PARAOXONASE ACTIVITY AND ANTIBODIES TO OXIDIZED-LDL IN CHRONIC RENAL FAILURE PATIENTS ON RENAL REPLACEMENT THERAPY

P. R. Krishnaswamy, Anjali Rao*, Murali.W. and H. Sudarshan Ballal

* Department of Biochemistry, Kasturba Medical College, Manipal, Karnataka
Manipal Hospital, Bangalore, Karnataka

ABSTRACT

Serum paraoxonase (PON1) and antibodies to oxidized-LDL (anti ox-LDL) were measured in chronic renal failure subjects on renal replacement therapy such as hemodialysis (HD) peritoneal dialysis (PD) and transplantation (Txp). Paraoxonase activity was significantly lower in HD and PD group ($P < 0.001$) than in control subjects. In transplant patients, paraoxonase activity was not significantly different from that of controls. Antibodies to ox-LDL was significantly higher in HD, PD and Transplant patients ($P < 0.0001$) compared to control subjects. High titers of antibodies were observed in the HD group compared to the PD and Transplant subjects. A decrease in paraoxonase activity and high titers of Antibodies to ox-LDL in the dialysis group suggest a decreased cardio protective effect of HDL and enhanced risk of premature cardiovascular complications. Whereas in case of transplant subjects, there seems to be restoration of PON1 activity, but elevated levels of anti-oxLDL could still be a potential atherogenic factor. Hence, we propose that estimation of these two parameters can be used as a useful index to measure the cardiac risk in the above patient category

KEY WORDS

Atherosclerosis, HDL-cholesterol, dialysis, oxidative stress, transplantation.

INTRODUCTION

Cardiovascular diseases represent the major cause of mortality in dialysis and transplant patients. Oxidized LDL (ox-LDL) plays a major role in the development and progression of atherosclerotic lesions. It has been suggested that high-density lipoprotein has the capacity to reduce the oxidative modifications of LDL due to the presence of enzyme Paraoxonase (PON1.).

Serum Paraoxonase 1 (PON1) is an esterase (aryldialkylphosphatase, EC 3.1.8.1) –synthesized and secreted by the liver, associated with a specific HDL particle also containing clusterin and apolipoprotein (apo) A1 that hydrolyzes organophosphate compounds, aryl esters, oxidized phospholipids and lipid peroxides (1, 2). The enzyme is found

to protect both HDL and low-density lipoproteins (LDL) against peroxidation, which suggests a possible involvement of PON1 in the antiatherogenic properties of HDL. It has been shown that in vitro purified paraoxonase decreased LDL lipid peroxidation (1) and that purified paraoxonase significantly reduced the ability of mildly oxidized LDL to induce monocyte-endothelial interactions (3). Thus, a protective role of paraoxonase against atherosclerosis has been suggested, as well as an explanation of the protective role of HDL against LDL oxidation. Low serum PON1 activity is associated with several risk factors for coronary heart diseases, including diabetes, hypercholesterolemia and smoking (1). Previous studies have indicated a low paraoxonase activity in CRF patients (4, 5). Oxidation of LDL is recognized as a key stage in the early development of atherosclerosis (6). Increased LDL susceptibility to oxidation has been reported in CRF, a condition characterized by accelerated atherogenesis and derangement of lipoprotein metabolism (7).

The aim of the present study was to evaluate the paraoxonase activity and antibodies to oxidized LDL across the above category of patients and possible utility of these parameters as cardiovascular risk index among the three groups.

