CROATIAN MEDICAL JOURNAL

42(2):146-150,2001

CLINICAL SCIENCES

Serum Paraoxonase Activities in Hemodialyzed Uremic Patients: Cohort Study

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Aim. To determine whether paraoxonase activity, paraoxonase phenotypes, and lipid status are altered in uremic patients on long-term hemodialysis treatment as compared to healthy population.

Methods. Patients (n=69) and control subjects (n=145) were from the area of Slavonski Brod, Croatia. Paraoxon was used as a substrate for measuring basal or sodium chloride-stimulated (NaCl-stimulated) paraoxonase activity, and phenylacetate for measuring arylesterase activity. The double substrate method was used to assign phenotypes. Cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-cholesterol) were determined by methods routinely used in medical-biochemical laboratories. Enzyme activities are expressed as international units per liter of serum or per mmol of HDL-cholesterol (HDL-standardized activities).

Results. Basal and NaCl-stimulated paraoxonase activity, as well as arylesterase activitiy expressed per serum volume, were significantly lower in the hemodialyzed uremic patients compared to the controls; 69% ($p<0.001$), 73% ($p<0.001$) and 49%, ($p<0.001$), respectively. However, basal and NaCl-stimulated paraoxonase activity standardized for HDL-cholesterol concentrations were not significantly reduced in the hemodialyzed uremic patients as compared to controls (86%, $p=0.614$ and 87%, $p=0.720$, respectively), contrary to arylesterase activity, which remained significantly lower (72%, $p<0.001$). The distribution of paraoxonase phenotypes in hemodialyzed uremic patients and controls was as follows: AA 45% and 39%, AB 37% and 48%, BB 18%, and 13%, respectively.

Conclusion. Patients on long-term hemodialysis have decreased paraoxonase/arylesterase activitiy, which might indicate a greater risk of premature atherogenesis.

Key words: *cholesterol; esterase; hemodialysis; lipoproteins, HDL cholesterol; phenotype; triglycerides*

Paraoxonase (aryldialkylphosphatase, EC 3.1.8.1) is a serum esterase synthesized in the liver. The enzyme hydrolyzes organophosphorous compounds, such as paraoxon, including esters of phosphonic and phosphinic acids, as well as aromatic carboxylic acid esters. The enzyme was originally found to be responsible for the hydrolysis of paraoxon (O,O-diethyl-O-p-nitrophenylphosphate), a catabolite of the insecticide parathion. Paraoxon is toxic to humans because it irreversibly inhibits acetylcholinesterase (1).

Paraoxonase exhibits two-sequence polymorphisms, Arg(192)→Gln and Met(55)→Leu, the former being responsible for distinct catalytic activity of the two corresponding allozymes against paraoxon. The amino acid Arg at the position 192 of the protein specifies high activity paraoxonase (B), whereas Gln at that position specifies low-activity paraoxonase (A). Three phenotypes and genotypes – homozygous AA (low activity), heterozygous AB (intermediate activity), and homozygous BB (high activity), which is the least common, have been defined. Paraoxonase has also arylesterase activity, which does not exhibit activity polymorphism (2,3).

Paraoxonase circulates as a high-density lipoprotein (HDL) component in the blood of humans and other vertebrates. The enzyme is tightly bound with the hydrophobic N-terminal domain to apoA-I of HDL. The presence of paraoxonase in HDL may be a major contributor to the antiatherogenicity of this lipoprotein (4,5).

Oxidation of low-density lipoproteins (LDL) is recognized as a key stage in the early development of atherosclerosis, leading to the uptake of LDL by the macrophage scavenger receptor and hence to the formation of foam cells (6). Recently, paraoxonase has been shown to inhibit the oxidative modification of LDL (4). Furthermore, paraoxonase can destroy active lipids in mildly oxidized LDL, thus preventing the induction of inflammatory responses in arterial wall cells (5). Increased LDL susceptibility to oxidation has been reported in chronic renal failure, a condition characterized by accelerated atherogenesis and derangement of lipoprotein metabolism (7,8).

