ALTERATIONS OF PARAOXONASE AND PLATELET-ACTIVATING FACTOR ACETYLMYDROLASE ACTIVITIES IN PATIENTS ON PERITONEAL DIALYSIS

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Running Head: PON1 and PAF-AH in Peritoneal Dialysis

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ABSTRACT

**Objective:** A more atherogenic lipid profile seen in peritoneal dialysis (PD) patients cannot fully explain the increased incidence of atherosclerosis in this population. Oxidative modification of low-density lipoproteins (LDL) is considered to play a central role in the atherogenic process, whereas high-density lipoprotein (HDL) protects LDL from oxidation. On the other hand, it has been suggested that LDL and HDL from PD patients are more resistant to oxidation than those from control subjects, while PD-HDL equally protects LDL from oxidation compared to control-HDL. Two HDL-associated enzymes have been shown to protect both LDL and HDL from oxidation: paraoxonase (PON1) and HDL-associated platelet-activating factor acetylhydrolase (HDL-PAF-AH). Furthermore, low PON1 activity and high total plasma PAF-AH concentration, which mainly represents the LDL-associated enzyme, have been shown to be independent risk factors for coronary artery events in the general population. However, there are limited data regarding possible alterations of these enzymes in PD patients. The aim of our study was to examine the possible alterations of PON1 and PAF-AH activities in patients undergoing PD.

**Design:** A cross-sectional study.

**Setting:** University Medical Center.

**Participants:** Fifty-six PD patients of Caucasian origin and 86 matched controls were studied.

**Measurements:** In all subjects, serum PON1 activity toward paraoxon (paraoxonase) and phenylacetate (arylesterase), as well as total serum and HDL-associated PAF-AH
activities were measured, while PON1 genetic polymorphisms known to influence PON1 activity (Q192R and M55L) were determined.

**Results:** PD patients exhibited significantly increased serum PON1 (paraoxonase) and PON1 (arylesterase) activities compared to controls, regardless of the PON1 polymorphisms or the levels of HDL cholesterol. Additionally, PD patients had significantly elevated activities of total serum PAF-AH and HDL-associated PAF-AH independently of the levels of LDL or HDL cholesterol, while the ratio of HDL-PAF AH/total PAF-AH, which has been recently suggested to be a potential marker of atherogenicity, was decreased in these patients compared to controls. Moreover, no difference in the prevalence of PON1 polymorphisms between PD patients and controls was found.

**Conclusion:** The elevated activities of PON1 and HDL-PAF AH could explain the increased resistance of PD-HDL to oxidation, while the higher activity of total PAF-AH and the decreased HDL-PAF AH/total PAF-AH ratio could contribute to the increased incidence of atherosclerosis in these patients.

**Key words:** Paraoxonase, Platelet-activating factor acetylhydrolase, Lipoprotein-associated phospholipase A2, Peritoneal dialysis, CAPD, Lipids, Renal failure, Atherosclerosis.
INTRODUCTION

Patients undergoing peritoneal dialysis (PD) have a high incidence of accelerated atherosclerosis and coronary artery disease (CAD) [1,2]. Studies have shown a higher prevalence of dyslipidemic risk factors associated with an increased risk of CAD, such as elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and lipoprotein (a) [Lp(a)], and decreased levels of high-density lipoprotein cholesterol (HDL-C) in PD patients [3-6]. However, these abnormalities cannot fully explain the increased incidence of CAD in this population. In this context, recent reports support a role for non-traditional cardiovascular risk factors, such as the elevated plasma concentrations of C-reactive protein [7], or the effect of the apolipoprotein E gene polymorphisms [8].

Oxidative modification of LDL is considered to play a key role in the atherogenic process [9]. On the other hand, it is well established that HDL plays a protective role against atherosclerosis [10], while oxidized HDL loses its anti-atherogenic potential [11]. In addition to its role in reverse cholesterol transport, HDL also exhibits anti-inflammatory and anti-oxidant properties. Two HDL-associated enzymes have been shown to contribute to these HDL properties, thus protecting both LDL and HDL from oxidation: paraoxonase (PON1) and HDL-associated platelet-activating factor acetylhydrolase (HDL-PAF AH). However, despite the higher incidence of atherosclerosis in PD patients, both LDL and HDL from these patients have been found to be more resistant to in vitro oxidation than lipoproteins from control subjects, while PD-HDL and control-HDL were equally effective in preventing cooper-mediated LDL oxidation [12]. Surprisingly, there are limited data regarding possible alterations of HDL-associated anti-oxidant enzymes in PD patients.

