Serum Paraoxonase (Arylesterase) Activity in Chronic Renal Failure

BALASUBRAMANIAM GAYATHRI,[1] ANAND USHA,[2] DURAISAMY VIJAYA[3]

Abstract:

Background: Mortality rates are higher among patients with chronic renal failure with a good proportion of this excess burden being attributable to cardiovascular disease. This excess risk is not entirely explained by elevated traditional risk factors. Among the non-traditional risk factors, serum paraoxonase (Arylesterase) activity is an important one.

Materials and Methods: It was a case-control study which included total 127 subjects, 44 were non-dialysis CKD patients, 33 Heamodialysis dependent patients and 50 healthy controls. HDL-C, Apo-A and serum paraoxonase (Arylesterase) activity was estimated and compared with normal control population.

Results: Serum Arylesterase activity, a measure of PON was significantly lower in CRF (on conservative management and on hemodialysis) compared to controls.

Conclusion: Measurement of anti oxidant capacity of HDL-C may improve the predictive accuracy of atherosclerotic coronary artery disease, providing new strategies for the prevention and treatment of accelerated atherosclerosis in chronic renal failure.

Keyword: Serum paraoxonase (Arylesterase), chronic renal failure, Atherosclerosis,

Introduction

Paraoxonase (aryldialkylphosphatase, EC 3.1.8.1) is a calcium dependent polymorphic enzyme with three activities, paraoxonase, arylesterase and dyazoxonase. [1] It is widely distributed in liver, kidney, intestine and also in serum. In serum, paraoxonase (PON) circulates as a high density lipoprotein cholesterol (HDL-C) component, tightly bound with the hydrophobhic N-terminal domain to apo A1 of HDL-C. Biochemical studies have shown that anti oxidant activity of HDL - C is shown to reside in its enzymes, particularly paraoxonase and hence a major contributor to its anti atherogenic role. [2]

Oxidation of low density lipoprotein Cholesterol (LDL -C) plays a pivotal role in the early development of atherosclerosis. An increase in susceptibility for oxidation of LDL -C has been reported in chronic renal failure (CRF). [3] Accelerated atherosclerosis and altered lipoprotein metabolism is responsible for increased cardiovascular events and cardiac death in chronic renal failure. [4] Inverse correlation is shown between HDL - C concentration and the development of atherosclerosis. PON associated with HDL- C is important in determining the capacity of HDL- C against oxidative modification and in turn to protect against atherosclerosis in chronic renal failure. [5]

Recently it has been reported that significant reduction of serum paraoxonase activity occurs in chronic renal failure, explaining the cause for high and premature incidence of atherosclerosis in these individuals. [6] The phenotypic distribution of PON can be used to subdivide the population into three groups AA represents low, AB intermediate and BB high enzyme activity. [7] Arylesterase activity is a measure of PON activity, in which p- nitro phenyl acetate is used as a substrate. The aim of this study was to measure serum PON - arylesterase activity and phenotypic distribution in patients with chronic renal failure (both on conservative management and on hemodialysis) and healthy individuals.

Materials and Methods:

Subjects

The study was carried out on 44 CRF (mean age-53,range 24-73) patients on conservative management and on 33 CRF patients on hemodialysis , 3 times/week for 4.5 hours 9 mean age – 50, range 24-72)and 50 healthy controls (mean age - 43,range 26-69), in the department of Biochemistry , PSG IMSR , Coimbatore. Samples were collected from proved cases of CRF (patients having GFR < 30mL/min and serum creatinine >1.6mg/dL for more than three months, along with clinical and serological findings were considered having

1. Assistant Professor, Department of Biochemistry, PSG Institute of medical sciences and Research. Coimbatore, India
2. Professor, Department of Biochemistry, PSG Institute of medical sciences and Research. Coimbatore, India
3. Professor ,Department of Biochemistry, PSG Institute of medical sciences and Research .Coimbatore, India

Correspondence: Anand Usha, Professor, Department of Biochemistry, PSG Institute of medical sciences and Research. Coimbatore, India.

CRF) maintained on conservative management and those on hemodialysis, from the department of nephrology, PSG Hospitals, Coimbatore.

