Abdolkarim Mahrooz, PhD¹

Mehryar Zargari, PhD¹

Omid Sedighi, MD²

Hamed Shaygani, MD¹

Ghorban Gohari, MSc¹

¹ Department of Clinical Biochemistry and Genetics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

² Department of Internal Medicine (Nephrology Division), Imam Teaching Hospital, Mazandaran University of Medical Sciences, Sari, Iran

Increased oxidized-LDL levels and arylesterase activity/HDL ratio in ESRD patients treated with hemodialysis

Abstract

Purpose: Investigations, in which oxidized-low density lipoprotein (ox-LDL), serum paraoxonase (PON1) and homocysteine (Hcy) are considered together as important agents involved in the development of oxidative and atherogenic events in non-diabetic hemodialysis (HD) population, are limited. This case-control study was designed to evaluate these parameters in the patients and control subjects and to determine the correlations among the factors.

Methods: Forty-nine age- and sex- matched subjects, including 28 non-diabetic HD patients (paired pre-and post-dialysis samples) and 21 control subjects, were enrolled. Ox-LDL and Hcy levels were measured with ELISA and EIA methods, respectively. Arylesterase activity of PON1 was measured by spectrophotometric assay.

Results: Compared with the control group, ox-LDL levels were significantly increased both before ($p=0.001$) and after HD ($p=0.036$). Arylesterase activity-to-HDL ratio in HD patients was significantly higher than control subjects ($p=0.003$). Homocysteine levels in the ESRD patients were higher than control subjects both in pre-dialysis and post-dialysis. There was a significant positive correlation ($r=0.25$, $p=0.026$) between ox-LDL and homocysteine in samples obtained before HD. Logistic regression analysis revealed ox-LDL levels ($OR=3.02$, $p<0.001$) and arylesterase activity/HDL ratio ($OR=2.43$, $p=0.01$) to be associated with the increased risk of ESRD.

Conclusions: Ox-LDL levels and arylesterase activity/HDL ratio indicated the strongest association with ESRD risk. These factors, especially ox-LDL as an indicator of oxidative stress, may be biomarkers in evaluating the status of non-diabetic ESRD patients. Because of the pathogenic relationship between ox-LDL and homocysteine as nontraditional risk factors of atherosclerosis, therapeutic strategies adopted to reduce them may be useful in decrease of high prevalence of cardiovascular mortality in dialysis patients. In addition, measurement of PON1 activity to HDL ratio is possibly a more valuable biomarker than arylesterase activity alone in non-diabetic ESRD.

Correspondence to:

Abdolkarim Mahrooz, Ph.D.

Department of Clinical Biochemistry and Genetics,

Faculty of Medicine, Mazandaran University of Medical Sciences

Khazar-Abad Road, Sari, Iran, 4847191971

Email 1: kmahrooz2@gmail.com Email 2: kmahrooz@yahoo.com

Manuscript submitted 20th February, 2012

Manuscript accepted 2nd May, 2012

Clin Invest Med 2012; 35 (3): E144-E151.

Atherosclerotic events are the leading causes of mortality in patients with end-stage renal disease (ESRD) [1, 2] and these events are responsible for nearly 55% of mortalities among the patients [3]. Further understanding of the basis of these events could be useful in decreasing both the mortality and the frequent complications in ESRD patients and improve their survival [1]. Atherogenic lipid and lipoprotein abnormalities, such as decreased high-density lipoprotein (HDL) levels, are frequent in patients with chronic renal failure [2]. It has been established that low plasma HDL concentration is one of the strongest risk factors for cardiovascular disease [4, 5]. Decreased HDL levels have been identified as a marker for the presence of small and dense low-density lipoprotein (LDL) particles in the circulatory system. These particles increase the risk of atherosclerosis because of their susceptibility to oxidation and lower clearance [7]. Oxidized-LDL (ox-LDL), as an indicator of *in vivo* peroxidation of LDL, is believed to be a key molecule in the process of atherosclerosis [5, 8]. Ox-LDL plays a critical role in several proatherogenic activities such as immunogenic and cytotoxic events and accumulation of cholesterol in macrophages [9]. HDL-associated enzymes, especially serum paraoxonase (PON1), are essential for the protective effect of it on LDL and impeding LDL oxidation [6, 10]. PON1 is a calcium-dependent esterase/lactonase that resides on HDL and accounts, in part, for an important part of the antioxidative activity of HDL [11-14]. PON1 is a multifunctional enzyme that can not only decrease ox-LDL but can also hydrolyze and inactivate homocysteine thiolactone (HTL) [15, 16]. HTL as a reactive and toxic metabolite of homocysteine (Hcy) could bind to proteins and damage protein structure [16]. These homocysteinylated proteins could induce autoimmune responses and play a role in pathogenesis of atherosclerosis [16]. Several studies have shown that there are elevated Hcy levels in patients with ESRD [17]; however, there is no proven evidence for increased Hcy in renal disease. Perhaps, performing of the direct experiments on homocysteine-metabolizing enzymes could be helpful in revealing the cause of hyperhomocysteinemia in renal disease [17].