PON1 is an esterase that is exclusively associated with HDL (especially with a HDL subclass which also contains apolipoprotein AI and clusterin). PON1 can hydrolyze
oxidized phospholipids and cholesteryl ester hydroperoxides formed during lipoprotein peroxidation [13]. The PON1 gene is located on the long arm of chromosome 7. Two main genetic polymorphisms that determine PON1 activity have been described, one at position 55 (methionine/leukine, M55L) and the other at position 192 (glutamine/arginine, Q192R) [14]. The substrates for PON1 that have been mostly used in assays in vitro are paraoxon and phenylacetate. The latter is used to measure the arylesterase activity of PON1, which is considered to be a more accurate estimate of the PON1 mass. Interestingly, low serum PON1 activity toward paraoxon has recently been shown to be an independent risk factor for coronary events in high-risk men [15].

Studies in patients undergoing hemodialysis have revealed a decreased serum PON1 activity in this population, even if it was corrected for HDL-C levels or PON1 genotypes [16-18]. Furthermore, decreased PON1 activity toward paraoxon, but not phenylacetate, was found in the only study which examined the serum PON1 activity in PD patients [18].

PAF-AH (also termed lipoprotein-associated phospholipase A2) hydrolyzes short acyl groups at the sn-2 position of PAF and oxidized phospholipids [19]. In human plasma, PAF-AH is primarily associated with LDL particles, whereas a small proportion (<20% of total enzyme activity) is associated with HDL [19]. There has been much controversy concerning the possible role of the LDL-associated PAF-AH in atherogenesis [19]. However, recent studies suggest that plasma levels of PAF-AH, which are proportional to its activity and mainly reflect the LDL-associated enzyme, represent an independent risk factor for coronary artery disease [20,21]. On the contrary, HDL-associated PAF-AH (HDL-PAF AH) has been shown to protect LDL from oxidation and inhibit the biological activity of oxidized LDL, thus exhibiting atheroprotective properties [19,22]. In the only study examining the PAF-AH activity in PD patients, it was shown that total plasma, as
well as HDL-associated PAF-AH activities were significantly increased in these patients compared to the control population [23].

Thus, we undertook the present study to further examine possible alterations of PON1 activity in relation to its genetic polymorphisms, as well as of total and HDL-associated PAF-AH activities in patients undergoing PD compared with control subjects.
MATERIALS AND METHODS

Study population

Our study population consisted of 56 Caucasian patients originating from Northwestern
Greece on chronic ambulatory peritoneal dialysis (PD) for at least six months before their
inclusion in the study. PD patients performed 4 exchanges per day with 2-liter solutions of
1.86 or 3.86% glucose, depending on the individual need for ultrafiltration. All patients
had been free of peritonitis or any other infection at least 3 months preceding blood
sampling. The adequacy of the dialytic treatment was evaluated by the Kt/V ratio, which
ranged from 1.9 to 2.0 (weekly), while in all patients the residual renal function estimated
by the average of the residual creatinine and urea clearances was <5.0 ml/min. The renal
diagnosis was chronic glomerulonephritis in 11 patients, hypertensive nephropathy in 11
patients, chronic pyelonephritis in 12 patients, polycystic kidney disease in 6 patients and
obstructive uropathy in 7 patients, while it remained unknown in the remaining 9 patients.
Patients with a known family history of primary dyslipidemia, excessive alcohol
consumption, diabetes mellitus [fasting serum glucose >6.93 mmol/l (126 mg/dl)], obesity
(body-mass index >30 Kg/m²), liver disease, systemic illness, thyroid disorders, or any
other metabolic or endocrine disorder were excluded from the study. Patients received no
other medications except erythropoietin, multivitamins, calcitriol, iron, phosphate binders
other than sevelamer HCl, angiotensin-converting enzyme inhibitors and calcium channel
blockers. Patients taking lipid-lowering drugs or any other medication known to affect
serum lipids (e.g b-blockers) were also excluded from the study. Furthermore, 86
Caucasian healthy individuals from the same region matched for age, sex and smoking
habits with the PD patients were also studied. These subjects were consecutive healthy
unrelated individuals who underwent a regular check-up in our outpatient internal
medicine clinic. None of these individuals were receiving drugs affecting lipid profile or
renal function. Smoking habit was defined as smoking currently. All participants gave
informed consent for genetic analysis, and the study protocol was approved by the ethic
committee of our university hospital. In all participants blood samples were obtained after
a 14-hour overnight fast for gene genotype detection, as well as for the determination of
serum laboratory parameters. All patients were dialyzed during night with a 2-liter solution
of 1.86% glucose. Blood samples were centrifuged for 30 min (3600g) and then the serum
was separated and stored at -80 °C for analysis of laboratory parameters.

