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ORIGINAL ARTICLE

Lipids, Apolipoproteins, Lipid Oxidation and Paraoxonase Enzyme Activity In Diabetic And Non-Diabetic End Stage Renal Disease Patients

Solati M, Raiszadeh F, Azizi F

Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University (MC), Tehran, I.R. Iran

Paraoxonase (PON) is a serum esterase, associated with HDL-C. It decreases the oxidation of LDL-C. Serum PON1 activity has been shown to diminish in several diseases. We investigated the serum PON1 activity and lipid profiles in diabetic and non-diabetic end stage renal disease (ESRD) patients and controls as well as serum PON1 activity pre and post dialysis.

Materials and Methods: For this study we recruited 92 patients with ESRD on hemodialysis, and 46 healthy controls. The patients were assigned into diabetic and non-diabetic groups (each, n=46 each). Serum lipid profiles including total cholesterol, triglycerides, HDL-C, apolipoprotein A-1, apolipoprotein B, Lipoprotein (a) serum PON activity, oxidized LDL-C and total antioxidant capacity were compared among groups. Furthermore, pre and post dialysis serum PON activity were also compared.

Results: Serum levels of total cholesterol, HDL-C, LDL-C and apoA-I were lower in the ESRD patients, compared to controls. While serum PON activity was significantly lower in the ESRD than in controls, it did not differ significantly between diabetic and non-diabetic patients with ESRD. Serum PON activity was observed to rise significantly postdialysis, compared to predialysis, 69±48 vs. 72±50 IU/ml and 47±32 vs. 53±37 IU/ml in diabetic and non- diabetic patients respectively, the increase being significantly correlated with the quality of hemoidalysis.

Conclusion: To conclude, reduced serum paraoxonase activity in diabetes and non-diabetes ESRD may further predispose lipids to oxidation. Enhancement of the quality of hemodialysis can increase serum PON activity in hemodialysis patients.

Correspondence: Fereidoun Azizi, M.D. Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University, (MC), Tehran, I. R
E-mail azizi@endocrine.ac.ir

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Introduction

Oxidation of LDL-C in the artery wall is considered as a key stage in the development and progression of atherosclerosis[1,2] which increases the risk of ischemic heart disease. HDL-C appears to prevent the oxidation of LDL-C in vitro by a mechanism that is probably enzymatic in part.[3] Human paraoxonase is a serum esterase which is synthesized by the liver and is associated with HDL-C as its carrier and site of action.[4,5] Serum HDL-associated PON inhibits lipid peroxidation of LDL-C[6] and thus decreases the risk of atherosclerosis. PON1 has 2 amino acid polymorphisms with different protective capacities.[7] Individuals with a low PON activity phenotype seem to have a greater risk in developing atherosderosis.[8] Serum PON activity has been reported to be

low in diseases such as diabetes mellitus [9-12] and renal failure,[13-15] both conditions in which ischemic heart disease is increased.

Cardiovascular disease is considered as a major cause of morbidity in patients with chronic renal failure (CRF), which may be related to alterations in lipoprotein concentrations such as decreased HDL-C and elevated lipid peroxidation in the vessel walls.[16,17] PON activity has been demonstrated to diminish in patients with CRF[15,18] which predisposes them to premature atherosclerosis whereas renal transplantation seems to restore PON activity.[15] Itahara et al. found the enzyme activities of PON1 to be decreased in hemodialysis patients, independent of the genetic polymorphism;[19] despite the findings, the influence of hemodialysis on the serum PON activity is not well delineated.

Diabetes mellitus, is one of the leading causes of CRF in western countries. Studies indicate the low serum PON activity in diabetic population which may give rise to atherosclerosis in affected patients.[8] Abbott et al. showed the serum PON activity to be low in diabetics and found that this finding was not the result of phenotypic differences in the diabetic population nor was it due to a lower PON concentration in diabetics.[8]

This study was designed to compare serum PON activity and other antioxidant markers in diabetic and non-diabetic hemodialysis patients as well as the influence of hemodialysis on serum PON1 activity in this population.

Materials and Methods

Subjects

In this case-control study, 138 Iranian subjects, aged 20-75 years were enrolled; 46 diabetic patients with end-stage renal disease (ESRD), 46 non-diabetic patients with ESRD and 46 healthy control subjects were studied between March 2000 and September 2001.