There is a 10- to 20-fold elevated risk of cardiovascular mortality in patients with ESRD [18]. Oxidative stress and atherothrombotic vascular disease are associated with high mortality in patients treated with hemodialysis (HD) [1, 19]. Alterations of ox-LDL, PON1 activity and homocysteine levels are important participating agents in the initiation and progression of oxidative and atherogenic events. Given the importance of the parameters in cardiovascular disease and the role of PON1 in the metabolism of ox-LDL and Hcy, these three factors were chosen for evaluation in non-diabetic ESRD patients

treated with HD. In most previous studies on ESRD patients, diabetes, as an important confounding factor, was not excluded. Therefore, one of the main objectives of this study was to evaluate the study parameters in non-diabetic ESRD patients. Studies in which all three parameters are considered together are very limited and some findings on PON1 levels in hemodialysis patients are controversial [2, 20]. The present study was therefore designed 1) to evaluate ox-LDL, Hcy and PON1 activity levels in patients with ESRD treated with hemodialysis, 2) to compare these levels with those assayed in control subjects and 3) to evaluate which parameter is the best biomarker for the assessment of atherosclerotic disease in the HD population. In addition, the correlations between the study factors were determined.

Materials and Methods

Subjects

A total of 49 age- and gender-matched subjects, including 28 non-diabetic hemodialysis patients (15 males and 13 females) and 21 control subjects (11 males and 10 females), were examined in the case-control study. Informed consent was obtained from each of the subjects. The study protocol was approved by the university's local ethics committee and was performed in accordance with the principles of the Helsinki Declaration. Because diabetes can affect the study factors, especially PON1 activity, patients with this disease were excluded. Subjects with a history of cardiovascular disease, active infection, neoplasms, hepatitis, AIDS and/or a smoking habit were also excluded. Patients had been on regular HD for at least 3 months and were dialyzed thrice weekly for 4 h per session. HD patients were hemodialyzed on polysulfone membrane dialysators with a bicarbonate dialysis solution. The *kt/v* (adequacy of dialysis) was 1.2 ± 0.1 . Patients were not receiving immunosuppressive medication, lipid-lowering drugs or ACE inhibitors. Vitamin E and C supplements were not administered to patients before the study. Some patients with hypertension were treated with the drug amlodipine. The antihypertensive drug was suspended for at least 24 h before the start of the study. Control subjects were selected from a healthy population with no history of renal disease or diabetes. Blood samples were collected in heparinized tubes and plasma was isolated by low-speed centrifugation. The samples were stored at -70 °C until evaluation.

Methods

Oxidized-LDL was measured in the plasma samples by a commercially available capture ELISA kit (Mercodia, Uppsala, Sweden). The antibody used in the kit was the murine mono-

clonal antibody, 4E6. The assay was based on the direct sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B moiety of LDL [21]. Absorbance values were read spectrophotometrically at 450 nm. In this study, the coefficient of variation (CV) and the detection limit were 7.8% (n=3) and <1 mU/L, respectively.

HDL-C, LDL-C and triglyceride levels were determined using commercially available kits (Pars Azmoon Inc., Tehran, Iran).