The causes for CRF in patients with conservative management were diabetes mellitus -34%, hypertension - 32%, chronic glomerulo nephritis – 20%, other causes -14% and on patients under going hemodialysis were diabetes mellitus – 40%, hypertension – 36% and chronic glomerulo nephritis – 16% and other causes – 8%.

No antioxidant medications were taken by the cases and all were on renal diet (50g of protein and 5g salt/day). The healthy controls were age and sex matched, were not on any kind of prescribed medications or dietary restrictions. The study was approved by the institutional ethical committee and informed consent was obtained from all the subjects.

Biochemical Assay

The blood samples were collected using commercially available (BD vacutainers) serum gel tubes under strict aseptic conditions from antecubital veins after an over night fast for 12 hours and before hemodialysis. For preparation of venous serum, the blood was allowed to clot for 30 minutes and after which the blood was centrifuged 3000 rpm for 15 minutes and the clear serum was used for biochemical analysis.

Lipid Measurement

Levels of HDL -C, Apo-A were determined immediately in Roche Auto analyser (Immuno turbidimetric method) using dedicated kits. Levels of serum creatinine were determined Roche Auto analyser [Jaffe's Method] using dedicated kits. GFR was calculated by the modified diet and renal disease [MDRD] formula. The stages of chronic kidney disease were classified according to stages;

Stage 1 CKD

Slightly diminished function: Kidney damaged with or relatively high GFR [>90 mL/min/1.73mL]

Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies.

Stage 2 CKD

Mild reduction in GFR [60-89mL/min/1.73m^2] with kidney damage. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies.

Stage 3 CKD

Moderate reduction in GFR [30-59mL/min/1.73m^2]

Stage 4 CKD

Severe reduction in GFR [15-29mL/min/1.73m^2]

Stage 5 CKD

Kidney failure [GFR <15mL/min/1.73m^2 or permanent renal replacement therapy (RRT)]

Serum PON assay –

This enzyme was estimated spectrophotometrically using 5.5 mM   4-nitrophenylacetate as the substrate in 20 mM Tris-HCl buffer at a pH of 8.0. The increase in absorbance due to the formation of the yellow 4-nitro-phenol was monitored at 412 nm for three minutes.   For each sample basal PON activity as well as salt-stimulated PON activity was determined as described below. [6] Basal PON –This was estimated by using the Tris-HCl buffer containing only 1mM calcium chloride. Salt Stimulated PON - This was estimated by using the same Tris-HCl buffer which however contained 1mM calcium chloride as well as 1M NaCl. PON Activity was calculated after corrections were made for non-enzymatic hydrolysis. PON was taken to be 1 U/L when the rate of formation of the product 4-nitrophenol was 1 ìmol/ minute under the assay conditions.

Chemicals: - 4-nitrophenol acetate was procured from Sigma Chemical Company (St. Louis, MO,USA). All other chemicals used for the assay of PON were of analytical grade.

Paraoxonase Phenotype Distribution

The Pearson stimulation of PON was calculated as

$$\frac{\text{SALT STIMULATED PON ACTIVITY - BASAL PON ACTIVITY}}{\text{BASAL PON ACTIVITY}} \times 100\%$$

Individuals were classified for PON phenotype using the antimode of PON as proposed by Eckerson et al. [8] Individuals below the limit of 60% stimulation were considered AA phenotype, between 60-200% AB phenotype, and above the level 200% stimulation BB phenotype.

Statistical Analysis

The results were expressed as mean ± standard deviation. p value of < 0.05 was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS 10). Student't' test was used to compare mean values. Pearson's Correlation coefficient analysis was used to find the degree of correlation between parameters.

Results

51% of the CRF patients on conservative management belong to stage 5 chronic kidney disease. The remaining 30% patients fall into stage 4 CKD,14% of the patients in stage 2 and 5% of patients in stage 1 CKD.

Serum Arylesterase activity a measure of PON was significantly lower (p<0.05) in CRF (on conservative

management and on hemodialysis) compared to controls. (Table-I)

Paraoxonase Phenotype distribution
In patients with chronic renal failure, both on conservative management and on hemodialysis 43, 30 subjects belong to AA Phenotype (low Paraoxonase activity) respectively. This explains the cause for accelerated atherosclerosis in chronic renal failure. In the control group, 17 belong to AA Phenotype which also adds to the cause for increased incidence of atherosclerosis in the general population

Lipid Parameters
HDL- C concentration was significantly lower in the CRF patients on conservative management and on hemodialysis ($p<0.05$) compared to the control group.