Patients included 92 subjects with ESRD on three hemodialysis sessions per week at medical centers affiliated to the Shaheed Beheshti University of Medical Sciences. They were assigned to diabetic (n=46) and non-diabetic (n=46) groups according to WHO criteria of diabetes mellitus or existence of chronic complications of the disease.

Patients with a history of hospitalization, surgery, acute myocardial infarction in the 4 weeks preceding the study, pregnancy, an over 3-fold increase of liver enzymes and a history of thyroid disorders were excluded, as were those taking lipid-lowering medication (HMG CO-A reducates, nicotinic acid, bile acid-binding resins during the 6 weeks prior and fibrates in the 12 preceding weeks), steroids, alcohol, or vitamin C and E supplements.

The control group consisted of 46 non-diabetic healthy individuals without renal failure or nephrotic syndrome, and not taking any medication. They were selected from relatives of non-diabetic patients as well as the staff of medical centers, and were matched to the patient groups by age and sex. Demographic variables and past medical histories were collected by a questionnaire. Coronary artery disease (CAD) was defined as a history of ischemic heart disease and cerebrovascular accident (CVA) as an ischemic or hemorrhagic accident in cerebrals' vascular.

In the case of patients, ten milliliters of venous blood was obtained after an overnight fast for at least 10-12h, and, in the case of patients, immediately before dialysis; another blood sample was drawn from patients, 2 minutes following the end of dialysis sessions.

Blood samples were drawn into tubes containing EDTA and heparin and sent to the research laboratory of the Endocrine Research Center, where all laboratory measurements were performed. Serum was separated by centrifugation (2500 rpm, 30 min, 4^{o}C) and multiple aliquots were frozen at -80 oc before further analysis. Serum total cholesterol, triglycerides, HDL-C, apolipoproteins (AI and B), lipoprotein (a) and oxidized LDL were measured in the blood samples obtained before dialysis whereas serum PON activity and total antioxidant capacity were

determined in the samples taken before and as after dialysis.

KT/V and URR (Urea Reduction Ration) were measured by standard formula.[20]

Laboratory methods

Serum TC and TGs were determined by commercially available enzymatic reagents (Pars Azmon, Iran) adapted to the Selectra autoanalyzer. HDL-C was measured after precipitation with phosphotungstic acid MgC12. LDL-C was calculated using Friedwald's formula for serum samples with TG values less than 400 mg/dL. In patients with triglycerides over 400 mg/dL, LDL-C was not calculated. Concentrations of apo A-I and apo B were measured with a modified commercially available immunoturbidometric assay (Pars Azmon, Iran). Paraoxonase activity was determined by adding 15 µl serum to 285-µl Tris-Hcl buffer (100 mM, PH=8.0) containing 1mM CaC12 and 1mM paraoxon (Sigma Chemical Company, D9286). The generation of p-nitrophenol was measured at 405 nm, and at 25°C with an autoanalyzer (Selectra 2, Netherlands). Routine quality control measures and comparison with standard control serum were employed to ensure the accuracy of the measurements throughout the study. Coefficients of variation for measurement of apolipoprotein A-I were 1.81% at a concentration of 36 mg/dL, 1.66 % at 86 mg/dL, and 3.65% at 152 mg/dL and those of apolipoprotein B were 2.63% at concentration of 24 mg/dL, 2.43 % at 95 mg/dL, and 2.18% at 156 mg/dL. The recovery of apo A-I was between 92.5 and 99.4% and that of apo B was between 97.3 and 101.4%. Coefficients of variation for paraoxoxnase assays were 5.6% at 16 U/mL, 5.8% at 69 U/mL and 4.4% at 130 U/mL.

The plasma total antioxidant capacity was determined according to the ferric reducing ability of plasma (FRAP method) using a Cobas Fara centrifugal analyzer (Roche Diagnostics, Basel, Switzerland). In brief, the Frap assay measures changes in absorbance at 593 mm owing to the formation of a blue colored FeII-tripyridyltriazine compound from the colorless oxidized FeIII from the action of the electron donating antioxidants. Inter- and intra-assay coefficients of variation were 1 and 3% respectively.

