organophosphorus compounds like pesticides, arylesters and paraoxon. A genetic polymorphism of PON activity which determines high versus low paraoxon hydrolysis in human populations, may determine sensitivity to parathion poisoning [4]. Its ability to detoxify organophosphate insecticides and nerve gases has been established earlier but the physiologic role in human body has not been discussed completely [5]. Chronic ill health reported by farmers may be due to organophosphate exposure and PON1 Q192R polymorphism associated with lower rates of paraoxon hydrolysis [6]. Turkey, chicken and most other birds, lack paraoxonase activity and are very susceptible to organophosphates [7]. The enzyme is a calcium-dependent esterase and resides on circulating HDL-cholesterol in association with apolipoprotein-A1 [8]. In human being the main action is hydrolyzing lipid peroxides and preventing LDL-cholesterol oxidation [2]. Therefore, the risk of ischemic strokes may be decreased in the adult population. Both enzymes, PON1 and PON2, have similar function in the human beings. There are some different polymorphisms in PON1 but there are only two common and important polymorphisms in the coding region [1]. Glutamine to arginine substitution at position 192 (Q192R) makes the first important polymorphism and the second substitution is leucine (L allele) to methionine (M allele) at position 55 (M55L) [2]. The highest paraoxon hydrolytic activity is related to the PON1 192RR and 55LL and the lowest activity is correlated with the PON1 192QQ and 55MM [4]. The position 192 polymorphism is the major determinant of the PON1 activity polymorphism. However, the position 55 polymorphism also modulates activity [9]. In animal models, ablation of the PON1 gene activity produces inflammatory and atherogenic processes while over-expression of PON1 has anti-inflammatory and anti-atherogenic characteristics. It seems that in diabetes mellitus, glycation has major negative impact on PON1 activity and contributes to the typical inflammatory process of diabetes, leading to the excess atherosclerosis in patients with cerebrovascular diseases [10]. The protective role of PON1 due to its antioxidant activity, against the development of various diseases, such as atherosclerosis, Parkinson's disease, multiple sclerosis and stroke had been discussed elsewhere but the results are inconsistent especially for stroke [1,3,11–13]. Several studies have not found an association between PON and cerebrovascular disorders [2,14–17].

Atherosclerosis is increasingly recognized as an inflammatory disease and stroke is the leading cause of disability worldwide [18].

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However, ischemic strokes are occurring approximately 1 decade earlier in Iran than in other countries [19] and lacunar infarctions (LI) constitute up to 25% of ischemic strokes [20]. In lacunar infarctions small penetrating branches of the cerebral arteries may become occluded, and the resulting infarcts may be so small to cause any symptoms. A type of lipohyalin degeneration and occlusion in the first part of the small vessels is the main proposed mechanism in those groups of patients. It may be associated with PON1 activity. The objective of our study was to assess the distribution of PON1 polymorphisms and its serum activity in patients with lacunar infarctions and matched control subjects in a population of south-eastern, Iran and to measure the association between genotype and lipid profiles in both groups.

Materials and methods

Patient selection

Based on the World Health Organization definition, stroke is defined as "acute focal or global disturbance of cerebral function lasting 24 hours or more or symptoms lasting <24 hours if a brain imaging study showed an ischemic lesion appropriate to the symptoms due to a cerebral vascular insufficiency" [21,22]. A total of 275 stroke patients were admitted to our neurology department during March 2009 to April 2010. Patients with other underlying cause for the ischemic stroke such as systemic diseases, cancer, migraine, cerebral venous infarction, sickle cell anemia, protein C and protein S deficiencies were excluded. After clinical examination, patients were classified as having small vessel lacunar infarctions or cortical stroke and/or large vessel stroke. The diagnosis was according to the patient's history and physical examination and it was confirmed by T1, T2 and diffusion weighted sequences of brain MRI. We distinguished four types of lacunar syndromes including pure motor, pure sensory, sensori-motor and ataxic hemiparetic syndrome [23]. Our patients were mainly classified into the pure motor type. Blood samples for measuring cholesterol, triglyceride, and paraoxonase and arylesterase activities were taken from the patients during the first three days after admission. Although there are more ethical difficulties in getting consent and blood samples in very old and ill cases for entering them into the study but in this research we tried to include all of them. Thirty-seven patients and 53 normal healthy people without any history of cardiovascular or cerebrovascular disease were asked to participate in the study. Normal controls were enrolled consecutively in the study and patients were entered into the study at least one month after the incident of the stroke. The study was approved by the research ethics committees of Zahedan University School of Medicine. All participants were unrelated individuals of Persian origin.