Apo A concentrations was significantly lower in the CRF patients on conservative management and on hemodialysis ($p<0.005$) compared to the control group

HDL standardized Paraoxonase activity
Since HDL- C concentrations and Arylesterase enzyme activity were lower in CRF patients, arylesterase activity was standardized for HDL cholesterol concentrations. It was found that HDL Standardized arylesterase activity was significantly reduced in the CRF patients than in the control subjects.

Table-I

Parameter	mean±SD	P value*
Basal arylesterase activity (U/L)		
Control	114.35± 35.68	
CRF on conservative Management	89.27± 20.64	0.003**
CRF on Hemodialysis	39.05± 10.35	0.001**
Salt Stimulated arylesterase activity(U/L)		
Control	137.5± 45.29	
CRF on conservative Management	103.4± 26.34	0.006**
CRF on Hemodialysis	122.3±36.10	0.033**
HDL Cholesterol		
Control	52.97±35.87	
CRF on conservative Management	34.60± 12.83	0.005**
CRF on Hemodialysis	29.89±11.20	0.001**
Apo A Control	151.30±51.89	
CRF on conservative Management	110.58±29.83	0.05**
CRF on Hemodialysis	83.66±32.76	0.03**
Basal Arylesterase/HDL - C ratio		
Control	2.54±1.00	
CRF on conservative Management	3.20±2.46	0.001**
CRF on Hemodialysis	4.59±3.27	0.002**
Salt Stimulated Arylesterase/HDL -C ratio		
Control	3.05± 1.28	
CRF on conservative Management	3.73± 3.02	0.047**
CRF on Hemodialysis	5.19± 3.49	0.002**

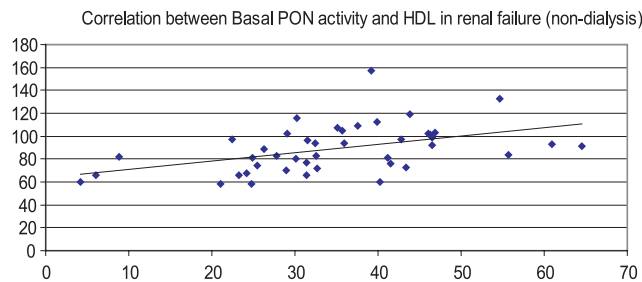* Student t test, ** Significant

Fig.-1: *Correlation between Basal PON activity and HDL in renal failure (non-dialysis)*
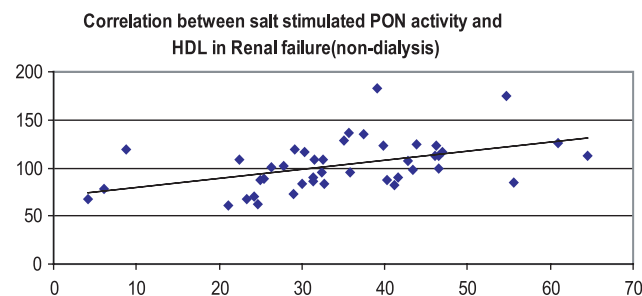

Fig.-2: *Correlation between salt stimulated PON activity and HDL in Renal failure(non-dialysis)*

Discussion
Chronic Renal failure is accompanied by a complex pattern of altered lipoprotein metabolism and structure. The key changes include oxidative modification of LDL-C, which accelerates the development and progression of atherosclerosis. [9] It was well established that HDL-C protects against LDL-C induced oxidative modification and thereby prevents progression of atherosclerosis. The anti oxidant capacity of HDL-C depends on the enzymes associated with it, particularly PON.[10]

Our current findings have shown that serum arylesterase activity was lower in CRF (both on conservative management and on hemodialysis) than in the control group ($p <0.01$). In addition, the serum levels of HDL-C and Apo A were also lower in CRF group than in the control group ($p <0.05$). Low concentrations of HDL-C, Apo A and Arylesterase activity increase the susceptibility of LDL-C against oxidative modification and consequently atherosclerotic coronary heart disease.[11, 12]