The aim of this study was to investigate serum paraoxonase/arylesterase activity in uremic patients under long-term hemodialysis and healthy population of the Slavonski Brod area, Croatia. High prevalence of endemic nephropathy has been documented in this area and patients with this diagnosis usually have to undergo hemodialysis (9).

Subjects and Methods

Subjects

The patient group included 69 uremic patients on long-term hemodialysis. There were 32 men and 37 women; their mean age was 39 years (range, 13-74 years). The most common cause of renal failure was endemic nephropathy (n=31), followed by pyelonephritis (n=15), glomerulonephritis (n=10), diabetes mellitus (n=3), polycystic kidneys (n=4), and other degenerative diseases (n=6). All patients had been on regular hemodialysis for an average of 7.7 years (range 1-20) and had been dialyzed three times a week for 4.5 hours. The blood was collected from patients after an overnight fast, immediately before the first hemodialysis session of the week at the Nephrology and Dialysis Unit, Department of Internal Medicine, Dr Josip Benčević General Hospital, in Slavonski Brod.

Control group comprised 145 apparently healthy individuals – volunteer blood donors – who were not on any medication. There were 78 men and 67 women with mean age of 36 years (range 14-77 years). The inclusion criteria for the control group were living in similar socioeconomic environment as patients (rural area, diet) and not being related to the patients. The blood was collected from donors after an overnight fast at the Department of Transfusion Medicine, Dr Josip Benčević General Hospital, Slavonski Brod.

The groups studied were of similar age and sex. All subjects were informed about the aims and procedure of the study and gave their consent. The Hospital Research Ethics Committee approved the study.

Serum Samples

Blood samples were collected by venipuncture from the fasting subjects. After the blood was centrifuged, 2 mL aliquots of each subject's serum were frozen at -20°C before further analysis, usually done within 5 weeks.

Enzyme Activity Assays

Paraoxonase assays were performed in the absence of sodium chloride (NaCl) (basal activity) and in the presence of 1 mol/L NaCl (NaCl-stimulated activity) (10,11). Initial rates of hydrolysis of paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co, London, UK) were determined by measuring liberated p-nitrophenol at 405 nm at 37°C on a Technicon RA-1000 autoanalyzer (Bayer, Milan, Italy).

The basal assay mixture included 2.0 mmol/L paraoxon and 2.0 mmol/L of calcium chloride ($CaCl_2$) in 0.1 mol/L Tris-HCl buffer, pH 8.0. To 350 μL of the reagent mixture 10 μL of serum was added.

For the NaCl-stimulated assay, 1 mol/L NaCl was added into the above described mixture.

Phenylacetate was used as a substrate to measure arylesterase activity (12). Initial rates of hydrolysis were determined spectrophotometrically by detecting the increase in phenol concentration at 270 nm. The reaction mixture contained 2.0 mmol/L phenylacetate (Sigma) and 2.0 mmol/L $CaCl_2$ in 0.1 mol/L Tris-HCl buffer, pH 8.0.

Enzyme activities were expressed in international units (or kilounits) per liter of serum or per mmol of HDL-cholesterol (HDL-standardized activities).

Paraoxonase Phenotype Distribution

The phenotype distribution of paraoxonase activity was determined by double substrate method. The ratio of the hydrolysis of paraoxon in the presence of 1 mol/L NaCl to the hydrolysis of phenylacetate for each individual was calculated (13).

The cumulative distribution of the activity ratios was used to assign individuals to one of the three possible phenotypes: AA (homozygous, low activity), AB (heterozygous, intermediate activity) or BB (homozygous, high activity).

Lipid and Lipoprotein Determinations

Serum triglycerides were measured enzymatically with glycerol phosphate oxidase (GPO-PAP method, HD dijagnostika, Sisak, Croatia). Total serum cholesterol and HDL-cholesterol after precipitation of lower density lipoproteins were determined by the enzymatic CHOD-PAP method (HD dijagnostika).

