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Serum Paraoxonase Activity and Protein Thiols in Chronic Renal Failure Patients

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Abstract: Serum paraoxonase is known to prevent low-density lipoprotein oxidation and atherogenesis. Relation of paraoxonase with severity of renal failure and protein thiols are studied in the current research. Serum paraoxonase, protein thiols, lipid hydroperoxides, creatinine and urea were estimated by spectrophotometric methods in chronic kidney disease patients on conservative management and in healthy controls. Lipid hydroperoxides, creatinine and urea levels were higher, protein thiols and paraoxonase activity were lower in patients compared to healthy controls. Creatinine correlated positively with lipid hydroperoxides and negatively with protein thiols and paraoxonase activity. In conclusion, paraoxonase activity is decreased with increase in severity of renal failure.

Key words: Chronic renal failure, paraoxonase, lipid hydroperoxides, oxidative stress, protein thiols, uremia

INTRODUCTION

Serum paraoxonase (PON1) is a calcium dependent high-density lipoprotein (HDL) associated enzyme (Durrington *et al*., 2001). PON1 is proposed to protect low-density lipoprotein (LDL) from oxidation and the biological role of HDL is attributed to the presence of PON 1 associated with it (Baskol *et al*., 2006). Recently the natural substrate and biological role of PON1 was reported indicating the role of PON1 in hydrolysis of homocysteine thiolactone into homocysteine (homocysteine thiolactonase activity) (Jakubowski, 2000a). Homocysteine thiolactone is unstable compound and can bind to proteins at lysine residues. This N-homocysteinylation of proteins alters protein structure and increased susceptibility to proteolysis. N-homocysteinylation of PON1 (or other component of HDL regulating its activity like apolipoprotein A1) decreases its activity. This, decrease in PON1 activity may initiate a positive feedback mechanism, since reduced PON1 activity will cause further accumulation of homocysteine thiolactone and may augment protein homocyteinylation (Beltowski, 2005; Jakubowski, 2000b).

In this context the reducing property of PON1 and the donor of reducing equivalents to PON1 have to be clarified. In recent times enzyme PON1 is considered as the better marker of lipid peroxidation than lipid hydroperoxides (Baskol *et al*., 2005). This expanding role of PON1 in our body created considerable current interest in this enzyme in recent times. The SH groups present on protein are considered as major antioxidants *in vivo* and most of them are present over albumin. The levels of protein SH in the body indicate antioxidant status and low levels of protein SH correlated with increased levels of Advanced Oxidation Protein Products (AOPP) (Himmelfarb *et al*., 2000). Decreased

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levels of protein SH were found in many disease conditions including chronic renal failure (Prakash *et al*., 2004). There very few studies available to date correlating PON1 with protein SH and severity of chronic renal failure.

In the current study, serum PON1 activity was estimated along with urea, creatinine, protein-SH, lipid hydroperoxides in CRF patients on conservative management compared with healthy controls.

MATERIALS AND METHODS

Subject

The study was carried out on 69 CRF patients on conservative management and 42 healthy controls in the Department of Biochemistry, Kasturba Medical College, Manipal, India in December 2006. Samples were collected from the proved cases of CRF patients maintained on conservative management from the Department of Nephrology, Kasturba Medical College Hospital, Manipal. Acute inflammation in all the cases and controls is ruled out by assaying C-Reactive Protein. Patients having GFR <30 mL min^{-1} and serum creatinine >1.6 mg dL^{-1} for more than three months along with clinical and sonological findings were considered having CRF. The cause of CRF was chronic glomerulonephritis (54 cases), diabetic nephropathy (11 cases) and unknown (4 cases). None of the patient groups received any form of antioxidant medication; all of them were on renal diet (50 g protein and 5 g salt/day). The healthy controls were age and sex matched; were not on any kind of prescribed medication or dietary restrictions. This study was approved by institutional review board and informed consent was obtained from all subjects involved in the study.