**Analytical methods**

Concentrations of total cholesterol and triglycerides were determined enzymatically on
the Olympus AU600 clinical chemistry analyzer (Olympus Diagnostica, Hamburg,
Germany). High-density lipoprotein cholesterol (HDL-C) was determined in the
supernatant, after precipitation of the apolipoprotein B-containing lipoproteins with
dextran sulphate-Mg²⁺ (Sigma Diagnostics, St. Louis, MO, USA) (HDL-rich serum). Low-
density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula if
fasting triglycerides were <4.52mmol/L (400 mg/dl) [24], while the non HDL cholesterol
was calculated by the equation: non HDL-C=Total cholesterol – HDL-C. Apolipoproteins
A1, B, E and the lipoprotein (a) [Lp(a)] were measured with a Behring Nephelometer
BN100 using reagents (antibodies and calibrators) from Date Behring Holding GmbH
(Liederbach, Germany). The ApoA1 and ApoB assays were calibrated according to the
International Federation of Clinical Chemistry (IFCC) standards. Finally, serum
interleukin-6 (IL-6) levels were measured by ELISA (R&D Systems Europe, Abingdon,
Oxon, UK) in 21 randomly selected PD patients and 32 controls matched for age, sex, BMI
and smoking habits.
**PON1 assay**

Both PON1 (paraoxonase) and PON1 (arylesterase) activities in serum were determined in the presence of 2 mM Ca\(^{2+}\) in 100 mM Tris-HCl buffer (pH 8.0) for paraoxon as a substrate, and in 20 mM Tris-HCl buffer (pH 8.0) for phenylacetate as a substrate, respectively, as previously described [25].

**PAF-AH assay**

PAF-AH activity was measured by the trichloroacetic acid precipitation procedure [26] using as a substrate 1-O-hexadecyl-2-[\(^3\)H-acetyl]-sn-glycero-3-phosphocholine ([\(^3\)H]-PAF) (10 Ci/mm; DuPont-New England Nuclear, Boston, MA) at a final concentration of 100 µM [26]. Fifty µl of either serum diluted 1:50, v/v with HEPES buffer, pH 7.4, or the HDL-containing supernatant after treatment of serum with magnesium chloride-dextran sulfate (HDL-rich serum) (diluted 1:3, v/v with HEPES) were mixed with HEPES in a final volume of 90 µl and used as the source of the enzyme. The reaction was performed for 10 min at 37 °C, and PAF-AH activity was expressed as nmol PAF degraded per min per ml of serum.

Reproducibility of PON1 (paraoxonase), PON1 (arylesterase) and PAF-AH assays was determined by intra-assay determination of coefficients of variation (CVs). The CVs for PON1 (paraoxonase) and PON1 (arylesterase) assays ranged between 6 to 8%, while the CVs for PAF-AH assays ranged between 4 to 5%.

**Determination of PON1 genotypes**

Genomic DNA was obtained from leukocytes using standard procedures. The PON1 Q192R and M55L polymorphisms were detected using a previously reported protocol [27]. Briefly, primers for amplification of a 99-bp DNA that contains the coding sequence for position 192 were 5’TAT TGT TGC TGT GGG ACC G3’ and 5’CAC GCT AAA G3’. After an initial denaturation step of 5 min at 95 °C, the PCR
was carried out for 40 cycles, with each cycle consisting of 60 sec of denaturation at 94 °C, 45 sec of annealing at 56 °C and 45 sec of extension at 72 °C. PCR product was digested with 5U of Alw I restriction enzyme, for 3 hours at 37 °C.

For genotyping the M55L polymorphism, the primers for amplification of 144-bp DNA encoding codon 55 were 5’GAG TGA TGT ATA GCC CCA GTT TC3’ and 5’AGT CCA TTA GGC AGT ATC TCC G3’. An initial incubation for 5 min at 95 °C was followed by the step of amplification that was carried out for 40 cycles, with each cycle consisting of 1 min of denaturation at 94 °C, 45 sec of annealing at 61 °C and 45 sec of extension at 72 °C. PCR product was digested with 5U of Hinf I restriction enzyme for 24 hours at 37 °C.

**Statistical analysis**

Statistical analysis was performed with STATISTICA 6.0 statistical software. Allele frequencies were estimated by the gene-counting method. Chi square-test was used to compare gene frequencies, while Students’ t-test for independent samples and Mann-Whitney U test were used to test the differences of parametric and non-parametric data, respectively, between the two study populations. The effect of the PON1 gene polymorphisms on laboratory parameters was tested using one-way analysis of variance (one-way ANOVA) followed by the LSD test for pairwise comparisons in case of significant results, except for serum PON1 (Paraoxonase), PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI, where the Kruskal-Wallis ANOVA median test was used followed by the Mann-Whitney U test for pairwise comparisons in case of significant results. Multiple linear regression analysis was performed to test the overall effect on PON1 and PAF-AH activities of genetic and other factors shown to influence these activities in univariate analysis. Log transformation of PON1 (paraoxonase) activity and serum IL-6 levels was applied because of their skewed distribution. Finally, linkage
disequilibrium coefficients for Q192R and M55L genetic variants of PON1 were calculated using the DnaSP 4.0 software package.
RESULTS