Address for Correspondence:

Dr. Murali.W
Senior clinical Biochemist, Manipal Hospital,
No.98, Rustom bagh, Airport Road, Bangalore-560 003.
E-mail : muraliwoodi@yahoo.com

MATERIALS AND METHODS

The study consisted of three groups of CRF patients on renal replacement viz, stable Hemodialysis (HD) (n=25; males=14, females=11); duration: 2-5yrs, peritoneal dialysis (PD) (n=25; males=13, females=12); duration: 1-5 years and stable Transplant (Txp) (n=25; males=15, females=10); 2-9 years post transplant without any chronic rejection. The age group of patients was 35 – 65 yrs. These patients were being treated at Manipal Institute of Nephrology and Urology either as inpatients or as outpatients. Prior consent was obtained from the patients and the hospital ethics committee. The control group consisted of master health check up individuals (n=25; males=13, females=12) in the age group of 35 – 65 yrs. Fasting venous blood was collected from the patients and serum separated and frozen at -20° C till analysis.

Serum paraoxonase was determined by the method of Hagen et al (8) using p-nitro phenyl acetate as the substrate. Briefly 0.250 ml of serum was added to 2.0 ml of buffered p-nitro phenyl acetate and the rate of generation of p-nitro phenol (25°C) as yellow compound was measured at 402 nm in Genisys 10 UV spectrometer over a period of 1 minute with a delay time of 30 seconds. The yellow colour formed is proportional to the enzyme esterase activity expressed in international units (U) per milliliter of serum.

Antibodies (IgG) to oxidized LDL was measured in the semi quantitative mode which was solid phase enzyme labeled immunosorbent assay (ELISA) from Immco Diagnostics, USA. Micro wells were coated with purified native LDL and/or ox LDL antigen. Each sample was run on LDL and ox-LDL wells. Absorbance value on native-LDL is subtracted from the absorbance obtained on ox-LDL for calibrators, controls, and patient samples. The concentration of anti-oxLDL is determined from the calibration curve. Results are expressed in EU/ml.

HDL cholesterol was measured by immuno-inhibition spectrophotometric assay on Dade dimension-RxL (USA)-automated chemistry analyzer.

Statistical analysis: Data are presented by descriptive analysis (case number, mean/median, standard deviation). The comparison between groups was performed by unpaired t-test and analysis of variance using online statistical programme. $p < 0.05$ was accepted as significance level.

RESULTS

Table. 1 gives comparison of serum paraoxonase activity and HDL- cholesterol levels and anti ox-LDL levels between controls and patients. The mean PON1 activity of dialysis – HD and PD patients is significantly reduced compared to the controls ($p < 0.001$). Paraoxonase activity in transplant patients was comparable with the control group and the difference was not significant. A comparative analysis (ANOVA) between the transplant and the dialysis group was significant ($p < 0.001$). With respect to HDL-cholesterol levels, HD and PD groups showed significant reduction compared to controls. In Txp group the values are comparable to controls and the difference was not significant. The anti ox-LDL levels were significantly higher in all the three patient groups ($p < 0.001$) than the controls. The mean values in case of HD and Txp is higher than the PD group. Highest in the HD group compared to controls.

Table 1

Parameter	Controls (n = 25) mean $\pm$ SD	Patients (n = 25) mean $\pm$ SD
Paraoxonase (U/ml)	1.37 $\pm$ 0.187	HD = 0.880 $\pm$ 0.144 ^c PD = 0.994 $\pm$ 0.143 ^c TXP = 1.300 $\pm$ 0.165 ^{ns}
HDL cholesterol (mg/dl)	44.9 $\pm$ 7.21	HD = 34.6 $\pm$ 5.83 ^b PD = 38.3 $\pm$ 7.5 ^a TXP = 42.3 $\pm$ 6.8 ^{ns}
Anti-ox LDL (EU/ml)	12 $\pm$ 8.5	HD = 33.5 $\pm$ 24.7 ^c PD = 25.1 $\pm$ 17.2 ^c TXP = 29.7 $\pm$ 15.7 ^c

n = sample size; ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ - compared to control group, ns-not significant