Statistical Analysis

Depending on the normality of data distribution, either Mann-Whitney rank sum-test or Student t-test was used. Differences between activity distribution profiles were tested by the Kolmogorov-Smirnov two-sample test, which is based upon distances between cumulative frequency distribution functions. The values $p<0.05$ were considered significant. SigmaStat program for Windows, version 2.0, Jandel Corporation (San Rafael, CA, USA) was used for statistical analysis.

Results

Paraoxonase Activity

Serum paraoxonase activity showed a polymodal distribution in both groups, whereas arylesterase activity was unimodally distributed. Basal and NaCl-stimulated paraoxonase activity, as well as arylesterase activity were significantly lower in the hemodialyzed uremic patients compared to the controls. Significant differences between activity distribution profiles were found by the Kolmogorov-Smirnov two-sample test ($D=0.282$, $p<0.001$; $D=0.268$, $p=0.002$; and $D=0.711$, $p<0.001$, respectively) (Fig. 1).

The median of basal and NaCl-stimulated paraoxonase activity represented only 69% ($p<0.001$) and 73% ($p<0.001$) of the control values, respectively. Arylesterase activity in sera of the hemodialyzed uremic patients were also lower than in the controls (49%, $p<0.001$) (Table 1).

Paraoxonase Phenotype Distribution

The double substrate method showed a trimodal repartition in our control population as well in the hemodialyzed uremic patients. According to this method, 39% individuals in the control group classified as AA phenotype, 48% as AB phenotype, and 13% as BB phenotype. In the group of hemodialyzed uremic patients, there were 45% AA, 37% AB, and 18% BB phenotypes (Fig. 2).

Table 1. Serum paraoxonase activity in patients on hemodialysis (n=69) and control subjects (n=145)

Paraoxonase	Mean	SD	Median	Interquartile range	p
Basal paraoxonase activity (U/L):					
control	251	143	201	236	
hemodialysis	167	105	138	150	<0.001[a]
NaCl-stimulated paraoxonase activity (U/L):					
control	542	337	411	587	
hemodialysis	365	254	299	373	<0.001[a]
Arylesterase activity (kU/L):					
control	106	38	109	53	
hemodialysis	52	18	51	28	<0.001[b]

[a]Mann-Whitney rank sum-test.
[b]Student t-test.

Lipid Parameters

Cholesterol and HDL-cholesterol were significantly decreased (87%, p<0.001 and 71%, p<0.001, respectively), whereas triglycerides were significantly increased (195%, p<0.001) in the group of hemodialyzed uremic patients compared to control mean values (Table 2).

HDL-standardized Paraoxonase Activity

Since HDL-cholesterol concentrations and enzyme activities were lower in hemodialyzed uremic patients than in the controls (Tables 1 and 2), paraoxonase and arylesterase activities were standardized for HDL-cholesterol concentrations. HDL-standardized basal or NaCl-stimulated paraoxonase activities expressed as medians in the group of hemodialyzed uremic patients were not significantly reduced compared to control values (86%, p=0.614 and 87%, p=0.720, respectively). HDL-standardized arylesterase activity was significantly decreased, to 72% of control mean value (p<0.001) (Table 3).

Discussion

Our study demonstrated that serum paraoxonase and arylesterase activities expressed per serum volume were lower in the hemodialyzed uremic patients from the area of Slavonski Brod than in the control group. Reduced activities of a similar level for both enzymes were also found in hemodialyzed uremic patients from other countries (10,14,15). This indicates that uremia or dialysis probably induces changes in the enzyme activity, enzyme concentration, or its connection with HDL. Reduced paraoxonase activity may cause decreased HDL antioxidant capacity in hemodialyzed uremic patients and would therefore be expected to contribute to the increased risk of premature atherosclerosis found in these patients (7,8,16). Enzyme activities and lipid status in healthy individuals from the same area and with same living and eating habits were selected as a baseline. Dietary habits, such as lipid levels in the diet, have been reported to affect paraoxonase and arylesterase activities (14,17-20).