Table 1 shows the clinical and laboratory characteristics of the study population. Control subjects and PD patients were well-matched with respect to age, gender, smoking habits, body-mass index (BMI) and serum glucose concentration. The observed genotype frequencies of Q192R and M55L polymorphisms did not deviate from those predicted by the Hardy-Weinberg equation, while there was a strong linkage disequilibrium between them (Lewontin’s $D'$ coefficient of 0.75 in the control subjects and 0.71 in PD patients, $p<0.0001$ for both groups). Furthermore, frequencies of Q192R and M55L gene alleles were not different between the two groups. On the other hand, PD patients exhibited significantly lower serum albumin levels and a more atherogenic lipid profile, consisting of significantly increased levels of total cholesterol, LDL-C, non HDL-C, triglycerides, apolipoproteins B and E, as well as elevated concentration of Lp(a), and decreased levels of HDL-C.

Table 2 demonstrates the effect of Q192R genotypes on PON1 activity in PD patients. QQ individuals exhibited the lowest activity toward paraoxon, even when corrected for HDL-C or ApoAI levels. QR patients had intermediate PON1 (paraoxonase) activity, while RR patients showed the maximum activity. PON1 activity toward phenylacetate was not influenced by the Q192R polymorphisms. Table 3 presents the effect of M55L genotypes on PON1 activity in PD patients. LL patients had the highest PON1 (paraoxonase) and PON1 (arylesterase) activities compared to LM and MM subjects, which had intermediate and minimum activities toward paraoxon and phenylacetate, respectively. The same trend remained true even after correction of PON1 activities for HDL-C or ApoAI levels. Finally, neither Q192R nor M55L polymorphisms had any effect on the levels of serum lipid parameters (data not shown).
PD patients had significantly increased activities of PON1 (paraoxonase), as well as of PON1 (arylesterase) compared to control subjects (Fig. 1A and 1B). This difference persisted even after correction of PON1 (paraoxonase) and PON1 (arylesterase) activities for HDL-C and ApoAI levels (Fig. 2A and 2B), suggesting that the increase in PON1 activities in PD patients is unrelated to the alterations of HDL-C or ApoAI levels seen in this population. Furthermore, because of the powerful effect of PON1 genotypes on PON1 activities, different genotype combinations could result in different PON1 activities between the two groups. However, as shown in figure 3, median PON1 (paraoxonase) activity remained significantly higher in PD patients, even if certain PON1 combined haplotypes between the two groups were compared. Interestingly, the most pronounced differences were observed between subjects and patients carrying the high-activity haplotypes (RL/RL). The same observations were also made for the PON1 (arylesterase) activity, as well as for the corrected PON1 (paraoxonase) and PON1 (arylesterase) activities (data not shown). Therefore, PON1 activities are increased in PD patients independently of PON1 genotypes or HDL-C and ApoAI concentrations.

Moreover, PD patients exhibited significantly increased serum IL-6 levels compared with the controls subjects (5.3±3.9 vs 1.4±0.9 ng/L, respectively, p<0.001 by the Mann-Whitney U test). Moreover, a significant association between serum Log IL-6 levels and Log PON1 (paraoxonase) activity (r=0.27, p=0.03), as well as between serum Log IL-6 levels and PON1 (arylesterase) activity (r=0.31, p=0.01) was found in PD patients, but not in the control subjects (r=0.11 and 0.12, respectively, p=NS). Additionally, no correlation between Log PON1 (paraoxonase) or PON1 (arylesterase) activities and glomerular filtration rate was found in our patient group (r=0.03 and 0.09, respectively, p=NS).

In a multiple linear regression model, Log PON1 (paraoxonase) activity in PD patients was independently associated with the Q192R polymorphism (b=0.51, p=0.007), M55L
polymorphism (b=0.46, p=0.022) and the duration of dialysis (b=0.34, p=0.049), but not with gender, age, BMI, smoking habit and ApoAI levels. In another model, PON1 (arylesterase) activity (which is considered to be an accurate estimate of PON1 mass) was independently associated with the M55L polymorphism (b=0.46, p=0.035) and the duration of dialysis (b=0.42, p=0.030), but not with the Q192R polymorphism, gender, age, BMI, smoking habit and ApoAI levels.

Moreover, as shown in table 4, PD patients had significantly elevated levels of total serum PAF-AH activity compared with the control subjects. This increase persisted after correction of total PAF-AH activity for the levels of LDL-C, ApoB and non HDL-C. The HDL-associated PAF-AH activity was also more increased in PD patients than in the controls, which insisted even after adjustment for the HDL-C and ApoAI levels (table 4). Finally, the ratio of HDL-PAF AH/total serum PAF-AH activities, which has been proposed to be a potential marker of atherogenicity [28], was significantly decreased in PD patients compared to the control population. In addition, no significant associations between total serum PAF-AH or HDL-PAF AH activities and glomerular filtration rate or serum IL-6 levels were found in our patient group (data not shown).