DISCUSSION

From the study it is evident that decrease in paraoxonase activity is marked and significant in HD followed by peritoneal dialysis. Also decrease in HDL levels was more pronounced in HD group compared to the PD group which could be responsible for decreased paraoxonase activity. Evidence is strong that low HDL cholesterol is a marker for the presence of small, dense, cholesterol-depleted LDL in the circulation, which itself increases the risk of atherosclerosis, probably because of its susceptibility to oxidation (9). It has been shown that PON1 is sensitive to oxidants and is inactivated by oxidized lipids (10). Thus a decreased paraoxonase activity may cause decreased HDL antioxidant capacity in uremic

patients and would therefore be expected to contribute to the increased risk of premature atherosclerosis found in these patients (11). In transplant patients, the HDL associated paraoxonase activity and HDL cholesterol is less altered compared to HD/PD patients. This could be due to the fact that kidney function in transplantation is normalized abolishing most of the metabolic factors which sustain the lipid abnormalities associated with uremia.

It is evident that the titers of antibodies to ox-LDL are high in all the three patient groups. And as expected the HD group shows highest titer with PD group faring better. The antibody titer in transplant patients is close on heels with HD patients. The presence of oxidized LDL, deriving from lipid abnormalities and oxidative stress is strongly supported by an increase in anti-oxidized LDL antibodies in plasma of uremic patients (7). HD-induced LDL oxidative modification is a multifactorial process including (i) qualitative abnormalities of LDL, (ii) oxidative stress and (iii) decrease in HDL protection against LDL oxidation. Enhanced oxidative stress due to overproduction of reactive oxygen species (ROS) by activated monocytes and impairment in antioxidant defence mechanisms (12) has been observed in HD patients (13,14) and may contribute to the accelerated hemodialysis-induced atherogenesis by oxidatively modified proteins and lipids. The same may be true with peritoneal dialysis. In case of renal transplant subjects it could be anticipated that successful transplantation would restore the balance between reactive oxygen species production and defense mechanisms but on the contrary persistence of oxidative stress after successful transplantation was reported by several authors (15, 16). Long term immunological stimulation by the allograft and maintenance treatment with immunosuppressive drugs like cyclosporine (CsA) may enhance oxidative stress. In vitro, CsA has been shown to increase lipid peroxidation in microsome systems (17, 18). The results of the elegant studies by van den Dorpel et al (19) suggest that CsA may cause lipoprotein oxidation in transplant recipients. It has been shown that increased oxidative stress leads to lipid peroxidation and oxidative modification of LDL resulting in the formation of oxidized LDL, which plays a major role in the development and progression of atherosclerosis lesions (20, 21).

Our study clearly demonstrates a decrease in HDL associated paraoxonase activity in dialysis subjects which could offset its protective role against lipid peroxidation of LDL and as a consequence we find elevated titers of antibodies to ox-LDL in HD followed by PD group. This could be in part due to the blood components coming in contact with bioincompatible membranes of dialysis machine (hemodialysis) and dialysate

solutions, though PD group gets some respite as the exchange takes place in vivo (intraperitoneal). This could be the reason for slightly better HDL values and low anti-ox LDL titers in PD group. With respect to transplant though near normal values for PON1 are observed, high antibody titers still could be a potential risk factor for accelerated development of atherosclerosis.

In conclusion, decreased paraoxonase and increased levels of ox-LDL as indicated by increased titers of anti ox-LDL could be risk indicators in HD and PD groups. In case of transplant patients, though HDL-paraoxonase association is normal, an increased titer of anti ox-LDL could still be a potential risk factor.

