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CLINICAL STUDY

Paraoxonase Activity in Glomerulonephritic Patients

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Background. Cardiovascular disease is the most common cause of morbidity and mortality in patients with chronic renal failure. Glomerulonephritic patients have an increased risk for cardiovascular disease, but its etiology is unclear. It is known that an increase in oxidizability of apolipoprotein B-containing lipoproteins has a key role in the initiation of atherosclerosis, and paraoxonase enzyme activity particularly has a preventive role against atherosclerosis. The aim of the present study was to evaluate the oxidizability of apolipoprotein B-containing lipoproteins, serum, and urinary paraoxonase/arylesterase activities in glomerulonephritis patients who had normal lipid parameters and creatinine levels. *Methods*. Thirty-two patients with glomerulonephritis and 22 healthy controls were included in this study. A total of 32 patients (including nine with membranous GN, eight with immunoglobulin A nephropathy, eight with mesangial proliferative GN, five with focal-segmental glomerulosclerosis, one with diffuse proliferative GN, and one with minimal chance disease having biopsy proven GN) were enrolled into the study. We compared serum and urinary paraoxonase, arylesterase, serum lipids, urea, creatinine, hemoglobin, total protein and albumin values between groups. *Results*. Serum urea, creatinine, total protein, albumin, uric acid, hemoglobin, and lipid parameters were similar in the glomerulonephritis and control groups ($p > 0.05$). PON1 activity was significantly lower in GN group than controls, but there was no statistically significant difference on arylesterase activity between groups. Oxidizability of apolipoprotein B-containing lipoproteins was significantly higher in GN group than controls. *Conclusion*. Our study shows that the findings of normal serum levels of creatinine, lipids, and proteins increased the oxidizability of apolipoprotein B-containing lipoproteins, and any decrease in PON1 activity in patients diagnosed with GN should be considered important. Hence, the immediate commencement of preventive as well as curative treatment in other to avoid the risk of cardiovascular and renal problems would be a correct approach.

Keywords paraoxonase, glomerulonephritis, oxidation, lipid, urinary paraoxonase

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INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of morbidity and mortality in patients with chronic renal failure (CRF).[1] In patients with glomerulonephritis

(GN)—which is one of the most important causes of CRF, particularly in cases with nephrotic syndrome (NS)—dyslipidemia is a common feature. While there is not so much information about lipid profiles in patients with GN having normal renal functions, high triglycerides (Tg), low high-density lipoproteins (HDL), normal low-density lipoproteins (LDL), and an increase of atherogenic small-dense LDL subgroups are classical findings in uremic patients.[2] Additionally, the results of different prooxidant agents' exposure in patients with GN are increased in lipoprotein oxidation. The response of the immune system in GN patients to this increase is more remarkable than the normal population.[3] Several lines of evidence suggest that reactive oxygen species (ROS) and oxidant-antioxidant imbalance play a role in the pathophysiologic processes of renal disease. The abundance of polyunsaturated fatty acids makes the kidney an organ particularly vulnerable to ROS damage. Among the possible effects of the oxidant stress in chronic kidney disease, it may contribute to increase the atherogenic risk.[4] However in GN patients, lipid abnormalities and an increase in oxidation are far away from explaining the progression of renal injury; thus, other possible causative factors are to be considered.

Paraoxonase 1 (PON1) is an esterase that hydrolyzes organophosphates (e.g., paraoxon), aromatic carboxylic acid esters (e.g., phenyl acetate), oxidized phospholipids, and lipid peroxides. Much of the paraoxonase activity in the serum of humans has been found to be associated with HDL.[5] Enzyme activities may vary by 10–40 times in individuals due to different ethnicity, which leads to gene polymorphism. This may explain the differences in the susceptibility of individuals to atherosclerosis and coronary artery disease. Apart from genetic factors, diet, acute phase reactants, pregnancy, and hormonal factors, cigarette smoking and simvastatin therapy also affect PON1 activity.[6] PON1 activity has been shown to be low in patients with myocardial infarction, diabetes mellitus, and familial hypercholesterolemia, as well as dialysis or predialysis chronic renal failure patients.[7–9] It is known that an increase in the oxidizability of apolipoprotein B-containing lipoproteins has a key role in the initiation of atherosclerosis, and PON1 enzyme activity particularly has a preventive role against atherosclerosis.[7,10]

The effect that primary GN has on PON1 enzyme activity and the oxidizability of apolipoprotein B-containing lipoproteins before the onset of renal failure or the deterioration of lipid profiles and renal functions in these patients is not known. The aim of this study is to investigate the oxidizability of apolipoprotein B-containing lipoproteins and PON1 activities, which are risk factors for arteriosclerosis in glomerulonephritis patients with similar renal and lipid parameters, and compare them with healthy voluntaries.