In a multiple linear regression model, total PAF-AH activity was independently associated with the levels of ApoB (b=0.927, p=0.0000), but not with the levels of triglycerides, gender, age, BMI, smoking habit, residual renal function and duration of dialysis. On the other hand, HDL-associated PAF-AH activity was independently associated only with triglyceride levels (b=-0.60, p=0.020), but not with gender, age, duration of dialysis, BMI, residual renal function or levels of ApoAI.
DISCUSSION

Our study clearly shows that peritoneal dialysis is associated with increased activities of PON1 (paraoxonase) and PON1 (arylesterase) compared with the control population, regardless of PON1 genetic polymorphisms or HDL-C and ApoAI concentrations. Furthermore, PD patients exhibited elevated levels of total serum and HDL-associated PAF-AH activities, independently of possible alterations in serum lipid and lipoprotein levels, as well as a lower ratio of HDL-associated/total PAF-AH activity.

This study is the first to determine PON1 genotypes (Q192R and M55L) in PD patients. No difference in the allele frequency between PD patients and control subjects was found. The effect of these polymorphisms on PON1 activities did not differ from that reported in healthy populations [14], in patients undergoing hemodialysis [29], or in kidney transplanted recipients [30]. In summary, both polymorphisms significantly influenced PON1 (paraoxonase) activity in PD patients, but only M55L polymorphism affected PON1 (arylesterase) activity, and therefore PON1 mass [30,31]. Finally, no effect of PON1 polymorphisms on levels of serum lipid parameters was noticed in PD patients, in disagreement with one study in Alberta Hutterites (a genetically isolated population) [32], but in agreement with other studies in renal patients [30].

The increased PON1 (paraoxonase) and PON1 (arylesterase) activities in PD patients in our study contradicts the previous observations of Dantoine et al, who found that serum PON1 activity towards paraoxon or 4-nitrophenyl acetate was significantly lower in PD patients compared to control subjects, whereas there was no difference in serum PON1 activity towards phenylacetate [18]. However, only a small number of PD patients (n=22) were included in that study, while no PON1 genotyping was performed, and no correction of PON1 activities for the levels of HDL-C or ApoAI was made. Thus, because of the small number of PD patients in that study, it is possible that even small differences in
genotype distribution or in HDL-C levels between patients and controls could have led to these different results between the two studies.

The reasons for the observed increase in PON1 (paraoxonase) activity and estimated PON1 mass [PON1 (arylesterase) activity] in PD patients are not well understood. Firstly, liver is the main source of circulating PON1, and PD has been associated with an enhanced nonspecific hepatic protein production as a consequence of protein losses in the dialysis fluid and increased glucose load to which these patients are exposed [1,33]. Therefore, an increased hepatic production of PON1 could be the underlying mechanism. Furthermore, interleukin-6 (IL-6) has been recently shown to up-regulate PON1 mRNA expression by hepatocytes [34], while PD has been shown to induce an increased production of IL-6 by peripheral blood mononuclear cells [35]. Additionally, serum IL-6 levels were increased in our PD patients compared to the control subjects, while both PON1 (paraoxonase) and PON1 (arylesterase) activities were significantly associated with serum IL-6 levels in the patient group. Therefore, IL-6-mediated increase in PON1 production may also contribute to the elevated PON1 activity observed in PD patients. On the other hand, no significant association between PON1 activities and glomerular filtration rate was found, suggesting that these activities were not altered by the residual renal function.

The markedly increased total serum and HDL-associated PAF-AH activities observed in our PD patients compared to control subjects are in agreement with our previous observations [23]. In that study, we had shown that plasma PAF-AH activity was not associated with creatinine clearance in patients with mild to moderate chronic renal failure, and PAF-AH activity was more increased in PD patients than in those undergoing hemodialysis [23]. Furthermore, no significant association between PAF-AH activity and residual renal function neither in univariate nor in multivariate analysis was found in our study. These observations almost exclude the possibility that the observed increase in PAF-
AH activity is due to a decreased catabolism/clearance of the enzyme by the kidneys. On the other hand, total PAF-AH activity has been shown to correlate with LDL-C and ApoB levels, being preferentially located in small-dense LDL particles [28]. Therefore, the increased levels of LDL-C and ApoB in our PD patients could, at least in part, explain the observed increase in total PAF-AH activity in this population, as it is also shown by the results of multiple linear regression analysis (see above). However, the above proposed mechanism does not explain why total PAF-AH activity remains higher in the PD population than the control subjects even if it was adjusted for the levels of LDL-C and ApoB. Therefore, an increased production of PAF-AH protein in PD patients from its main cellular sources (macrophages and liver cells) may be another mechanism for the observed increase in PAF-AH activities in these patients. Indeed, it has been reported that platelet-activating factor (PAF) levels are increased in patients with renal failure [36], while PAF has been shown to directly stimulate the synthesis and secretion of PAF-AH by the liver [37]. Additionally, the increased hepatic protein production in PD patients may contribute to the elevated PAF-AH activity. Furthermore, we cannot exclude the possibility that elevated circulating LDL particle numbers in such patients could enhance PAF-AH secretion from its cellular sources [19,38]. In addition, levels of oxidized LDL are increased in PD patients [39], and oxidized LDL contains PAF [40], which can in turn stimulate liver PAF-AH secretion, as previously discussed [37]. Consequently, high LDL plasma levels, typical of PD patients, may augment secretion of PAF-AH from both macrophages and liver cells.