Serum paraoxonase/arylesterase activity, and AA (low activity), AB (intermediate activity), and BB (high activity) phenotype distribution of the

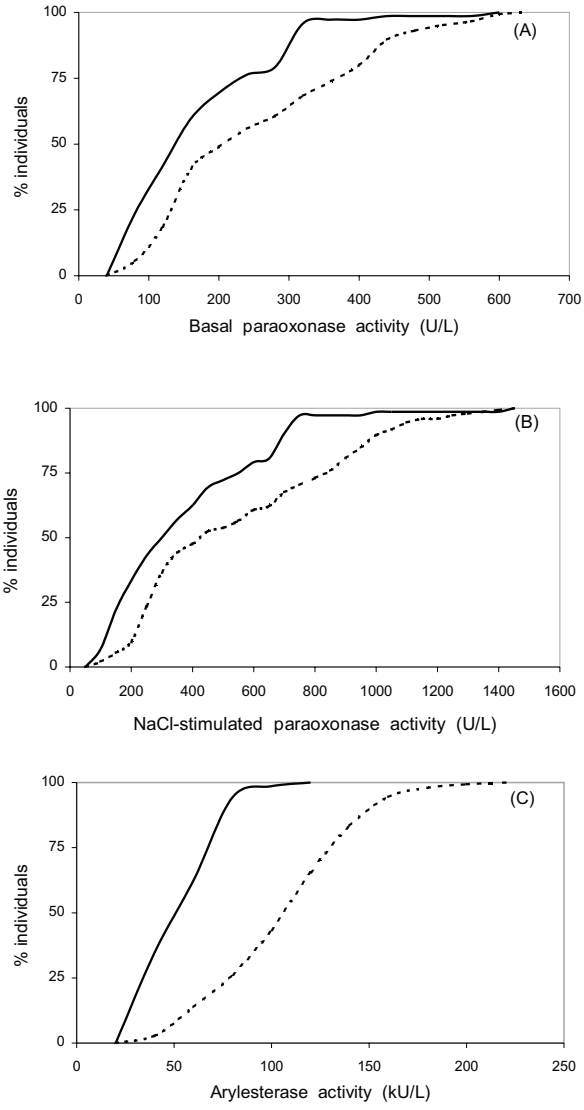

Figure 1. Cumulative frequency distribution profiles of (A) basal paraoxonase activities, (B) NaCl-stimulated paraoxonase activities, and (C) arylesterase activities in sera of hemodialyzed uremic patients (full line) and control individuals (broken line). Differences tested by the Kolmogorov-Smirnov two-sample test for the distribution profiles A, B, and C were D=0.282, p<0.001; D=0.268, p=0.002; and D=0.711, p<0.001, respectively.

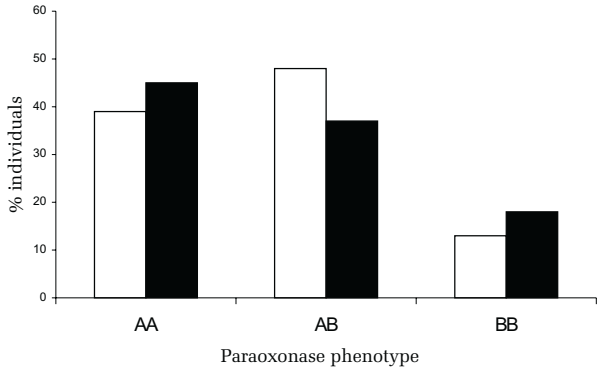

Figure 2. Paraoxonase phenotypes in hemodialyzed uremic patients (closed bars) and control individuals (open bars).

control individuals were similar to those reported for Caucasian healthy populations (21,22). Furthermore, our study did not demonstrate any statistically significant difference in distribution of the AA, AB, and BB phenotypes between the patient and control group. Thus, our results proved that lower serum paraoxonase activity in hemodialyzed uremic patients could not be assigned to different paraoxonase phenotype distribution. As it is not possible to completely dismiss a methodological bias that arises from the double substrate method, genotyping of the paraoxonase DNA polymorphism should be done to confirm the phenotype distribution in both controls and hemodialyzed uremic patients (14,15,23,24).