PATIENTS AND METHODS

Subjects

Thirty-two patients (17 males and 15 females, aged 43.7 ± 13.8 years) having biopsy-proven primary GN with stable renal function (i.e., had a change in their proteinuria and serum creatinine of no more than 20% in the last three months before enrolment) were included in this study. The patients with GN were not taking lipid-lowering drugs and did not have hepatic or respiratory diseases, acute coronary syndrome, diabetes mellitus, alcohol consumption, antioxidant vitamin supplementation, or clinical instability with physical examination. The mean follow-up was 80 ± 79 (range 6–300) months. Thirty-two patients diagnosed histologically as having immunoglobulin A nephropathy (n = 8), mesangial proliferative GN (n = 8), membranous GN (n = 9), focal-segmental glomerulosclerosis (n = 5), diffuse proliferative GN (n = 1), and minimal chance disease (n = 1) were enrolled into the study. Three patients had nephrotic (>3.5 g/day), 16 had nephritic (0.3–3.5 g/day), and 13 had microalbuminuria (0.03–0.3 g/day) ranges of proteinuria. A total of 22 healthy volunteers (12 males and 10 females, mean age 46 ± 9 years), without clinical and laboratory evidence of any disease, were included in the present study as the control group.

The study was performed in accordance with the guidelines of the Uludag University Medical Faculty ethics committee. Participants were informed about the aims and the procedure of the study and gave their written consent. Table 1 summarizes the characteristics and clinical data of the patients and the controls.

Table 1
Characteristics of the study groups

Parameter	Controls	Patients with GN	*p*
Age (years)	46.1 ± 8.9	43.7 ± 13.8	NS
Gender (M/F)	12/10	17/15	NS
BMI (kg/m^2)	26.4 ± 3.4	26.1 ± 5.0	NS
Hemoglobin (g/dL)	13.1 ± 1.4	13.0 ± 2.1	NS
Urea (mg/dL)	28.2 ± 10.4	41.3 ± 19.1	NS
Creatinine (mg/dL)	0.9 ± 0.1	1.1 ± 0.4	NS
Uric acid (mg/dL)	5.5 ± 1.7	5.9 ± 2.0	NS
Total protein (g/dL)	7.3 ± 0.9	6.9 ± 0.8	NS
Albumin (g/dL)	4.4 ± 0.5	4.2 ± 0.7	NS
Creatinine clearance (mL/min)	89 ± 17	89 ± 32	NS

Values are expressed as mean ± SD.

Abbreviations: NS = $p > 0.05$, GN = glomerulonephritis, BMI = body mass index.

Blood Sampling

Venous blood was collected within the plain and EDTA-containing tubes after an overnight fast. Serum or plasma was obtained by low speed centrifugation and assayed on the same day or stored at −20°C until the assay.

Methods

Serum levels of total cholesterol, HDL-cholesterol and triglycerides were determined using enzymatic assays on an Aeroset autoanalyzer. LDL cholesterol concentrations were calculated according to the Friedewald's formula.[11] Apo AI, apo B, and lipoprotein (a) [Lp (a)] were assayed by immunonephelometry (Dade Behring Marburg GmbH, Germany). Other parameters (hemoglobin, urea, uric acid, creatinine, albumin, and total protein) were determined by routine laboratory methods.

Paraoxonase activity was measured by spectrophotometry using two synthetic substrates: paraoxon (diethyl-p-nitrophenol phosphate) (paraoxonase activity) and phenyl acetate (arylesterase activity).

The rate of the hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm and 25°C due to the formation of p-nitrophenol. Enzymatic activity was calculated from the molar extinction coefficient at pH 10.5, which was 18290 M^{-1} cm^{-1}. Paraoxonase activity was expressed as U/L, with one unit of paraoxonase activity being defined as 1 μmol p-nitrophenol generated per minute.

Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated using the molar extinction coefficient 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated per minute under the above conditions and expressed as in units per milliliter.[11]

To study the oxidizability of apolipoprotein B-containing lipoproteins, this fraction was precipitated with dextran sulfate-magnesium chloride, and EDTA was then removed. Cholesterol concentration of apolipoprotein B-containing lipoprotein fraction was adjusted to 200 μg/mL with phosphate-buffered saline. As a measure of lipid peroxidation, the malondialdehyde (MDA) production was evaluated by measuring the thiobarbituric acid reactive substances. MDA, produced by the hydrolysis of lipid hydroperoxides heated under acid conditions, reacts with thiobarbituric acid to form a complex that absorbs maximally at 532 nm. The complex was measured after extraction into butanol and was quantified against MDA standards generated from 1, 1′, 3, 3′ tetraethoxypropane, which yields equimolar amounts of MDA under the same reaction conditions. Final results were given as nmol MDA/mg cholesterol. MDA level of apolipoprotein B-containing lipoprotein fraction was measured before (basal) and after 3-h incubation with copper sulfate (final concentration 50 μmol/L) at 37°C. The basal value was subtracted from the 3-h value to obtain ΔMDA. Basal MDA represents the basal oxidative status of the apolipoprotein B-containing lipoprotein fraction, whereas ΔMDA represents the degree of oxidative modification (capacity for peroxidation).

Paraoxonase phenotype distribution was determined by a double substrate method, which calculates the ratio of salt-stimulated paraoxonase activity and arylesterase activity.[12] The phenotypic distribution in groups was described as AA (homozygous low-activity phenotype), AB (heterozygous phenotype), and BB (homozygous high-activity phenotype).

The creatinine clearance was calculated with Cockcroft and Gault formula:

$$(140 - \text{age}) \times \text{weight in kilograms/serum creatinine} \times 72$$

In the case of females, the coefficient was 0.85.

Statistical Analysis

All statistical analyses were performed by using SPSS 13.0 software. Convenience of the values of variables to the normal distribution was tested by One-Sample Kolmogorov-Smirnov test. Analyses were done with parametric and non-parametric tests. Comparison of the groups was done by Student t, Mann Witney-U, and Kruskall Wallis tests for continuous variables. Categorized variables were compared by chi-square test between groups. The relations of the variables were analyzed by Pearson correlation analysis method. The values were given as mean ± standard deviation. A p value < 0.05 was considered statistically significant.

RESULTS

Clinical and laboratory data of patients and controls are summarized in Table 1. Serum urea, creatinine, creatinine clearance, total protein, albumin, uric acid, and hemoglobin levels did not differ significantly between the groups. There were also no significant differences in total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, and Lp (a) concentrations between the study and control groups. Serum apo A1 and apo B levels of the patient group were significantly higher than that of controls (see Table 2).

Table 2
Serum lipid and apolipoprotein concentrations in patient with GN compared to healthy control subjects

Parameter	Controls	Patients with GN	*p*
Total cholesterol (mg/dL)	198 ± 61	197 ± 43	NS
HDL cholesterol (mg/dL)	46 ± 8	51 ± 21	NS
LDL cholesterol (mg/dL)	128 ± 57	122 ± 37	NS
Triglyceride (mg/dL)	118 ± 51	131 ± 64	NS
Lipoprotein (a) (mg/dL)	20 ± 22	20 ± 20	NS
Apolipoprotein AI (mg/dL)	120 ± 20	184 ± 34	<0.001
Apolipoprotein B (mg/dL)	77 ± 24	139 ± 33	<0.001
PON activity/ HDL-chol	4.1 ± 1.9	2.8 ± 1.2	<0.01

Values are expressed as mean ± SD.

Abbreviations: NS = not significant, PON activity/HDL-chol = paraoxonase activity/high-density lipoprotein-cholesterol, LDL = low-density lipoprotein.

Basal MDA and ΔMDA levels of apolipoprotein B-containing lipoproteins were significantly higher in the GN group than in the control group (see Figure 1).

Paraoxonase activity was significantly reduced in the GN group as compared to controls (see Figure 2). HDL-standardized paraoxonase activity (PON1/HDL ratio) in GN group was significantly lower than that of controls. The hydrolysis of phenylacetate (arylesterase activity) in GN group was not significantly different than that in controls.

The double substrate method showed a trimodal repartition in the controls, as well as in the glomerulonephritis patients. The percentage of phenotypic distribution between glomerulonephritis patients and controls were AA: 56%, AB: 40%, BB: 4%; and AA: 59%, AB: 36%, BB: 5%, respectively. According to this method, paraoxonase phenotype distribution was not significantly different between groups.

When the groups with case numbers greater than four were compared with each other in the GN group, none of the parameters was significantly different between the groups ($p > 0.05$).

With bi-variety correlation analysis, PON1 activity in the GN had no correlation with urea, creatinine, total protein, albumin, and uric acid.

