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Paraoxonase activity in patients with chronic renal failure and hepatic insufficiency

Siddiqua Jamall[1], Mohammad Ishaq[1], Junaid Mahmood Alam[2], Sana Hussain[2] and Syed Muhammad Waqar Hussain[1]
[1]Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan,
[2]Department of Biochemistry and Chemical Pathology, Liaquat National Hospital, Karachi, Pakistan

Abstract: Paraoxonase (PON), a high density lipoprotein (HDL) associated enzyme, is believed to protect against the oxidation of low density lipoprotein (LDL) and hence affects the risk of vascular disease. PON is sensitive to oxidants and is inactivated by oxidized lipids, and thus it can be postulated that increased oxidative stress may decrease plasma PON activity in patients with chronic renal failure (CRF) and hepatic insufficiency (HI). Moreover, in CRF and HI patients, in contrast to normal individuals, higher levels of plasma biochemical parameters and liver enzymes had an inverse correlation with PON activity. In this study we aimed to investigate PON activity, total bilirubin, creatinine, urea and liver enzymes alanine aminotransferase and alkaline phosphatase that are the index of renal and hepatic insufficiency. We have analyzed plasma from pre-dialysis patients and compared the results with the normal individuals. We observed a positive association of PON activity with that of the disease state i.e. the activity of this enzyme was significantly lower in the patients ($p < 0.001$). Furthermore, the indicators of renal and hepatic insufficiency were significantly elevated as compared to the normal subjects. Based on our results we conclude that in CRF and HI, in contrast to normal individuals, higher levels of plasma biochemical parameters and liver enzymes had inverse correlation with PON activity. Collectively, these findings may add details to the understanding of the role that PON plays in chronic renal failure and hepatic insufficiency.

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***Author for Correspondence:** sidjam@hotmail.com

INTRODUCTION

Paraoxonase (PON)[1] is synthesized in the liver it is an ester hydrolase present in blood, liver and kidneys. It is an enzyme associated with high-density lipoprotein (HDL) that is believed to protect against the oxidation of low-density lipoprotein (LDL)[2,3]. It has been shown that PON prevents the transformation of low-density lipoproteins (LDL) into biologically active, atherogenic particles[4-6]. It has already been established that HDL possesses antioxidative potential[7] by the enzymatic removal of lipid peroxides accumulating on the LDL particles[8]. PON may also confer protection against coronary artery disease by destroying proinflammatory oxidized lipids present in oxidized low density lipoproteins[3,9,10]. It hydrolyzes phospholipid hydroperoxides and cholesterol ester hydroperoxides, degrades hydrogen peroxide and reduces lipid hydroperoxides thereby protecting plasma membranes from free radical injury[11-13].

Paraoxonase activity was found to be lower in patients with some degree of renal insufficiency, chronic hemodialysis, and chronic peritoneal dialysis while renal transplantation seemed to restore paraoxonase activity[3,13-15]. Moreover, it has been reported that there is considerably low paraoxonase activity in the patients of renal failure[16-18]. It has been reported that the serum of predialysis patients contains increased concentrations of oxidants and compounds that generate advanced glycation end-products (AGE)[19] that lower PON[20] activity.

The chronic renal failure patients are often suffering from hepatic insufficiency (HI). In hepatic insufficiency, plasma alanine aminotransferase, alkaline phosphatase and uremia-associated substances (creatinine and urea) and bilirubin, had significant positive correlation with the disease state and was found to increase the oxidative stress, thereby further reducing the activity of the PON[21-23]. There is considerable evidence indicating that the severity of hepatic damage in individuals with cholestatic liver disease is associated with the extent of intrahepatic oxidative stress[24]. Increased levels or accelerated generation of reactive oxygen species and toxic degradative products of lipid peroxidation have been reported in the plasma of individuals with chronic liver disease[24]. This may be due to lower activity of hepatic and serum paraoxonase.

We hypothesized that paraoxonase activity is inhibited in the uremic milieu that contributes to the lower activity seen in CRF and HI patients. We have investigated the role of PON in relation to uremic toxins such as urea and creatinine and enzymatic markers of cellular liver damage, such as alanine aminotransferase and alkaline phosphatase.

MATERIALS AND METHODS

Collection of samples

The blood samples were collected from 50 pre-dialysis patients with chronic renal disease and hepatic insufficiency with their prior consent from Local Hospitals in Karachi. The blood samples were also collected from 50 age and sex matched healthy individuals with their prior consent from the general population. The plasma was prepared and then stored at -20°C until further processed.

Determination of total and direct bilirubin, creatinine, urea and total proteins in plasma

Determination of total and direct bilirubin and creatinine were carried out by colorimetric methods[25,26]. The urea was measured by enzyme kinetic method[27]. The total plasma protein was estimated by Lowry's method[28].

Alanine aminotransferase and alkaline phosphatase

Estimation of alanine aminotransferase and alkaline phosphatase were carried out by UV method[29] and colorimetric method[30] respectively.

Determination of PON activity

PON activity towards phenyl acetate was quantified spectrophotometrically at 270nm using 20 mmol Tris/HCl buffer of pH-7.0, containing 1mmol $CaCl_2$ and 1mmol phenyl acetate. The reaction mixture contained 2 ml of Tris/HCl buffer, 0.5 ml of $CaCl_2$ and 0.5 ml of phenyl acetate. For control, 30 µl of distilled water was added while in case of test, 30 µl of 1:5 or 1:10 diluted plasma sample was added. The reaction was then monitored for 3 minutes at 25°C.