Data were analyzed by SPSS software (version 10.0.1, SPSS Inc., Chicago, USA). The results of quantitative variables are presented as mean ± SD and the results of qualitative variables as percentage. To compare quantitative and qualitative variables between groups, t test and Chi square tests were applied, respectively. Two-tailed ANOVA followed by post-hoc testing with Tukey's multiple comparison tests was used for comparison of laboratory variables in diabetic and non-diabetic patients with ESRD as well as controls. P values less than 0.05 were considered significant.

Results

In this study, 138 subjects were enrolled and assigned into three groups with 46 subjects each, i.e. ESRD with diabetes, ESRD without diabetes and controls.

There was no significant difference among the groups with regard to sex and age. Body mass index was significantly lower in diabetic and non-diabetic patients with ESRD than in the controls, whereas no significant difference was observed between diabetic and non-diabetic patients with ESRD (Table 1).

Table 1. Characteristics of ESRD patients and control subjects (mean±SD)

	Age (Years)	N (M/F)	BMI (kg/m^2)	Waist/Hip ratio	CAD (%)	CVA (%)	HTN (%)
Diabetic ESRD (n=46)	61.8±10.1	20/26	23.1±3.9*	0.99±0.06	26.1	15.2*, †	80.4‡, †
Non-diabetic ESRD (n=46)	61.5±9.9	21/25	22.7±3.5*	0.98±0.07	23.9	2.2	69.6‡
Controls (n=46)	60.8±10.0	22/24	25.4±30	1.0±0.1	19.6	0	19.6

*P<0.005 vs. Controls, † P<0.01 vs Non-diabetic ESRD, ‡ P<0.001 vs Controls; CAD, Coronary artery disease; CVA, Cerebrovascular accident

In the patients with ESRD, the mean serum creatinine level before hemodialysis was significantly higher in non-diabetics than in diabetics (5.1±0.9 vs. 4.8±0.9 mg/dL, P<0.05). The patients with ESRD had lower serum levels of albumin as compared with the controls (Table 2).

The serum levels of lipid, lipoprotein and apolipoprotein profiles in the population are given in table 2. Serum levels of total cholesterol, HDL-C and LDL-C were significantly higher in control subjects than in diabetic and non-diabetic patients with ESRD (in all, P<0.001); however they did not differ significantly between diabetic and non-diabetic patients with ESRD.

There was no significant difference between the three groups with regard to serum triglycerides and lipoprotein (a). Serum apo A-I was found to be significantly higher in the controls than in diabetic and non-diabetic patients with ESRD (183±22, 152±40 and 143±25 mg/dL, respectively, P<0.001).

The controls had significantly higher levels of serum apo B as compared with ESRD patients with diabetes (100±30 and 87±29 mg/dL, respectively, P<0.05), while no significant difference was observed between serum apo B patients with diabetes and non-diabetes ESRD.

Table 2. Serum creatinine, lipid profiles, apolipoproteins and PON activity and other variables in DM-ESRD, Non DM-ESRD and controls

	Diabetic ESRD (n=46)	Non-diabetic ESRD (n=46)	Controls (n=46)
FBS (mg/dL)	151.3±67.6* †	96.8±21.3	81.9±12.3
Creatinine (mg/dL)	4.8±0.9*	5.1±0.9*	1.3±0.4
Uric acid (mg/dL)	5.4±1.3*	5.5±0.9*	4.5±1.14
BUN (mg/dL)	57.3±19.3*	56.3±15.6*	15.7±3.5
Albumin (g/dL)	4.1±0.5*	4.2±±0.4*	4.9±0.4
TC (mg/dL)	166±43*	160±45*	232±47
TG (mg/dL)	182±99	168±100	179±91
HDL-C (mg/dL)	43±12*	45±24*	62±14
LDL-C (mg/dL)	88±39*	81±38*	135±34
Apo A-I (mg/dL)	152±40	143±25	183±22
Apo B (mg/dL)	87±29†	89±26	100±30
Lp (a) (mg/dL)	45±37	45±35	38±20
PON activity (IU/mL)	59.5±48.6*	47.2±32.2*	86.4±62.5
Oxidized LDL (mg/dL)	94.5±31.7	81.6±29.0	86.2±34.7
TAC (µmol/L)	1.5±0.3	1.5±0.4	1.5±0.3

* P<0.001, † P<0.05 vs. Controls; TC, Total cholesterol; TG, Triglycerides; LP(a), Lipoprotein (a) ; Apo, Apolipoprotein , PON, Paraoxonase

Apo A-I / apo B ratio was significantly depressed in the non-diabetic ESRD patients as compared with the control subjects (0.26±0.13 vs. 0.12±0.13, P<0.05). No significant difference was observed in the lipid and lipoprotein profiles between diabetic and non-diabetic patients with ESRD (Table 2).

