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Original Research Article

Study of Serum Paraoxonase Activity in Chronic Renal Failure

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ABSTRACT

Background: Paraoxonase is an enzyme associated with high density lipoprotein. It has been shown to prevent atherosclerosis by inhibiting oxidation of low density lipoprotein. Patients with chronic renal failure are at increased risk for developing atherosclerosis. Cardiovascular disease is the main cause of mortality and morbidity in patients with chronic renal failure and in patients undergoing hemodialysis. Reduced activity of serum Paraoxonase is considered to play a role in the development of atherosclerosis and other cardiovascular complications in these patients.
Materials and Methods: 30 patients with chronic renal failure, 30 patients with chronic renal failure undergoing hemodialysis and 30 age and sex matched healthy control subjects were taken for the study. Serum Paraoxonase activity was estimated in these study groups. Blood urea, serum creatinine, uric acid were also analysed. Serum Paraoxonase activity was correlated with urea, creatinine and uric acid levels.
Results: There was a significant decrease in the serum Paraoxonase activity in patients with chronic renal failure compared to controls ($P<0.001$). There was also a significant decrease in the Paroxonase activity in patients with chronic renal failure undergoing hemodialysis compared to controls ($P<0.001$). There was a negative correlation between serum Paraoxonase activity and urea, creatinine and uric acid in patients with chronic renal failure and in patients undergoing hemodialysis.
Conclusion: Serum Paraoxonase activity is decreased in patients with chronic renal failure and in patients undergoing hemodialysis. Paraoxonase is an predictor of cardiovascular risk in chronic renal failure. Measurement of the enzyme activity is recommended for the prevention and treatment of cardiovascular complications in these patients.

Keywords: Paraoxonase, chronic renal failure, hemodialysis, atherosclerosis.

INTRODUCTION

The human serum Paraoxonase (PON) is a calcium dependent esterase synthesized in the liver. The PON family consists of Paraoxonase1 (PON1), Paraoxonase2 (PON 2), Paraoxonase3 (PON3). The most studied member of the family is PON1.[(1)] PON1 is associated with apolipoprotein A-1 present in high density lipoprotein (HDL). It has been shown to prevent atherosclerosis by inhibiting

oxidation of low density lipoprotein (LDL).[2]

Oxidation of LDL is recognized as the key stage in the early development of atherosclerosis leading to uptake of LDL by the macrophage scavenger receptor and hence to the formation of foam cells.[3] PON1 is principally responsible for the breakdown of lipid peroxides before they accumulate on LDL. It also inhibits macrophage foam cell formation which contributes to its antiatherogenic property.[4]

Patients with chronic renal failure (CRF) are at increased risk for developing atherosclerosis. An increase in the susceptibility for oxidation of LDL has been reported in CRF.[5] Accelerated atherosclerosis and altered lipoprotein metabolism is responsible for the increased cardiovascular events and cardiac death in chronic renal failure.[6] Patients with CRF have a 10-20 fold increased cardiovascular mortality compared to healthy population.[7] It has been reported that there is a significant reduction of serum PON1 activity in CRF explaining for the high incidence of atherosclerosis in these patients.[8]

Renal replacement therapy using dialysis has prolonged the lives of patients with CRF. Hemodialysis is often used to remove excess toxins, metabolic products and blood components from patients with CRF. However dialysis itself may further contribute to atherosclerosis by oxidative stress, cytokine stimulation and other events inherent to hemodialysis.[9]

Hemodialysis is associated with excess production of reactive oxygen species reflected by increased serum indices of lipid peroxidation.[10] Mortality still remains high among dialysis patients and many of these deaths involve cardiac disease. Cardiovascular disease is by far the leading cause of morbidity and mortality in dialysis patients, accounting for around 30% of hospitalizations and 50% of deaths.[11]

It was found that the PON1 activity was significantly reduced in hemodialysis patients compared to healthy controls.[12] Reduced activity could lead to accelerated atherosclerosis in these patients. The aim of this study was to measure serum PON1 activity in patients with CRF and in patients with chronic renal failure undergoing hemodialysis and to compare it with healthy controls.

MATERIALS AND METHODS

The present study was conducted on diagnosed patients of chronic renal failure attending the outpatient and inpatient Department of Nephrology at JSS Medical College and Hospital, Mysore.

The study was conducted on 30 patients with CRF (23 males,7 females), 30 patients with CRF undergoing hemodialysis (21 males,9 females) 3 times a week for 4 hours and 30 age and sex matched healthy controls(25 males,5 females). The age group was between 20-65 years. Patients with conditions that could affect the PON1 activity such as hepatic, respiratory, acute inflammatory disease were excluded from the study. An equal number of age and sex matched healthy subjects without any history of hepatic disease and other major illness formed the control group. This study was approved by the Institutional Ethical Committee and informed consent was taken from all the subjects involved in this study.