Arylesterase activity of PON1 was assayed using phenylacetate (Fluka, Heidelberg, Germany) as the substrate [22]. Serum was added to the reaction mixture containing 1 mM phenylacetate and 1 mM CaCl₂ in Tris/HCL buffer (0.1 M, pH 8.0). The enzyme activity was measured spectrophotometrically (UV 1800, Shimadzu, Japan) at 270 nm. Enzymatic activity was calculated from the molar absorptivity, 1310 M⁻¹ cm⁻¹. 1 U of arylesterase activity is defined as 1 μmol phenylacetate hydrolyzed/min/ml. In the present study, intraassay and interassay CV were 3.2% (n=7) and 4.1% (n=7), respectively.

The samples were assayed for homocysteine using the EIA method (Axis-Shield Diagnostics Ltd, Dundee, UK). This assay is based on competition between the S-adenosyl-L-homocysteine (SAH) in the sample and the SAH bound to the plate for binding sites on a monoclonal anti-SA-H antibody. Secondary antibody was rabbit anti-mouse antibody conjugated with the enzyme horse radish peroxidase (HRP). The peroxidase activity was assayed spectrophotometrically at 450 nm. The quantification limit (CV<20%) was 1.0 μmol/L.

Statistical analysis

Normal distribution of the data was evaluated using Kolmogorov-Smirnov test. Comparison between the patients and the control group was performed by t-test (quantitative variables) and Chi-squared test (qualitative variables). Pearson's correlation coefficient was used to test the association between the study parameters. Odds ratios (ORs) were determined by logistic regression analysis. P values less than 0.05 were considered to be significant. SPSS 16.0 software (SPSS Inc., Chicago, USA) was used for all calculations.

Results

Twenty-eight hemodialysis patients (15 men and 13 women; mean age: 56.1 ± 8.4 years) and 21 control subjects (11 men and 10 women; mean age: 53.4 ± 7.6 years) were studied. As shown in Table 1, there were no significant differences between control subjects and hemodialysis patients with respect to age,

Table 1. Age, sex, TG, HDL-C and LDL-C levels in hemodialysis patients and control subjects.

	HD patients	Control subjects
Number of patients	28	21
Age, years	56.1 ± 8.4	53.4 ± 7.6
Sex (male/female)	15/13	11/10
TG, mg/dl	118 ± 54.2	103.6 ± 59.1
HDL-C, mg/dl	28.3 ± 6.8*	40.2 ± 11.4
LDL-C, mg/dl	105.9 ± 23.9	97.6 ± 48.4

Hemodialysis (HD), triglyceride (TG), high-density lipoprotein-C (HDL-C) and low-density lipoprotein-C (LDL-C) were determined in the samples obtained before hemodialysis.

* Significantly different from control subjects (p= 0.02).

FIGURE 1. Ox-LDL levels in HD patients (before HD and after HD) and control group. Data are presented as mean ± SD. * Significant difference between Before HD and Control groups (p= 0.001). ** Significant difference between After HD and Control groups (p= 0.036). No significant difference between Before HD and after HD groups (p=0.19).

HD: Hemodialysis.

sex, triglyceride (TG) and LDL-C (p values > 0.05). As expected, HDL-C levels were significantly higher in the control group compared with the HD patients (p=0.02).

Compared with the control group, ox-LDL levels were significantly increased both before hemodialysis (p=0.001) and after hemodialysis (p=0.036) (Figure 1). There was no significant difference between the pre-dialysis and post-dialysis groups regarding ox-LDL concentrations. ox-LDL levels did not change significantly during HD but decreased after dialysis.

FIGURE 2. Arylesterase activity levels in HD patients (before HD and after HD) and control group. Data are presented as mean $\pm$ SE. No significant difference between Before HD and Control ($p=0.56$), After HD and control ($p=0.21$) and Before HD and After HD ($p=0.29$). HD: Hemodialysis.

Our findings showed that arylesterase activity to HDL ratio in HD patients was significantly higher than control subjects (1.86 ± 0.41 vs. 1.24 ± 0.34 ; $p=0.003$); however, a significant difference in arylesterase activity was not seen between pre-dialysis and post-dialysis groups. HD did result in a small increase of arylesterase activity (Figure 2).