Under aseptic conditions blood samples (5 mL) were drawn into plain vacutainers from ante-cubital veins of controls and CRF patients on conservative management. The collected blood was allowed to clot for 30 min and then centrifuged at 2000 g for 15 min for clear separation of serum. All assays were performed immediately after serum was separated.

Reagents

Special chemicals like paraoxone, 5 5′ dithio-bis (2-nitrobenzoic acid) (DTNB), xylenol orange were obtained from Sigma chemicals, St. Louis, MO, USA. All other reagents were of analytical grade.

PON was estimated spectrophotometrically by the method described elsewhere with minimal modifications (Schiavon *et al*., 1996). Briefly, the assay mixture consists of 500 μL of 2.2 mM Paraoxone substrate in 0.1 M tris-HCl buffer, pH 8.0 containing 2 mM $CaCl_2$ and 50 μL of fresh serum specimen. The absorbance was monitored at 405 nm at 25°C. One unit (IU) of PON activity is defined as 1 μmol of p-nitrophenol formed per min per litre at 25°C and activity was expressed as U L^{-1} of serum.

Serum protein thiols were measured by a spectrophotometric method using 5 5′ dithio-bis (2-nitrobenzoic acid) (DTNB) (Motchnik *et al*., 1994). The lipid hydroperoxide content of whole serum was determined with the FOX version II assay for lipid hydroperoxides (FOX_2) (Nourooz-Zadeh *et al*., 1999; Nourooz-Zadeh *et al*., 1995). Serum urea and creatinine levels were estimated by spectrophotometric methods using automated analyser (Hitachi 911).

Statistical Analysis

The results were expressed as mean±Standard Deviation (SD). A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-10, Chicago, USA). Student t-test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS

As depicted in Table 1, there was significant increase in urea and creatinine levels in chronic renal failure cases ($p<0.0001$). Serum PON activity was significantly decreased in cases compared to healthy controls ($p<0.0001$). Serum protein SH were decreased ($p<0.0001$) and lipid hydroperoxides were increased ($p<0.001$) in cases compared to healthy controls.

On applying the Pearson correlation serum creatinine correlated positively with lipid hydroperoxides ($r = 0.298$, $p<0.001$), urea ($r = 0.592$, $p<0.0001$) and negatively with PON1 activity ($r = -0.445$, $p<0.0001$) (Fig. 1) and protein SH ($r = -0.547$, $p<0.0001$) (Fig. 2). PON1 activity correlated positively with protein SH ($r = 0.676$, $p<0.0001$) and negatively with lipid hydroperoxides ($r = -0.292$, $p<0.001$). There was negative correlation between lipid hydorperoxides and protein SH ($r = -0.434$, $p<0.0001$).