While urinary PON1 was detected (as low) only in two cases of GN, it was undetectable in the control group. The patient with urinary PON1 level (1 U/L) was a 48-year-old woman having the diagnosis of focal segmental GN with a serum PON1 level of 61.7 U/L and with a proteinuria of <3.5 g/day. The other patient with urinary PON1 level (4 U/L) was a 39-year-old man having the diagnosis of membranous GN with a serum PON1 level of 173 U/L and microalbuminuria.

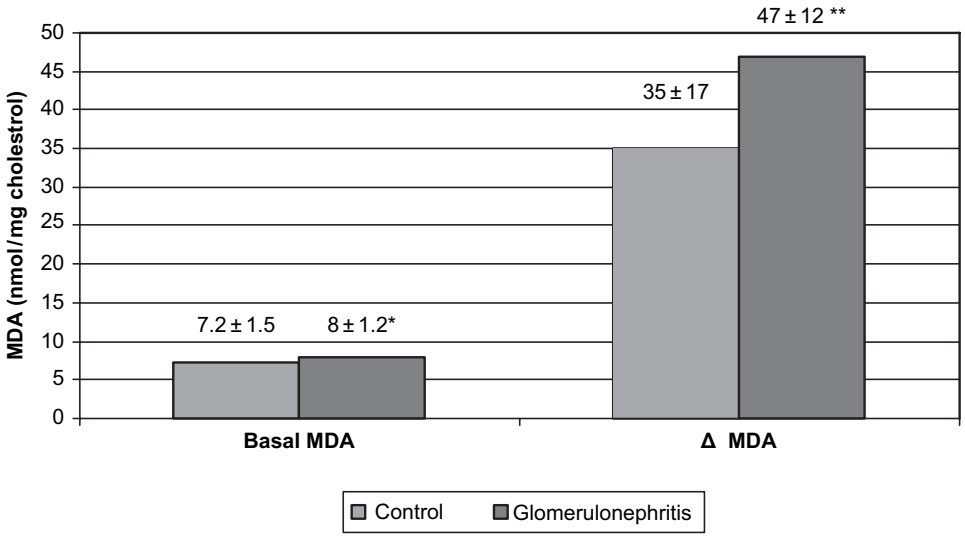

Figure 1. Basal malondialdehyde (MDA) and ΔMDA values (nmol/mg cholesterol) of apolipoprotein B-containing lipoproteins. ΔMDA value represents oxidizability of apolipoprotein B-containing lipoproteins and was obtained by subtracting basal MDA value (without incubation) from the 3-h (following incubation with copper sulfate) value. Results are expressed as mean ± SD. *$p < 0.05$, **$p = 0.01$.

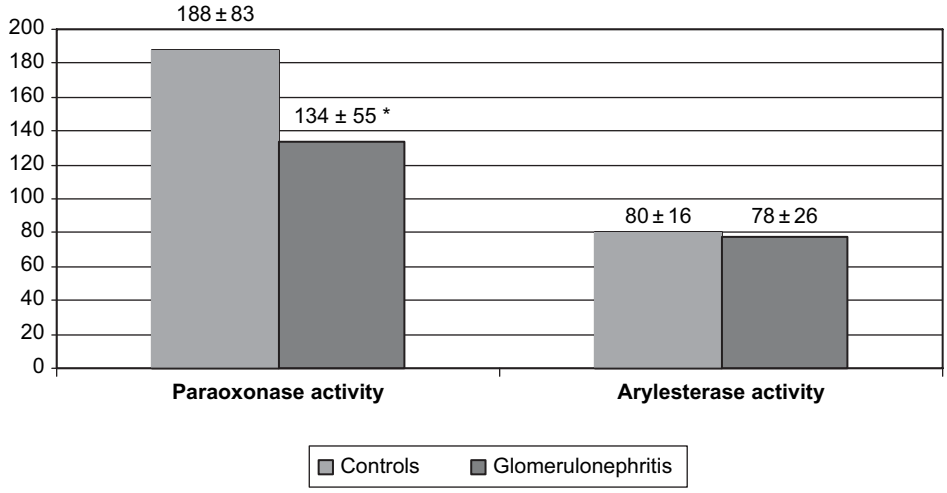

Figure 2. Serum paraoxonase (U/L) and arylesterase (U/mL) activities in the study groups. Values are mean ± SD. *Significantly different compared with the control group at $p < 0.05$.