As shown in Figure 3, homocysteine levels in the ESRD patients were higher than control subjects, both pre- and post-dialysis, but these differences were not statistically significant. HD was associated with a small, but non-significant reduction in Hcy concentrations.

There was a significant positive correlation ($r=0.25$, $p=0.026$) between ox-LDL levels and homocysteine concentrations in samples obtained before HD. Although there appeared to be weak negative correlations between arylesterase activity with ox-LDL and homocysteine levels, these correlations were not statistically significant.

To assess if the association among ox-LDL, Hcy and arylesterase activity/HDL ratio and ESRD was independent of other parameters, multivariate logistic regression was performed (Table 2). After controlling for other factors, ox-LDL (OR= 3.02, $p<0.001$) and arylesterase activity/HDL ratio (OR=2.43, $p=0.01$) indicated the strongest association with ESRD. There was a significant association between homocysteine, HDL-C and triglyceride levels with ESRD risk (at a lower degree of significance). Age, sex, LDL-C and arylesterase activity were not significantly associated with ESRD.

FIGURE 3. Homocysteine levels in HD patients (before HD and after HD) and control group. Data are presented as mean $\pm$ SE. No significant difference between Before HD and Control ($p=0.15$), After HD and Control ($p=0.51$) and Before HD and After HD ($p=0.23$). HD: Hemodialysis.

Table 2. Multivariate analysis of the study parameters.

Variable	Odds ratio (95% CI)	p value
Sex, male/female	0.98 (.091-1.02)	NS
Age, years	1.05 (0.97-1.15)	NS
Triglyceride	1.7 (1.09-2.41)	0.041
HDL-C	0.78 (0.39-1.29)	0.033
LDL-C	1.13 (0.96-1.6)	NS
Ox-LDL	3.02 (1.85-4.71)	<0.001
Homocysteine	1.8 (1.02-2.56)	0.038
Arylesterase activity	1.01 (0.99-1.03)	NS
Arylesterase activity/HDL-C	2.43 (1.06-4.01)	0.01

CI: confidence interval

NS: not significant

Discussion

Decreased HDL concentrations in HD patients represent the cause of reduction of antioxidant properties of this important lipoprotein [23, 24]. One result of the reduction is that ox-LDL production can be accelerated. Elevated oxidation of LDL is introduced as an important early stage in the develop-

ment of atherosclerotic disease [25]. Accumulation of ox-LDL in macrophages and promotion of inflammatory response, proliferation of smooth muscle cells, and differentiation of monocytes in the arterial wall by ox-LDL suggests a critical role for ox-LDL in atherosclerosis [26]. Meier et al. [27] indicated that elevated levels of ox-LDL increased the risk of atherosclerosis, even after adjusting for traditional risk factors. It has also been reported that the immunological response arising from LDL oxidation leads to the production of anti-ox-LDL autoantibodies and contributes to the development of atherosclerosis [28]. Concentrations of ox-LDL antibodies are significantly higher in patients who died from cardiovascular disease [28]. A study by Morena et al. reports that LDL isolated from HD patients show an elevated susceptibility to oxidation in vitro [29]. These data show the importance of ox-LDL levels in HD patients.

Since plasma ox-LDL concentrations may be an appropriate indicator of the actual balance between production and removal of ox-LDL in vivo [30], in this study, plasma ox-LDL levels were determined in the study subjects. Our findings showed that ox-LDL concentrations in pre- and post-dialysis were significantly higher than that found in control subjects. Meier et al. [27] and Castilla et al. [31] reported that ox-LDL levels in HD patients are significantly higher than in control subjects, in accordance with our study. According to Meier et al. [27], there is a significant increased ox-LDL concentration in HD patients, even though these patients were receiving a hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor or vitamin B and C supplements. The studies did not evaluate ox-LDL levels after HD. In our study, HD resulted in small decrease of ox-LDL levels. Bolton et al. [32] also showed that ox-LDL autoantibodies were increased in pre-dialysis group, but were decreased in dialyzed patients. In contrast, Kosch et al. [9] reported that ox-LDL concentrations are stable during dialysis therapy and attributed this stability to the rapid clearance of newly produced ox-LDL during HD [9].