The reasons for the observed enrichment of HDL with PAF-AH activity in PD patients are poorly understood. An altered enzyme glycosylation in PD patients could have resulted in this HDL enrichment, since it has recently been shown that the degree of enzyme glycosylation is a factor affecting the preferential association of PAF-AH with LDL versus
HDL particles [41]. Furthermore, the increased PON1 activity could have contributed to the observed increase in HDL-associated PAF-AH activity, since PON1 has been shown to additionally express PAF-AH-like catalytic activity [42].

The ratio of HDL-PAF-AH/total PAF-AH, which has been recently proposed to be a marker of the severity of hypercholesterolemia and atherogenicity [28], was decreased in our PD patients compared to the control subjects, suggesting that the increase of the total PAF-AH activity, which mainly represents the LDL-associated enzyme, exceeds that of the HDL-PAF-AH activity [19].

The elevated activities of PON1 and HDL-associated PAF-AH in our PD patients could explain why HDL from patients on short or long term PD treatment was more resistant to in vitro copper-mediated oxidation and auto-oxidation, while its capacity to protect LDL from oxidation was preserved [12,43]. In vivo, however, reduced levels of HDL in PD patients could result in decreased overall HDL antioxidant capacity.

The clinical significance of the above described alterations of PON1 and PAF-AH activities in PD patients in terms of atherogenicity and prevalence of coronary events is uncertain owing to the lack of appropriate clinical studies in this population. Results from studies in the general population revealed that low PON1 (paraoxonase) activity is an independent risk factor for coronary events in high-risk men [15]. These results suggest that PON1 has a direct protective effect against CAD by preventing the oxidation of LDL [13,15]. On the other hand, recent studies have shown that high total PAH-AH concentration is an independent risk factor for CAD in men [20], and in both women and men with low LDL-C levels [21]. The suggested pro-atherogenic role of the LDL-associated PAF-AH may be related to its preferential location in the small dense atherogenic LDL particles [26], and the PAF-AH-mediated release of lysophosphatidylcholine and oxidized fatty acids, which are known to exhibit
proatherogenic properties during LDL oxidation [19]. On the contrary, several lines of evidence suggest that HDL-associated PAF-AH activity, although much lower than that in LDL, may substantially contribute to the protection of LDL from oxidation and to the HDL-mediated inhibition of cell stimulation induced by oxidized LDL [19,22]. In this context, based on results obtained in patients with primary hypercholesterolemia [28] and in patients with unstable angina [44], our group has proposed that a decreased ratio of HDL-PAF AH/total PAF-AH could be a marker of atherogenicity [19].

One limitation of our study is that we do not provide data on other specific factors, which could have contributed to the described alterations of the enzymes’ activities in PD patients, such as glucose absorption, glucose homeostasis, calculated protein intake, nutritional status, inflammation markers, oxidative stress parameters and levels of antioxidants (e.g. beta-carotene and alpha-tocopherol). Thus, other possible causes, as well as the consequences of these alterations have not been completely analyzed.