Table 2. Serum cholesterol, triglycerides, and HDL-cholesterol concentrations in patients on hemodialysis (n=69) and control subjects (n=145)

Parameter	Mean	SD	Median	IQR[a]	p[b]
Cholesterol (mmol/L):					
control	5.33	0.89	5.40	1.10	
hemodialysis	4.68	1.14	4.65	1.61	<0.001
Triglycerides (mmol/L):					
control	1.27	0.35	1.20	0.56	
hemodialysis	2.48	1.37	2.06	1.30	<0.001
HDL-cholesterol (mmol/L):					
control	1.20	0.24	1.20	0.30	<0.001
hemodialysis	0.85	0.25	0.83	0.28	

[a]Interquartile range.
[b]Student t-test.

Table 3. Serum HDL-standardized paraoxonase activity in patients on hemodialysis (n=69) and control subjects (n=145)

Paraoxonase	Mean	SD	Median	IQR[a]	p
Basal paraoxonase activity/HDL-cholesterol (U/mmol):					
control	221	128	196	217	
hemodialysis	216	142	168	194	0.614[b]
NaCl-stimulated paraoxonase activity/HDL-cholesterol (U/mmol):					
control	477	300	403	511	
hemodialysis	472	338	350	457	0.720[b]
Arylesterase activity/HDL-cholesterol (kU/mmol):					
control	91	36	88	49	
hemodialysis	65	26	63	39	<0.001[c]

[a]Interquartile range.
[b]Mann-Whitney rank sum-test.
[c]Student t-test.

Variations in serum paraoxonase activity were associated with changes in serum triglycerides and HDL-cholesterol concentrations, observed in uremic patients on long-term hemodialysis (19,24). There was no significant difference between HDL-standardized paraoxonase activity of the hemodialyzed uremic patients and of controls in our study and in the studied groups from the Belfast area, Northern Ireland (15). Another two studies, from Hungary and Italy, demonstrated significantly decreased HDL-standardized paraoxonase activity in the hemodialyzed groups compared to their controls (10,23). These different results suggest that paraoxonase activity changes do not entirely depend on HDL concentration in hemodialyzed uremic patients, but they may be due to some other factors. Compared to our control group, triglycerides and HDL-cholesterol levels in the control group in the study from Hungary and Italy were in better accordance with the generally recommended values for the prevention of coronary heart disease in clinical practice (25). This may be related to different inclusion criteria concerning the lipid status in the selection of control subjects. Assuming the role of triglycerides in the HDL metabolism (26) and the fact that serum paraoxonase is mostly bound to the large apoA-I containing HDL particles (19), ratio of the HDL subgroups in hemodialyzed uremic patients and controls could be also different. Correlation between paraoxonase activity and HDL composition was previously demonstrated in patients with lipoprotein metabolism disorders (27-29).

In conclusion, we recommend that paraoxonase/arylesterase activities should be measured in patients on long-term hemodialysis because decreased activities indicate a greater risk of premature atherogenesis. In addition, genotyping of the paraoxonase DNA polymorphism and the assay of paraoxonase activity on HDL subgroups will give an insight into the decreased HDL antioxidant capacity in the hemodialyzed uremic patients.

Acknowledgments

This work was supported in part by the Ministry of Science and Technology of the Republic of Croatia (Grants No. 006311 and 00220104).

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Received: July 7, 2000
Accepted: February 2, 2001

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