As expected, in this study, HD patients had increased homocysteine levels. Although this increase was relatively mild, our study confirmed hyperhomocysteinemia in HD patients, in agreement with other authors [1, 33, 34]. Clinical and experimental evidence confirms that hyperhomocysteinemia is an independent risk factor for atherosclerosis [1]. It has been reported that Hcy levels higher than 12.1 $\mu\text{mol/L}$ can double the risk of atherosclerosis and cerebral or peripheral vascular diseases [35]. Bednarek-Skublewska and colleagues [36] indicated that elevated Hcy levels can predict vascular access consequents in patients on HD. Induction of oxidative stress and the promotion of inflammation and thrombosis are mechanisms by which the increased Hcy may be involved in advanced athero-

sclerosis [35]. Our results indicated that dialysis can remove Hcy, similar to the results reported in a review article by Urquhart et al. [17].

Various studies in the literature have showed that decreased PON1 activity is associated with higher risk of atherosclerosis; i.e., PON1 is an antiatherogenic enzyme [38-40]. In the current study, our results did not confirm alterations of the arylesterase activity of PON1 in pre- and post-dialysis patients as compared to control subjects; however, arylesterase activity/HDL ratios in control subjects were found to be significantly lower than patients following HD. Since PON1 is located on HDL, the assay of PON1 activity to HDL ratio may be more important than measuring the enzyme alone in the evaluation of the relationship between PON1 activity and ESRD.

In general, there are the conflicting data in the ESRD concerning PON1 activity. Although, most of studies show the decreased PON1 activity in the ESRD population [2, 23, 24], some studies suggest no difference in enzyme activity between patients and control subjects [20]. In a study by Gugliucci et al. [41], the free fraction of PON1 was the same for HD patients and control subjects. These conflicting results may be explained by the effects of several factors, such as diabetes, sample size, sex, age and PON1 polymorphisms, on PON1 activity. Studies have indicated that diabetes is associated with reduced PON1 activity [12]; therefore, to better assess changes in PON1 activity in the ESRD patients, diabetes as a confounding factor should be excluded. In the present study, patients with diabetes were excluded. In accordance with our study, Kalogerakis et al. [20] separately compared PON1 activity in the patients with ESRD and patients with type I diabetes with control subjects and showed that the paraoxonase activity of PON1 is significantly lower in the diabetic patients compared with the control subjects but does not change in the ESRD patients.

Gender and age can affect the activity of PON1. In humans, although gender is influenced by the genetic heterogeneity, few studies have shown that PON1 activity is higher in women than men [42]. Some authors have reported that there is a progressive reduction in PON1 activity in elderly persons [42]. Moreover, some investigations have linked this decline to the elevated prevalence of atherosclerosis with age [42]. Therefore, matching the study groups regarding age and gender may be important. Q/R 192 polymorphism is the most common coding polymorphism at position 192 [43]. The polymorphism is substrate-dependent and results in two different allozymes. While paraoxon is hydrolyzed faster by the R allozyme, phenylacetate hydrolysis is catalyzed at the same rate by both allozymes [13]. Also, the Q allozyme has lower paraoxonase activity towards paraoxon [43]. A study by Gugliucci et al. [2] indi-

cated that the significant reduction in the amount of enzyme activity seen in their study could be related to a different phenotype distribution among the ESRD patients and control subjects. Therefore, in studies that measure the enzyme activity, groups should ideally be matched for the polymorphism or at least for assays performed with phenylacetate as a substrate.

In addition, in HD patients, the duration of dialysis therapy can affect PON1 activity [44]. Feretti et al. reported that patients with a longer dialysis therapy had lower PON1 activity compared with patients with briefer dialysis therapy [44]. In general, given that many factors affect the enzyme activity, evaluation of PON1 activity to HDL ratio is possibly a better biomarker than measuring the enzyme activity alone in non-diabetic patients with ESRD.