The oxidation profile showed serum PON activity to be significantly lower in the diabetes and non-diabetes ESRD patients, than in the controls (59.5±48.6 vs. 47.2±32.2 vs. 86.4±62.5 IU/mL, respectively, p<0.001) while it did not differ significantly between diabetic and non-diabetic patients with ESRD. The oxidized LDL and total antioxidant capacity (TAC) were not significantly different among three groups (Table 2).

The means of KT/V and URR were 0.90±0.45 and 0.52±0.16 in the ESRD patients respectively, neither being significantly different between the diabetic and non-diabetic patients.

Serum PON activity was increased significantly after hemodialysis both in the diabetic and non-diabetic ESRD patients whereas total antioxidant capacity was not (Table 3).

Table 3. PON activity, TAC and Ox-LDL-C in the ESRD patients before and after hemodialysis

Patients (n=46)	PON (IU/mL)		TAC (µmol/L)	
	Before	After	Before	After
Diabetic ESRD	69.5±48.6	72.9±50.6*	1.5±0.3	1.5±0.2
Non-diabetic ESRD	47.2±32.2	53.1±37.5*	1.5±0.4	1.4±0.1

* P<0.001 compared with before hemodialysis

The increase in serum PON activity in the ESRD patients was significantly correlated with KT/V (r=0.3, P<0.005), and was significantly higher in those with a good quality hemodialysis (KV/V≥1) than in those with a poor quality hemodialysis (KT/V<1) (16.1±21.8 IU/mL and 4.4±12.3 IU/mL respectively, P<0.05) (Fig 1). Such a difference was not found in total antioxidant capacity.

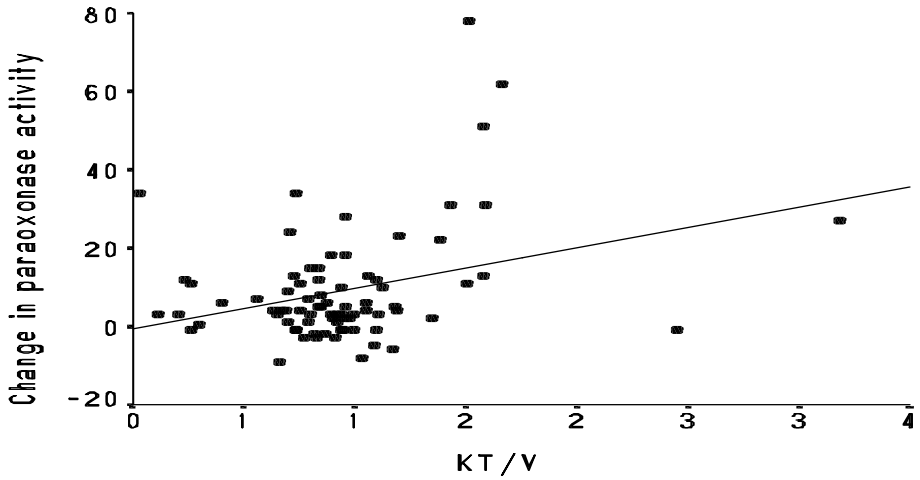

Fig. 1. Correlation between change in serum PON activity and KT/V in ESRD patients (r=0.3, P<0.005).

Discussion

Our results show that serum PON activity is decreased significantly in patients with ESRD as compared with the controls, which is consistent with previous studies.[8,14] While no difference was observed in serum PON activity between diabetic and non-diabetic patients with ESRD, hemodialysis increased serum PON activity and standardized PON activity (PON/ HDL ratio) while it had no effect on total antioxidant capacity. Postdialysis increase of serum PON activity was correlated positively with the quality of hemodialysis (defined as KT/V). We observed serum total cholesterol, HDL-C, LDL-C and apo A-I to be significantly decreased in the ESRD patients compared to controls, whereas no significant difference was found in the para-

meters between diabetic and non-diabetic patients with ESRD.