In conclusion, we have shown that PD patients exhibit enhanced activities of PON1, as well as total and HDL-associated PAF-AH, along with a decreased HDL-PAF AH/total PAF-AH ratio. Further prospective studies on the relationship between the altered activities of these enzymes and the occurrence of coronary events in this population, as well as on the effects of lipid-lowering drugs on these enzyme activities [45] are absolutely necessitated.
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| Age (years)                      | 60.3±17.2     | 61.4±13.4     | NS  
| Gender (M/F)                     | 49/37         | 31/25         | NS  
| Smoking (Yes/No)                 | 25/61         | 17/39         | NS  
| BMI (Kg/m²)                      | 25.4±3.1      | 25.1±2.9      | NS  
| Duration of dialysis (months)    | -             | 53.1±20.4     | NS  
| Glucose (mmol/L)                 | 5.70±1.28     | 5.87±1.74     | NS  
| (mg/dl)                          | (103.8±23.3)  | (106.8±31.7)  |  
| Creatinine (µmol/L)              | 81.3±11.5     | 839.8±247.5   | 0.0000  
| (mg/dl)                          | (0.92±0.13)   | (9.5±2.8)     |  
| Albumin (g/L)                    | 41.0±3.9      | 36.6±4.0      | 0.0000  
| T-CHOL (mmol/L)                  | 5.22±0.84     | 6.22±1.47     | 0.0000  
| (mg/dl)                          | (201.8±32.7)  | (240.1±57.0)  |  
| TRG (mmol/L)                     | 1.16±0.61     | 2.0±1.0       | 0.0001  
| (mg/dl)                          | (102.8±54.1)  | (177.7±89.9)  |  
| HDL-C (mmol/L)                   | 1.35±0.28     | 1.18±0.41     | 0.0001  
| (mg/dl)                          | (52.3±10.8)   | (45.6±15.9)   |  
| LDL-C (mmol/L)                   | 3.49±0.82     | 4.12±1.20     | 0.0000  
| (mg/dl)                          | (135.1±31.6)  | (159.2±46.5)  |  
| non HDL-C (mmol/L)               | 3.89±0.97     | 5.04±1.37     | 0.0000  
| (mg/dl)                          | (150.3±37.4)  | (194.6±53.0)  |  
| ApoAI (g/L)                      | 1.44±0.24     | 1.40±0.38     | NS  
| ApoB (g/L)                       | 0.96±0.21     | 1.20±0.26     | 0.0000  
| ApoE (mg/L)                      | 3.6±0.8       | 4.8±1.6       | 0.0001  
| Lp(a) (g/L)                      | 0.78 (0.08-5.6)| 2.26 (0.30-5.30) | 0.0001  
| Frequencies of Q/R alleles of    |               |               |  
| the Q192R PON1                    | 69.3/30.7     | 69.6/30.4     | NS  

Table1. Clinical and laboratory characteristics of the study population
| Frequencies of L/M alleles of the L55M PON1 polymorphism (%) | 66.7/33.3 | 68.7/31.3 | NS |

Values are expressed as mean±SD except for Lp(a), which is expressed as median (range). PD; peritoneal dialysis, BMI; body-mass index, M; male, F; female, T-CHOL; total cholesterol, TRG; triglycerides, HDL-C; high-density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, non-HDL-C; non-HDL cholesterol, Apo; apolipoprotein, Lp(a); lipoprotein (a). Values were compared using the Student’s t-test for independent samples, except for ApoE, Lp(a) and TRG, where the Mann-Whitney U test was used. Gene frequencies were compared by the chi-square test. NS; not significant
Table 2. Effect of PON1 Q192R genotypes on PON1 activity in patients undergoing peritoneal dialysis (PD)