Correlation studies showed that ox-LDL levels in HD patients correlated positively and significantly with Hcy concentrations. The positive correlation between these important risk factors for cardiovascular disease may be related to increased mortality in HD patients. Considering both ox-LDL and Hcy are involved in oxidative stress and inflammation [26, 45], the positive and significant relationship can be confirmed by the fact that increased oxidative stress and inflammation are common properties of ESRD [46]. Results of a study by Seo et al. indicated that there is a Hcy-induced LDL oxidation in atherosclerotic patients [35]. Therefore, it may be concluded that the pathogenic association between ox-LDL and Hcy plays an important role in atherosclerosis in the HD population.

In this study, logistic regression analysis showed ox-LDL levels and arylesterase activity/HDL ratio to be associated with the increased risk of ESRD. The analysis indicates that the observed difference in ox-LDL levels between ESRD patients and control subjects may not contribute to the higher LDL-C levels in the patients.

Our data suggest that the dialysis procedure has an effect on ox-LDL, Hcy and PON1 activity levels. HD decreased plasma levels of ox-LDL and Hcy, although they did not reach the normal level. PON1 activity in the ESRD patients was increased after dialysis, in agreement with other authors [19]. These changes did not appear to be due to differences in the dialysis procedure because the conditions such as kt/v were almost identical for all patients. Thus, an increase in the quality of dialysis could be useful in reducing the risk factors levels and increased activity of PON1.

In conclusion, ox-LDL levels and arylesterase activity/HDL ratio showed the strongest association with ESRD risk. These factors, especially ox-LDL as an indicator of oxidative stress, may be appropriate biomarkers in assessing the status of non-diabetic patients with ESRD. Elevated ox-LDL levels may

confirm that there is increased oxidative stress in HD patients. The pathogenic association between ox-LDL and homocysteine, as nontraditional risk factors, should be considered in the assessment of cardiovascular complications in ESRD patients treated with HD. Therefore, therapeutic strategies to reduce these risk factors could minimize the harmful complications arising from them. Studies such as that by Ando et al. [30], conducted with the aim of reduction of ox-LDL and Hcy levels in HD patients, may decrease the high prevalence of cardiovascular mortality in dialysis patients. In addition, the PON1 activity/HDL ratio is probably a better indicator than determining the enzyme activity alone for assessing the relationship between PON1 activity levels and ESRD. The present study highlights the importance of ox-LDL and arylesterase activity/HDL ratio in non-diabetic ESRD patients; however, interventional investigations and studies involving larger samples need to be done in order to design therapeutic strategies to decrease ox-LDL and Hcy levels and to help reduce the high prevalence of atherothrombotic cardiovascular mortality in HD population.

Acknowledgments

This study was funded by Mazandaran University of Medical Sciences, Sari, Iran.

References

1. Yean JY, Kaysen GA. C-reactive protein, oxidative stress, homocysteine, and troponin as inflammatory and metabolic predictors of atherosclerosis in ESRD. *Curr Opin Nephrol Hy* 2000; 9: 621-630.
2. Gugliucci A, Mehlhaff K, Kinugasa E, Ogata H, Hermo R, Schulze J, et al. Kimura S. Paraoxonase-1 concentrations in end-stage renal disease patients increase after hemodialysis: correlation with low molecular AGE adduct clearance. *Clin Chim Acta* 2007; 377: 213-220.
3. Prichard SS. Impact of dyslipidemia in end-stage renal disease. *J Am Soc Nephrol* 2003; 14: S315-S320.
4. Superko HR. Cardiovascular event risk: high-density lipoprotein and paraoxonase. *J Am Coll Cardiol* 2009; 54: 1246-1248.
5. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001; 21: 473-480.
6. Mackness MI, Mackness B, Durrington PN. Paraoxonase and coronary heart disease. *Atherosclerosis* 2002 Suppl 3: 49-55.
7. Nesto RW. Beyond low-density lipoprotein: addressing the atherogenic lipid triad in type 2 diabetes mellitus and the metabolic syndrome. *Am J Cardiovasc Drug* 2005; 5: 379-387.
8. Meier P, Spertini F, Blanc E, Burnier M. Oxidized low-density lipoprotein activate CD4+ T cell apoptosis in patients with end-