Statistical analysis

Statistically significant differences were determined by student's t-test using software Statistical Package for Social Sciences (SPSS).

RESULTS

Determination of PON activity

Figure 1 shows the PON activity in chronic renal failure and hepatic insufficiency patients. We observed a significantly lower activity of PON enzyme ($p<0.001$), 60.84±18.85 Units of E/ml in the patients' samples as compared to the normal controls 106±19.42 Units of E/ml.

Biochemical parameters of CRF and HI

Table 1 demonstrates that the level of creatinine (3.51 ± 2.83 mg/dl) in patients, was found to be significantly higher ($p < 0.001$) than normal individuals (0.79 ± 0.24 mg/dl at 95% C.I.). Furthermore, the level of urea (114.44 ± 62.12 mg/dl) in patients was also found to be significantly higher ($p<0.001$) than the normal individuals (23.82 ± 7.68 mg/dl at 95% C.I.).

The value of total bilirubin (6.47 ± 10.57 mg/dl) in patients with CRF and HI was significantly ($p<0.001$, Fig. 2) higher than the normal individuals (0.60 ± 0.38 mg/dl at 95% C.I). Furthermore the values of direct (4.8 ± 8.69 mg/dl) and indirect bilirubin (1.67 ± 2.10 mg/dl) were significantly higher in patients than the values in normal individuals, that is. 0.15 ± 0.203 mg/dl and 0.47 ± 0.28 mg/dl respectively.

As expected, the levels of alkaline phosphatase (184.64 ± 19.13 U/l) and alanine aminotransferase (293.94 ± 63.19 U/l) in the CRF and HI patients were significantly higher ($p < 0.001$) as compared to the controls. These high values were matched with the corresponding rise in the plasma bilirubin that reflects the hepatic cellular damage.

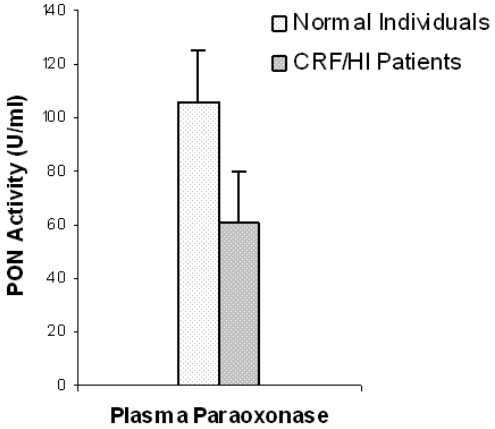

Figure 1: Paroxonase activity in plasma of CRF and HI patients. Each bar represents the mean±SD of n=50 normal and CRF and HI patients each. The PON activity was significantly reduced in the patients (*$p<0.001$) compared to normal individuals.

Table 1: Biochemical parameters in normal individuals and patients with chronic renal failure and hepatic insufficiency.

Parameters	Normal (n = 50)	Patients (n = 50)
Urea (mg/dl)	23.82± .68	114.44±62.12*
Creatinine (mg/dl)	0.79±0.24	3.51±2.83*
Total bilirubin (mg/dl)	0.60±0.38	6.47±10.57*
Direct bilirubin (mg/dl)	0.15±0.203	4.82±8.698*
Indirect bilirubin (mg/dl)	0.47±0.28	1.67±2.108*
GPT (U/L)	21.22±8.01	293.94±63.19*
ALP (U/L)	82.62±23.52	184.64±19.13*
Protein (gm/dl)	8.53±0.99	7.99 ± 1.82

Values are mean±SD of n=50. *$p<0.001$, The normal individuals were compared with the patients having chronic renal failure and hepatic insufficiency, the significance of difference is indicated by p-values calculated by independent t-test.

DISCUSSION

Oxidative stress reduces the antioxidant defence which is very critical in the patients with chronic renal failure and hepatic insufficiency. Uremia accompanied by oxidative stress further aggravates the disease state[31]. Increased oxidative stress has been reported in numerous studies in patients with

CRF[21,31-33] which lowers the anti-oxidant marker PON. Studies have shown that paraoxanase has protective effect against oxidative stress and can act as an antioxidant[34,35]. Lower activity of PON in the CRF patients may be the result of various factors such as the consumption of antioxidants during free-radical production and exposure to uremic toxins.

The patients population that we choose were pre-dialysis CRF and HI and the very high total bilirubin, creatinine and urea values indicated that the degree of renal insufficiency was very advanced. The important finding of our study is that patients with advanced CRF and HI who were not receiving renal replacement therapy had evidence of exposure to increased oxidative stress. We observed that the PON activity was significantly lower in patients compared to the normal controls and this correlates well with the previous studies[36-38].

Plasma bilirubin, creatinine and urea concentrations in our patients also showed significant inverse correlation with the PON activity. The negative correlation between PON and urea/creatinine indicated the true relationship between antioxidants and renal functions. The uremic retention solutes are retained when renal failure develops and are called uremic toxins. The retention of these uremic solutes results in the progressive failure of other organ system, in parallel with the failing function of the kidneys and most importantly affects the liver. The resulting clinical picture is the uremic syndrome.

Another characteristic feature of chronic hepatic insufficiency observed in our study was that the liver enzymes alanine aminotransferase and alkaline phosphatase were higher in patients with CRF and HI than in the controls. These findings correlate well with the previous studies reporting high values in these biochemical parameters following CRF and HI[39-43].

Based on our results we suggest that the retention of uremic toxins in the CRF and HI patients could play a role in reducing the PON activity. The lower values of PON activity can be used as a marker of advanced CRF and HI.

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