Enzyme activity assay

Paraoxonase activity assays were done in the absence (basal activity) and presence of 1 M NaCl (salt-stimulated activity) using paraoxon (diethyl-p-nitrophenyl phosphate) as a substrate in the serum of patients and healthy controls. It has been described previously [24]. Phenylacetate was used as a substrate to determine the arylesterase activity. The rate of phenol produced was continuously monitored at 270 nm at 37 °C. Arylesterase activity was determined using molar extinction coefficient of phenol (1310 M^{-1} cm^{-1}) and expressed as IU/L serum. Serum cholesterol, triglyceride and HDL were measured based on spectrophotometric method by using commercial kit Pars-Azmoon Co. (Tehran, Iran). Ultimately, LDL was calculated using Friedewald formula. Serum creatinine levels of all patients were under 2 mg/dL.

Genotype determination

Genomic DNA was extracted from peripheral blood (whole blood mixed with EDTA) based on the technique described elsewhere [25].

Briefly, 500 μL blood was transferred to 1.5-mL microfuge tubes, and 1 mL cell lysis buffer (10 mM Tris–HCl, 11% w/v sucrose, 5 mM $MgCl_2$, and 11% v/v Triton X-100) was added. Microfuge tubes were gently mixed and centrifuged for 2 min at 6000 rpm at room temperature, after which the supernatant was discarded. The procedure was repeated twice. Next, 300 μL buffer II (10 mM Tris–HCl, 10 mM EDTA, and 10 mM sodium citrate) and 40 μL 10% SDS were added, and the mixture incubated for 2 min at room temperature. Then, 100 μL saturated NaCl and 600 μL chloroform were added with gentle mixing, and the mixture was centrifuged for 2 min at 6000 rpm. The supernatant was transferred to a new microfuge tube, where 700 μL cold isopropanol was added, followed by gentle mixing and centrifugation for 1 min at 12,000 rpm for 2 min at 4 °C. The supernatant was discarded and 700 μL cold 70% ethanol was added. The suspension was gently mixed and centrifuged for 1 min at 12,000 rpm at 4 °C. Pellets were subsequently dried before dissolving in 100 μL distilled water. Polymorphisms were determined using tetra primer amplification refractory mutation system-PCR methodology described by one of the authors. The primers used were shown in Fig. 1. Polymerase chain reaction (PCR) cycling conditions for detection of L55M polymorphism, were as follows: 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 59 °C and 40 s at 72 °C; 10 min at 72 °C (Corbett research, Australia). For determination of Q192R polymorphism, PCR cycling conditions were; 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 40 s at 72 °C; 10 min at 72 °C. Each reaction was verified on a 3% agarose gel.

Statistical analysis

By using SPSS software for Windows, Version 15 (SPSS Inc., Chicago, IL, USA), median, arithmetic mean, and standard deviation values for different variables were calculated. Independent student *t*-test and chi-squared tests were applied to compare continuous (age, cholesterol and triglyceride level) and dichotomous variables (sex, genotype frequencies) between cases and controls, respectively. Dichotomous variables were analyzed using odds ratios (ORs) with 95% confidence intervals (CIs). Comparisons were labeled as statistically significant at the conventional *p*-value of less than 0.05. We used Lehr's formula for calculating the sample size for a power of 80%. A logistic regression model was used to evaluate the association

Primers	Q192R	L55M
Forward outer	5′-TGTTCCATTATAGCTAGCACGA-3′	5′-GGCTTTTGTACGTTTTGTG-3′
Reverse outer	5′- TTTCACCCCCTGAAAAATTA-3′	5′-CCGAAGAACACAAATATGCA-3′
Forward inner	5′-TTTCTTGACCCCTACTTCCA-3′	5′-CAGAAACTGGCTCTGAAGTCA-3′
Reverse inner	5′-CAAATACATCTCCCAGGCTC-3′	5′-TCCATTAGGCAGTATCTCGAA-3′

Fig. 1. Oligonucleotide primer sequences used for genotyping.