- stage renal disease through fas engagement. *J Am Soc Nephrol* 2007; 18: 331-342.
9. Kosch M, Levers A, Fobker M, Barenbrock M, Schaefer RM, Rahn K-H, et al. Dialysis filter type determines the acute effect of haemodialysis on endothelial function and oxidative stress. *Nephrol Dial Transplant* 2003; 18: 1370-1375.
 10. Mirdamadi HZ, Sztanek F, Derdak Z, Seres I, Harangi M, Paragh G. The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. *Br J Clin Pharmacol* 2008; 66: 366-374.
 11. Mahrooz A, Rashidi M-R, Nouri M. Naringenin is an inhibitor of human serum paraoxonase (PON1): an in vitro study. *J Clin Lab Anal* 2011; 25: 395-401.
 12. Aviram M, Rosenblat M. Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. *Curr Opin Lipidol* 2005; 16: 393-399.
 13. Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, et al. Paraoxonase status in coronary heart disease: Are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol* 2001; 21: 1451-1457.
 14. Cece H, Yazgan P, Karakas E, Karakas O, Demirkol A, Toru I, et al. Carotid intima-media thickness and paraoxonase activity in patients with ankylosing spondylitis. *Clin Invest Med* 2011; 34: E225-E231.
 15. Jakubowski H. Calcium-dependent human serum homocysteine thiolactone hydrolase. *J Biol Chem* 2000; 275: 3957-3962.
 16. Zimny J, Sikora M, Guranowski A, Jakubowski H. Protective mechanisms against homocysteine toxicity: the role of bleomycin hydrolase. *J Biol Chem* 2006; 281: 22485-22492.
 17. Urquhart BL, House A. Assessing plasma total homocysteine in patients with end-stage renal disease. *Perit Dial Int* 2007; 27: 476-488.
 18. Gritters M, Grooteman MPC, Schoorl M, Schoorl M, Bartels PCM, Scheffer PG, et al. Citrate anticoagulation abolishes degranulation of polymorphonuclear cells and platelets and reduces oxidative stress during haemodialysis. *Nephrol Dial Transplant* 2006; 21: 153-159.
 19. Kirschbaum B. Correlation studies of plasma paraoxonase activity and uric acid concentration with AAPH-induced erythrocyte hemolysis in hemodialysis patients. *Artif Organs* 2004; 28: 259-264.
 20. Kalogerakis G, Baker AM, Christov S, Rowley KG, Dwyer K, Winterbourn C, et al. Oxidative stress and high-density lipoprotein function in type I diabetes and end-stage renal disease. *Clin Sci* 2005; 108: 497-506.
 21. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998; 98: 1487-1494.
 22. Richter RJ, Jarvik GP, Furlong CE. Paraoxonase 1 (PON1) status and substrate hydrolysis. *Toxicol Appl Pharmacol* 2009; 235: 1-9.
 23. Vaziri ND, Navab K, Gollapudi P, Moradi H, Pahl MV, Barton CH, et al. Salutary effects of hemodialysis on low-density lipoprotein proinflammatory and high-density lipoprotein anti-inflammatory properties in patient with end-stage renal disease. *J Natio Med Associ* 2011; 103: 524-533.
 24. Horoz M, Kiykim AA, Cimen B, Erden A. The influence of hemodialysis membrane permeability on serum paraoxonase-1 activity and oxidative status parameters. *Artif Organs* 2011; 35: 923-929.
 25. Hasselwander O, Young IS. Oxidation of low-density lipoprotein and atherosclerosis in chronic renal failure. *Med Hypotheses* 1997; 49: 389-395.
 26. Ziouzenkova O, Asatryan L, Akmal M, Tetta C, Wratten ML, Loseto-Wich G, et al. Oxidative cross-linking of apoB100 and hemoglobin results in low density lipoprotein modification in blood. *J Biol Chem* 1999; 274: 18916-18924.
 27. Meier P, Golshayan D, Blanc E, Pascual M, Burnier M. Oxidized LDL modulates apoptosis of regulatory T cells in patients with ESRD. *J Am Soc Nephrol* 2009; 20: 1368-1384.
 28. Bayes B, Pastor MC, Bonal J, Junca J, Hernandez JM, Riutort N, et al. Homocysteine, C-reactive protein, lipid peroxidation and mortality in haemodialysis patients. *Nephrol Dial Transplant* 2003; 18: 106-112.
 29. Morena M, Cristol J-P, Dantoine T, Carbonneau M-A, Descomps B, Canaud B. Protective effects of high-density lipoprotein against oxidative stress are impaired in haemodialysis patients. *Nephrol Dial Transplant* 2000; 15: 389-395.
 30. Ando M, Sanaka T, Nihel H. Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. *J Am Soc Nephrol* 1999; 10: 2177-2184.
 31. Castilla P, Echarrri R, Davalos A, Cerrato F, Ortega H, Teruel JL, et al. *Am J Clin Nutr* 2006; 84: 252-262.
 32. Bolton CH, Downs LG, Victory JGG, Dwight JF, Tomson CRV, Mackness MI, et al. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. *Nephrol Dial Transplant* 2001; 16: 1189-1197.
 33. Borazan A, Aydemir S, Sert M, Yimaz A. The effects of hemodialysis and peritoneal dialysis on serum homocysteine and C-reactive protein levels. *Mediat Inflamm* 2004; 13: 361-364.
 34. Katko M, Kiss I, Karpati I, Kadar A, Matyus J, Csongradi E, et al. Relationship between serum nickel and homocysteine concentration in hemodialysis patients. *Biol Trace Elem Res* 2008; 124: 195-205.
 35. Seo H, Oh H, Park H, Park M, Jang Y, Lee M. Contribution of dietary intakes of antioxidants to homocysteine-induced low density lipoprotein (LDL) oxidation in atherosclerotic patients. *Yonsei Med J* 2010; 51: 526-533.
 36. Bednarek-Skubilewska A, Przywara S, Wronski J, Ksiazek A. Influence of homocysteine on the function of native arteriove-