Under aseptic precautions around 5ml of venous blood was collected in serum gel tubes. The blood was allowed to clot for 30 minutes and after which the blood was centrifuged at 3000 rpm for 15 minutes for the separation of serum. The serum was used for the various biochemical analysis.

Serum PON1 Assay

Paraoxonase activity was estimated spectrophotometrically using assay mixture consisting of 5.5mM 4-nitrophenylacetate as the substrate in 20mM Tris-HCL buffer

containing 1mM$CaCl_2$ at pH of 8.0 and 50µl of fresh serum sample. The increase in absorbance due to the formation of yellow 4-nitrophenol was monitored at 405nm for three minutes. PON1 was taken as 1 U/ml when the rate of formation of the product 4-nitrophenol was 1nanomol/ml of the serum under the assay conditions.[13] 4-nitrophenyl acetate was procured from Sigma Aldrich Chemicals. All other chemicals used for the assay of PON1 activity were of analytical grade.

Blood urea, serum creatinine and uric acid were estimated using Randox Daytona autoanalyzer. Blood Urea was estimated by Urease / Glutamate dehydrogenase method.[14] Serum Creatinine was estimated by modified Jaffe's method.[15] Uric acid was estimated by Uricase method.[16]

Statistical Methods

The statistical analysis was carried out by using the SPSS (Statistical package for social sciences) software. Independent sample 't' test was used to compare mean values. Pearson's correlation coefficient analysis was used to find out the degree of correlation between parameters.

RESULTS

The mean age of the patients with CRF, patients undergoing hemodialysis and healthy controls were 33.57±10.88 years, 39.03±11.58 years and 36.57±9.41 years respectively. The serum PON1 activity ,blood urea, serum creatinine, uric acid were estimated and expressed as mean ± SD, the results are depicted in Table-1.The mean PON1 activity was 33.38± 8.8U/ml in patients with CRF. The mean PON1 activity was 34.19±8.13U/ml in patients undergoing hemodialysis and 53.09 ±8.42U/ml in control group .The PON1 activity was significantly decreased in patients with chronic renal failure when compared to controls (P<0.001). The PON1 activity was also significantly decreased in patients undergoing hemodialysis when compared to controls (P<0.001).There was no significant difference in the PON1activity between the patients with CRF and in patients undergoing hemodialysis (p=0.713).

Table. 1. Serum PON1 activity, urea, creatinine, uric acid in patients with CRF, hemodialysis and healthy controls.

Parameters	Patients with CRF (n=30)	CRF patients on Hemodialysis (n=30)	Healthy Controls (n=30)
Age (years)	33.57±10.88	39.03±11.58	36.57±9.41
Sex(M/F)	23/7	21/9	25/5
PON1 activity(U/ml)	33.38±8.8 *	34.19±8.13*	53.09±8.42
Urea(mg/dl)	140.30±31.79*	129.97±34.15 *	24.23±3.41
Creatinine(mg/dl)	8.47±4.49*	10.02±4.19*	1.05±0.14
Uric acid(mg/dl)	9.25±2.64*	6.99±1.55*	4.15±0.67

*P < 0.001 compared to healthy control

The blood urea, serum creatinine and uric acid was significantly increased in patients with chronic renal failure and in patients undergoing hemodialysis when compared to controls (P<0.001).There was a negative correlation between PON1 activity and urea in CRF patients (p<0.001, r = -0.58) and patients undergoing hemodialysis (p<0.0001,r = -0.74) (Fig 1). There was a negative correlation between PON1 activity and creatinine in CRF patients (p<0.0001, r =-0.62) and patients undergoing

hemodialysis ($p<0.0001$, $r = -0.66$) (Fig 2). There was a negative correlation between PON1 activity and uric acid in CRF patients ($p<0.001$, $r = -0.59$) and patients undergoing hemodialysis ($p<0.001$, $r = -0.51$) (Fig 3).

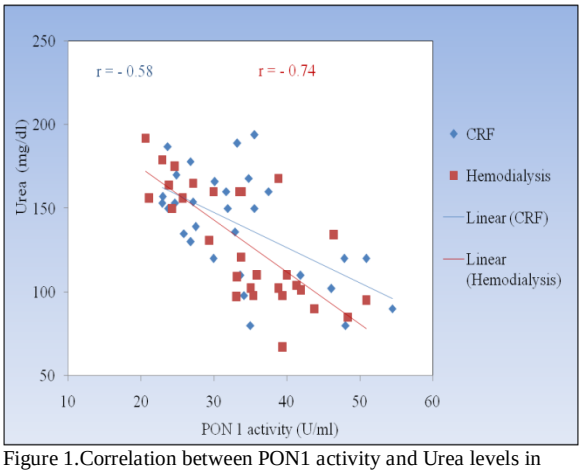

Figure 1.Correlation between PON1 activity and Urea levels in patients with Chronic Renal Failure and patients undergoing Hemodialysis.