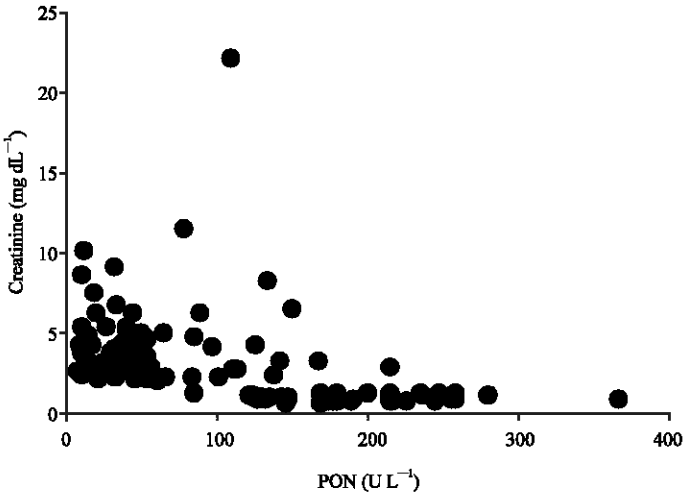

Fig. 1: Correlation between serum creatinine and serum PON activity

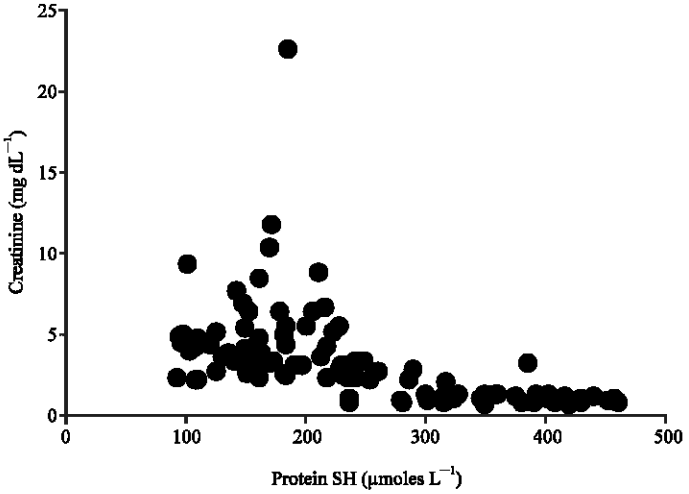

Fig. 2: Correlation between serum creatinine and serum protein SH

Table 1: Demographic, biochemical and oxidative markers of chronic renal failure patients and healthy controls, values expressed as mean±SD

Markers	Healthy controls (n = 42)	Renal failure cases (n = 69)
Age (years)	35.00±5.00	45.00±8.00
Sex (M/F)	31/11	58/11
Urea (mg dL^{-1})	31.19±5.80	96.80±48.7*
Creatinine (mg dL^{-1})	0.96±0.19	4.20±2.30*
Lipid hydroperoxides (μmoles L^{-1})	0.51±0.44	2.23±2.54**
Protein SH (μmoles L^{-1})	366.61±8.80	180.06±42.83*
PON activity (IU)	187.31±8.34	51.75±52.39*

*p<0.0001, **p<0.001 compared to healthy controls

DISCUSSION

PON1 activity is significantly decreased in chronic renal failure patients in present study. This findings support the findings of Schiavon *et al*. (1996) in uremic patients and Sutherland *et al*. (2004) in hemodialysis patients. This decrease in PON1 activity was attributed to the consumption of free SH groups in the active site of PON1. Among PON family, PON1 has shown to have antioxidant property (Mackness *et al*., 1998). The positive correlation of PON1 activity with HDL indicates the role of PON1 in biological function of HDL, although exact mechanism is not clear at present (Durrington, 2001).

The significant lower levels of protein SH in CRF patients and positive correlation between protein SH and PON1 activity indicates increased protein oxidation particularly albumin and increased consumption of protein bound SH groups for PON1 catalyzed reactions. The positive correlation of serum creatinine with PON1 and protein SH indicates increased consumption of protein bound SH groups and PON1 enzyme activity with increase in severity of renal failure. Several previous studies proved existence of oxidative stress in renal failure patients (Galle, 2001; Himmelfarb and Hakim, 2003) hence excess of oxidants may be responsible for the increased consumption of thiols and PON1 activity in this patient population.

There exists controversy over the better marker of lipid peroxidation between lipid hydroperoxides and PON1 activity (Baskol *et al*., 2005). Present study in line with previous report supports PON1 activity as better indicator of lipid peroxidation as there was decrease in PON1 enzyme activity according to the severity of renal failure and methodically it is simple to estimate compared to lipid hydroperoxides. Serum PON1 correlated positively with antioxidant protein SH and negatively with oxidant lipid hydroperoxides indicating itself as oxidative stress marker. However, further specially designed studies are required to know the exact relationship between protein thiols and the-SH group present in PON1.

In conclusion, PON1 activity decreased according to the severity of renal failure and it is correlated positively with protein SH.

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