- nous fistula in hemodialysis patients. *Dialysis Transplant* 2006; 35: 604-609.
37. Ducloux D, Klein A, Kazory A, Devillard N, Chalopin J-M. Impact of malnutrition-inflammation on the association between homocysteine and mortality. *Kidney Int* 2006; 69: 331-335.
 38. Davis KA, Crow JA, Chambers HW, Meek EC, Chambers JE. Racial differences in paraoxonase-1 (PON1): a factor in the health of southerners? *Environ Health Perspect* 2009; 117: 1226-1231.
 39. Soran H, Younis NN, Charlton-Menys V, Durrington P. Variation in paraoxonase-1 activity and atherosclerosis. *Curr Opin Lipidol* 2009; 20: 265-274.
 40. Mahrooz A, Rashidi M-R, Aslanabadi N, Qujeq D, Nouri M. Association between paraoxonase-1 activity and the extent of coronary stenosis. *J Cardiovasc Thorac Res* 2009; 1: 1-8.
 41. Gugliucci A, Schulze J, Kinugasa E, Ogata H, Kimura S. The free fraction of paraoxonase 1 is not increased in patients with end stage renal disease undergoing hemodialysis. *Clin Chim Acta* 2009; 402: 209-210.
 42. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol* 2005; 69: 541-550.
 43. Ng CJ, Shih DM, Hama SY, Villa N, Navab M, Reddy ST. The paraoxonase gene family and atherosclerosis. *Free Radic Biol Med* 2005; 38: 153-163.
 44. Ferretti G, Bacchetti T, Masciangelo S, Pallotta G. Lipid peroxidation in hemodialysis patients: effect of vitamin C supplementation. *Clin Biochem* 2008; 41: 381-386.
 45. Joseph R, Nath SG, Joseraj MG. Elevated plasma homocysteine levels in chronic periodontitis: a hospital-based case-control study. *J Periodontol* 2011; 82: 439-444.
 46. Locatelli F, Canaud B, Eckardt K-U, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 2003; 18: 1272-1280.