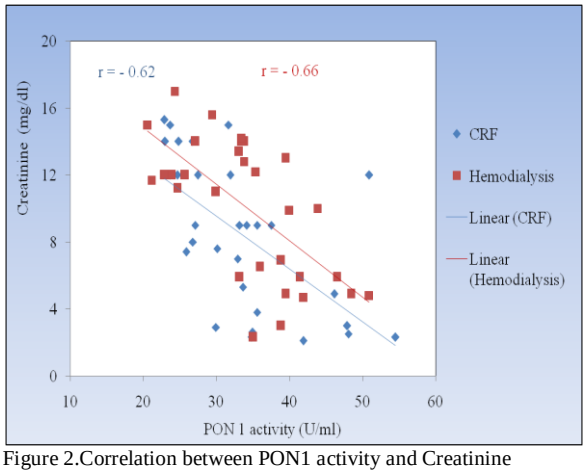

Figure 2.Correlation between PON1 activity and Creatinine Levels in patients with Chronic Renal Failure and patients undergoing Hemodialysis.

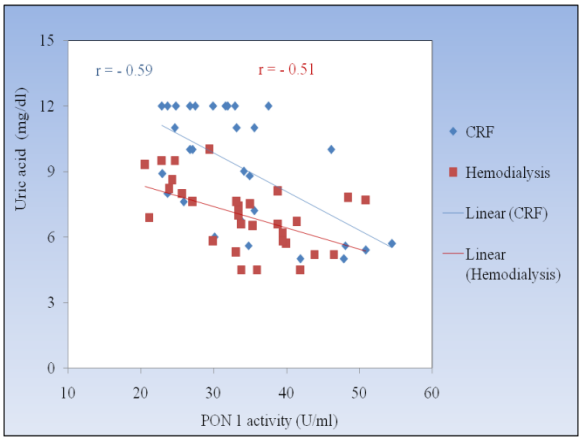

Figure 3.Correlation between PON1 activity and Uric acid levels in patients with Chronic Renal Failure and patients undergoing Hemodialysis.

DISCUSSION

Chronic renal failure is characterised by irreversible loss of renal function. Cardiovascular disease accounts for the majority of mortality and morbidity in these patients. Reduced PON1 activity is implicated in the development of atherosclerosis in this condition.

In the present study, the activity of PON1 was significantly reduced in patients with chronic renal failure compared to healthy controls. Our finding is comparable with the previous study done by Juretic et al who have demonstrated a significant decrease in PON1 activity in CRF patients.[17] Schiavon et al have also observed reduced PON1 activity in CRF patients.[8] Chronic renal failure is accompanied by a complex pattern of altered lipoprotein metabolism. It has been suggested that there is an enhanced oxidative stress and insufficient enzyme activities which contributes to the enhanced tendency to atherosclerosis in this condition.[18] Reduced activity of the enzyme in CRF may be the result of various factors such as the consumption of antioxidants during free radical production and exposure to uremic toxins.[19]

In the present study the PON1 activity was significantly reduced in patients undergoing hemodialysis compared to healthy controls. Our results are similar to that of Suehiro et al who have demonstrated significantly reduced PON1 activity in patients undergoing hemodialysis compared to controls.[12]

Gulcin et al have found that PON1 activity was lower in both the CRF patients and in patients undergoing hemodialysis and there was no significant difference in the enzyme activity between these groups.[20] Our findings were similar to this study in which there was no significant difference between the PON1 activity in CRF patients and in patients undergoing hemodialysis. Dantoine et al have found that the reduction of the enzyme activity was more pronounced in patients undergoing hemodialysis compared to patients with CRF.[21]

It has been proposed that there is an increased oxidative stress and enhanced lipid peroxidation in patients undergoing hemodialysis. Dialysis itself may further contribute to atherosclerosis by enhanced oxidative stress and changes in the enzyme activity.[9] Reduced PON1 activity is expected to contribute to the increased risk of premature atherosclerosis found in these patients.[21]

In the present study there was a negative correlation between PON1 activity and urea, creatinine, uric acid levels in patients with CRF and in patients undergoing hemodialysis. Our results were similar with the results obtained by Dirican et al who demonstrated a negative correlation between PON1 activity and urea, creatinine, uric acid.[22] Jamall et al have also demonstrated negative correlation between the enzyme activity and urea and creatinine.[23] They suggested that the retention of uremic toxins in CRF and hemodialysis could play a role in reducing the PON1 activity. It has been proposed that the enzyme activity is inhibited in the uremic environment.[19] The enzyme activity inhibition through posttranslational modification of Paraoxonase as a result of reactions with advanced glycation end products or urea derived cyanate remains possible.[24]

CONCLUSION

In the present study Paraoxonase activity was decreased in patients with chronic renal failure and in patients undergoing hemodialysis. This could lead to accelerated tendency for atherosclerosis in these patients. Paraoxonase activity could be a good predictor of the cardiovascular disease in these patients. Estimation of this enzyme activity is useful to predict and prevent cardiovascular complications arising from chronic renal failure.

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