<table>
<thead>
<tr>
<th></th>
<th>QQ (n=29)</th>
<th>QR (n=20)</th>
<th>RR (n=7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (Paraoxonase) (U/L)</td>
<td>50.0(25-160)</td>
<td>132(43-230)</td>
<td>214(170-259)</td>
<td>0.010</td>
</tr>
<tr>
<td>PON1 (Arylesterase) (U/ml)</td>
<td>83.2±25.9</td>
<td>89.8±19.4</td>
<td>85.5±23.3</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L) (mg/dl)</td>
<td>1.16±0.30 (44.9±11.9)</td>
<td>1.23±0.50 (47.5±19.5)</td>
<td>1.19±0.28 (46.0±10.8)</td>
<td>NS</td>
</tr>
<tr>
<td>ApoAI (g/L)</td>
<td>1.44±0.30</td>
<td>1.44±0.44</td>
<td>1.40±0.28</td>
<td>NS</td>
</tr>
<tr>
<td>PON1(Paraoxonase)/HDL-C (U/mg)</td>
<td>0.11(0.05-0.46)</td>
<td>0.32(0.06-0.60)</td>
<td>0.47(0.35-0.62)</td>
<td>0.019</td>
</tr>
<tr>
<td>PON1(Paraoxonase)/ApoAI (U/mg)</td>
<td>0.03(0.02-0.22)</td>
<td>0.11(0.02-0.16)</td>
<td>0.16(0.12-0.19)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein. Values are expressed as mean±SD, except for PON1 (Paraoxonase), PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI, which are expressed as median (range). Values were compared using the one-way ANOVA test followed by LSD test for pairwise comparisons in cases of significant results, except for PON1 (Paraoxonase), PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI, where the Kruskal-Wallis median test was used followed by Mann-Whitney U test for pairwise comparisons in cases of significant results. NS; not significant.
Table 3. Effect of PON1 M55L genotypes on PON1 activity in patients undergoing peritoneal dialysis (PD)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>LL (n=28)</th>
<th>LM (n=21)</th>
<th>MM (n=7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (Paraoxonase) (U/L)</td>
<td>152(43-320)</td>
<td>64(35-167)</td>
<td>44(25-47)</td>
<td>0.030</td>
</tr>
<tr>
<td>PON1 (Arylesterase) (U/ml)</td>
<td>101.9±21.1</td>
<td>83.6±15.4</td>
<td>67.0±17.3</td>
<td>0.019</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.23±0.49</td>
<td>1.21±0.32</td>
<td>1.21±0.25</td>
<td>NS</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>(47.8±19.0)</td>
<td>(46.9±12.4)</td>
<td>(46.8±9.7)</td>
<td></td>
</tr>
<tr>
<td>ApoAI (g/L)</td>
<td>1.46±0.04</td>
<td>1.47±0.31</td>
<td>1.47±0.10</td>
<td>NS</td>
</tr>
<tr>
<td>PON1 (Paraoxonase)/HDL-C</td>
<td>0.32(0.06-0.76)</td>
<td>0.12(0.05-0.41)</td>
<td>0.09(0.05-0.14)</td>
<td>0.030</td>
</tr>
<tr>
<td>(U/mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PON1 (Paraoxonase)/ApoAI</td>
<td>0.11(0.02-0.22)</td>
<td>0.04(0.02-0.12)</td>
<td>0.03(0.02-0.03)</td>
<td>0.030</td>
</tr>
<tr>
<td>(U/mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein. Values are expressed as mean±SD, except for PON1 (Paraoxonase), PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI, which are expressed as median (range). Values were compared using the one-way ANOVA test followed by LSD test for pairwise comparisons in cases of significant results, except for PON1 (Paraoxonase), PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI, where the Kruskal-Wallis median test was used followed by Mann-Whitney U test for pairwise comparisons in cases of significant results. NS; not significant.
Figure 1. Bar graph showing the median serum PON1 (Paraoxonase) [A] and the serum PON1 (arylesterase) (as mean ± SD) [B] activities in control subjects and peritoneal dialysis (PD) patients. a; p<0.05 compared with controls by the Mann-Whitney U test, b; p<0.01 compared with controls by the Student’s t-test for independent samples.
Figure 2. Bar graph showing the median ratios of serum PON1 (Paraoxonase)/HDL-C and PON1 (Paraoxonase)/ApoAI [A], as well as the ratios of serum PON1 (arylesterase)/HDL-C and PON1 (Arylesterase)/ApoAI (as mean ± SD) [B] activities in control subjects and peritoneal dialysis (PD) patients. HDL-C; high-density lipoprotein cholesterol, ApoAI; apolipoprotein AI, †; p<0.05 compared with controls by the Mann-Whitney U test, ‡; p<0.01 compared with controls by the Student’s t-test for independent samples.
Figure 3. Median PON1 (Paraoxonase) activity in control subjects and in patients undergoing peritoneal dialysis (PD) according to different PON1 Q192R and M55L genotype combinations. Values were compared using the Mann-Whitney U test. *p<0.05 PD vs controls; **p<0.01 PD vs controls, ***p<0.001 PD vs controls.
Table 4. Total serum and HDL-associated platelet-activating factor acetylhydrolase (PAF-AH) activities in control subjects and peritoneal dialysis patients (PD).

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=86)</th>
<th>PD (n=56)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PAF-AH (nmol/ml serum/min)</td>
<td>47.3±11.7</td>
<td>88.1±19.4</td>
<td>0.0000</td>
</tr>
<tr>
<td>Total PAF-AH/LDL-C (nmol/mg/min)</td>
<td>35.4±10.6</td>
<td>58.5±16.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>Total PAF-AH/ApoB (nmol/mg/min)</td>
<td>49.9±14.5</td>
<td>73.9±12.0</td>
<td>0.0000</td>
</tr>
<tr>
<td>Total PAF-AH/non HDL-C (nmol/mg/min)</td>
<td>36.7±8.7</td>
<td>47.0±11.4</td>
<td>0.0000</td>
</tr>
<tr>
<td>HDL-PAF AH (nmol/ml serum/min)</td>
<td>3.3±1.2</td>
<td>4.3±1.9</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-PAF AH/HDL-C (nmol/mg/min)</td>
<td>7.4±2.7</td>
<td>9.6±3.8</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-PAF AH/ApoAI (nmol/mg/min)</td>
<td>2.3±0.9</td>
<td>3.1±1.2</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-PAF AH/Total PAF-AH</td>
<td>0.07±0.03</td>
<td>0.05±0.02</td>
<td>0.002</td>
</tr>
</tbody>
</table>

HDL-C; high-density lipoprotein cholesterol, LDL; low-density lipoprotein cholesterol, Apo; apolipoprotein. Values were compared using the Student’s t-test for independent samples. NS; not significant.