Cardiovascular disease is a major cause of morbidity and the main cause of death in the patients with CRF. ESRD patients show some abnormalities in plasma lipids and lipoproteins, conditions called uremic dyslipidemia. In uremic dyslipidemia, plasma triglycerides and HDL-C levels are increased and decreased respectively while total cholesterol level is usually normal.[20]

In CRF, oxidative modification of lipoproteins is enhanced because of an imbalance between pro-oxidant and antioxidant systems which in turn increases the risk of atherosclerosis in CRF patients.[21]

Paraoxonase is a serum esterase, secreted by the liver and is associated with HDL-C. PON has been shown to decrease the oxidative modification of LDL-C in vitro which appears to represent a key step in the generation of atherosclerosis.[14,17,18] The metabolic abnormalities in chronic renal failure enhance the oxidation of LDL-C.[22] Therefore the decrease in PON activity in renal failure and diabetes mellitus predisposes affected patients to early atherosclerosis[7] following the increase in lipid peroxidation. Studying 119 patients with CRF and 110 healthy control subjects, Schiavon et al. showed serum PON was lower in the CRF patients than in the controls,[17] similar to the results of the Hasselwander et al. study,[18] and also concluded that the differences in PON activity between the groups investigated were not attributable to different phenotype distribution. Suehiro et al. revealed that the enzyme activity of PON was decreased in hemodialysis patients independent of the genetic polymorphism.[19] In our study, serum PON activity was also found to be lower in the CRF patients than in the controls. Low serum PON activity increases the risk of atherosclerosis by enhanced LDL oxidation in CRF population. Serum PON activity was not significantly different between diabetic and non-diabetic patients with ESRD. We speculate that irrespective of the etiology, ESRD gives rise to extensive metabolic changes in lipoprotein oxidation and antioxidant levels.

We found serum levels of LDL-C to be lower in the ESRD patients than in the controls which could be a consequence of malnutrition in the ESRD patients. Despite lower serum levels of LDL-C in the ESRD patients compared with controls, the serum levels of oxidized LDL-C were not different between the two groups, which shows enhanced oxidation of LDL-C in the ESRD patients. Our findings showed no difference in total antioxidant capacity (TAC) between the ESRD patients and the controls; as TAC just reflects water-soluble antioxidants, it cannot be considered as an appropriate index to evaluate the status of total serum antioxidants.

Although previous studies have shown increased plasma triglycerides with normal total cholesterol level.[20,22]

Post-dialysis increasing of paraoxonase activity was the most important finding of this study. Investigating 20 uremic patients, Schiavon et al. showed hemodialysis treatment induced a slight increase in PON activity, which paralleled that of the other parameters (cholesterol, TG, HDL-C, apo A-I, apo B), probably as a consequence of water loss.[17] Gugliucci et al[23] showed a 76% decrease in PON activity in 22 ESRD patients on hemodialysis; an increase activity of PON-1 was seen from 4 to 40% in all subjects. In his study Dirican et al[24] have found a reduction in PON activity in long term hemodialysis patients that correlate inversely with creatinine and urea levels. In our study, PON activity was observed to increase significantly post dialysis, as did serum HDL-C. PON/HDL ratio was found to be increased significantly post-dialysis. Intravenous Vitamin C administration in hemodialysis patients has been reported to increase PON activity.[25]

A limitation of this study was the low sample size in each subgroup; in addition, measurement of other oxidative variables as MDA and AGE should be done for the better evaluation of oxidative stress. There

were no significant differences in apolipoproteins A, B, Lp (a), TAC, oxidized-LDL and triglycerides between DM and non-DM ESRD patients and the control group in this study, which can be explained by low sample size.

We conclude that the increase in serum PON activity was more than increase in serum HDL-C postdialysis, showing that hemodialysis can raise serum PON activity partly independently of serum HDL-C. Serum PON activity was correlated positively with KT/V which reveals the effect of good quality hemodialysis on increasing serum PON activity and thus reduction in the risk of atherosclerosis.

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