DISCUSSION

In this study, serum PON1 activity was found to be low in non-uremic glomerulonephritis patients with normal lipid parameters as well as uremic predialysis and dialysis patients.[9,13–15]

The increase in frequency of CAD that is a major factor for mortality in patients with renal insufficiency is attributed to hyperphosphatemia and elevated concentrations of complement factor D, besides classical risk factors like diabetes mellitus, hypertension, dyslipidemia, impaired insulin sensitivity, and endothelial dysfunction. In cases with underlying renal pathology, a number of studies have been conducted to determine when the risk occurred to onset, and remarkable results were found. For example, the findings of hypertension and left ventricular hypertrophy in IgA nephropathy cases with normal inulin clearance; an increase in insulin resistance before the GFR decreases to values below 80 mL/min; and an increase in Lp (a), homocysteine and asymmetric dimethyl-L-arginine levels in cases with normal inulin clearance show that cardiovascular risk increases before renal failure develops even when the GFR values are normal.[16] In this study, we found that PON1, which may increase CAD risk, is decreased, and the oxidizability of apolipoprotein B-containing lipoproteins is increased in biopsy-proven glomerulonephritis patients with normal serum creatinine levels.

A decrease in HDL levels in these patients could lead to a decrease in serum PON1.[5] In our study, despite the fact that HDL was not different in control groups, the difference in standardized PON activity (PON1/HDL ratio) was statistically significant in GN group in parallel with previous studies. Therefore, in this group of patients, PON1 activity might be affected not only by differences in HDL levels, but also by other factors such as lipid peroxidation products, cytokines, and uremia.[6,8] In previous studies, the decrease in PON1 activity in patients with very high urea levels was related to uremia.[10,15] In one study, paraoxonase activity was found lower in both the conservatively managed CRF and HD patients than in controls, and there was no significant difference in paraoxonase activity between the CRF and HD groups.[13] In another study, paraoxonase and arylesterase activities were found reduced in both CRF and HD patients compared to controls, and the reduction was more pronounced in the HD group.[14] In one study comparing the uremic and kidney-transplanted patients with a healthy control group, it was shown that there is a correlation between the decrease in urea levels and the consequent increase in PON1 activity in kidney-transplanted patients.[15] In our study, serum urea, total protein, and albumin levels were normal, and the PON1 activity was low in GN patients.

In our study, serum arylesterase activity, which is the main component of the enzyme, was normal in glomerulonephritis patients while PON1 activity was detected as low. This result suggests that paraoxonase enzyme synthesis was not affected, but a functional deficit was detected. This could be because of lipid peroxidation products or cytokines.

The glomerulonephritis patient with or without cardiovascular risk factors has an increased tendency to the LDL oxidation, and the response of the organism to this

situation in these patients is more pronounced than in patients with normal renal functions.[3] In studies, ROS was found accumulating in sclerotic or mesangial sites in patients with renal failure.[17] At the end of this accumulation, cytotoxic substances released from foam cells leads to glomerular cell destruction, causing an increase in the already present renal disease.[18] Little is known about the biological events and molecular reactions promoting LDL oxidation in the damaged glomerular tuft. However, the present findings, the reactions that cause ROS production, are promoted by enzymatic activation of xanthine oxidase, mitochondrial oxidation, NAD(P)H oxidase, endothelial nitric oxide synthase in its uncoupled state, lipo-oxygenase or myeloperoxidase, and the transition of metals such as iron.[16]

In an experimental study, HDL did not prevent LDL-induced monocyte transmigration in PON1 knockout rats, and this in turn caused a significant increase in atherosclerotic lesions in the vessel wall.[19] Another study that deserves attention shows the association of PON1 activity and CAD cases with atherosclerosis and normal levels of total, HDL, and LDL cholesterol and triglycerides compared with healthy controls with similar serum lipid levels.[20] In that study, PON1 activity was found to be low. Similarly, in our study, glomerulonephritis patients with normal serum lipid levels were compared with healthy controls, and low PON1 activity was found. In the scope of these studies, the glomerulonephritis cases with low PON1 activity will have a decrease in the suppression of the oxidation with the beginning of GN, and as a result, the increased oxidizability of apolipoprotein B-containing lipoproteins may accelerate CAD, which is a major factor for mortality and morbidity besides the increase in renal damage and the deterioration of the renal functions.

We determined urinary PON1, not reported to be detected in the literature so far, in two of our patients. In this study, urinary PON1 was not associated with gender, age, proteinuria level, duration of the disease, and serum PON1 activity. Further studies are needed in order to evaluate the clinical and pathophysiological importance of urinary PON1 levels.

In conclusion, at the time of diagnosis, close follow-up of the patients with GN is necessary for the management of cardiovascular and renal risk, even if they have normal serum creatinine and lipid levels.

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