REFERENCES

1. Durrington, P.N., Arrol, S. and Mackness, M.I. (1991) Paraoxonase prevents accumulation of lipoperoxidase in low-density lipoprotein. *FEBS Lett.* 286, 152-154
2. Mackness, M.I., Abbott, C.A., Arrol, S. and Durrington, P.N. (1993) The role of High density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. *Biochem. J.* 294,829-835
3. Watson, A.D., Berliner, J.A., Hama, S.Y., La Du, B.N., Faull, K.F., Fogelman, A.M. and Navab, M. (1995) Protective effect of high density lipoprotein associated paraoxonase. *J. Clin. Invest.* 96, 2882-2891
4. Schiavon, R., De Fanti, E., Giavarina, D., Biasioli, S., Cavalcanti, G. and Guidi, G. (1996) Serum paraoxonase activity decreased in uremic patients. *Clin. Chim. Acta.* 247, 71-80
5. Gyorgy, P., Laszlo, A., Lidiko, S., Zoltan, B., Lajos, L. and Gyorgy, K. (1991) Serum paraoxonase activity changes in uremic and kidney transplanted patients. *Nephron* 83, 126-131
6. Witztum, J.L. and Steinberg, D. (1991) Role of oxidized low-density lipoprotein in atherogenesis. *J. Clin. Invest.* 88, 1785-92
7. Maggi, E., Bellazzi, R., Falaschi, F., Frattoni, A. and Perani, G., et al. (1994) Enhanced LDL oxidation in uremic patients: an additional mechanism for accelerated atherosclerosis? *Kidney Int.* 45, 876-83
8. Haagen, L. and Brock, A. (1992) A new automated method for phenotyping arylesterase based upon inhibition of enzymatic hydrolysis of 4-nitrophenylacetate by phenyl acetate. *Eur. J. Clin. Chem. Clin. Biochem.* 30, 391-395

9. Chait, A., Brazg, R., Tribble, D. and Krauss, R. (1993) Susceptibility of small, dense, low- Density Lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.* 94, 350-356
10. Aviram, M., Rosenblat, M. and Billecke, S. et al (1999) Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radic. Biol. Med.* 26, 892-904
11. Dantoine, T.F., Debord, J., Charmes, J.P. et al (1998) Decrease of serum paraoxonase activity in chronic renal failure. *J. Am. Soc. Nephrol.* 9, 2082-2088.
12. Paul, J.L., Sall, N.D., Soni, et al (1993) Lipid peroxidation abnormalities in hemodialysed patients. *Nephron* 64, 106-109
13. Cristol, J.P., Canaud, B., Rabesandratana, H., Gaillard, I., Serre, A. and Mion, C (1994) Enhancement of reactive oxygen species production and cell surface markers expression during hemodialysis. *Nephrol. Dial. Transplant* 9, 389-394.
14. Himmelfarl, J., Lazarus, M. and Hakim, R. (1991) Reactive oxygen species production by monocytes and polymorphonuclear leukocytes during dialysis. *Am. J. Kidney Dis.* 17, 271-276
15. Odetti, P., Garibaldi, S., Gurreri, G., et al (1996) Protein oxidation in hemodialysis and kidney transplantation. *Metabolism* 45, 1319
16. Cristol, J.P., Maggi, M.F., Vela, C., Descomps, B. and Mourad, G. (1996) Lipid metabolism and oxidative stress in renal transplantation: implications for chronic rejection. *Transplant Proc.* 28 (5), 2820.
17. Inselmann, G., Blank, M. and Baumann, K. (1988) Cyclosporine A induces lipid peroxidation in microsomes and effects on active and passive glucose transport by brush border membrane vesicles of rat kidney. *Res. Commun. Chem. Pathol. Pharmacol.* 62, 207.
18. Serino, F., Grevel, J., Napoli, K.L., Kahan, B.D. and Strobel, H.W. (1994) Oxygen radical formation by the cytochrome p 450 system as a cellular mechanism for cyclosporine toxicity. *Transplant Proc.* 26, 2916.
19. Wang, C. and Salahudeen, A.K. (1995) Lipid peroxidation accompanies cyclosporine nephrotoxicity: Effects of vitamin E. *Kidney Int.* 47, 917-934.
20. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., Witztum, J.L. and Beyong, C. (1989) Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320, 915-924.
21. Esterbauer, H., Gebicki, J., Puhl, H. and Jurgens, G. (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.* 13, 341-390.