HDL –C standardized enzyme activities (HDL-C/PON Arylesterase activity) were lower in CRF, $p <0.05$(Both on conservative management and on hemodialysis) (Table I). Similarly a significant positive correlation was determined between serum Basal PON activity, salt stimulated PON activity and HDL-C, Apo A levels in the CRF group on conservative management ($p<0.01$) fig 1,2. These data

suggest that PON activity changes are not entirely dependent on HDL-C concentration in the CRF patients. [13]

During renal failure, the accumulation of nitrogen derived products could decrease the paraoxonase activity by modifying the synthesis or secretion or by directly inhibiting the enzyme.[10] A reduction in serum PON activity as seen in the study, may increase the oxidative stress and makes not only LDL-C susceptible to oxidation but all other serum lipids, including HDL-C. HDL-C oxidation may impair its ability to induce cellular cholesterol efflux from macrophages. Hence, rather than the absolute levels of HDL-C, the quality of HDL-C is maintained by its protective enzymes eg. PON is altered in chronic renal failure. [14, 15, 16]

Our study demonstrated that majority of the patients in the CRF group belong to AA phenotype which has a lower paraoxonase activity. This may be an important reason for the increasing incidence of atherosclerotic coronary artery disease in the population.

Conclusion

The serum HDL-C level does not reflect and assess HDL functional property. Even with a normal level of HDL-C, a measure of anti oxidant capacity of HDL-C will yield additional information, which improves the predictive accuracy of atherosclerotic coronary artery disease in chronic renal failure. It may also provide new strategies for the prevention and treatment of accelerated atherosclerosis in chronic renal failure.

Conflict of Interest: None

References

1. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmacol 1998; 31**:** 329-336

2. Mackness MI, Durrington PN. HDL, its enzymes and its potential to influence lipid peroxidation. Atherosclerosis 1995; 115: 243-53

3. Saeed SA, Elsharkawy M, Elsaeed K, Fooda O. Paraoxonase-1 (PON1) activity as a risk factor for atherosclerosis in chronic renal failure patients. Hemodial Int 2008; 12(4): 471-9

4. Daniel E. Weiner, Hocine Tighiouart, Manish G. Amin et al. Chronic Kidney Disease as a Risk Factor for Cardiovascular Disease and All-Cause Mortality: A Pooled Analysis of Community-Based Studies. J Am Soc Nephrol 2004; 15: 1307–1315

5. Alan S. Go, Glenn M. Chertow, Dongjie Fan, Charles E. McCulloch, and Chi-yuan Hsu. Chronic Kidney Disease and the Risks of Death,Cardiovascular Events, and Hospitalization.. N Engl J Med 2004; 351:1296-305

6. Paragh G, Asztalos L, Seres I, et al. Serum paraoxonase activity changes in uremic and kidney-transplanted patients. Nephron 1999; 83(2):126-31

7. Dubravka Juretiæ, Milena Tadijanoviæ, Branka Rekiæ, Vera Simeon-Rudolf, Elsa Reiner, Martin Barièiæ . Serum Paraoxonase Activities in Hemodialyzed Uremic Patients: Cohort Study. CMJ 2001; 42: 146-150

8. Eckerson HW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet. 1983; 35(2):214–227

9. Thomas Quaschning, Vera Krane, Thomas Metzger, and Christoph Wanner. Abnormalities in Uremic Lipoprotein Metabolism and Its Impact on Cardiovascular Disease. Am J Kidney Dis 2001; 38 (4), 1: S14-S19

10. Thierry F. Dantoine, Jean Debord, Jean -Pierre Charmes, et al. Decrease of Serum Paraoxonase Activity in Chronic Renal Failure. J Am Soc Nephrol 1998; 9: 2082-2088

11. Paragh G, Sers I, Balogh Z, Varga Z, Kârpâti I, Mâtyus J, et al. The serum paraoxonase activity patients with chronic renal failure and hyperlipidemia. Nephron 1998; 80:166-170

12. Witztum JL, Daniel S. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1995; 88:1785-1792

13. Emin T Elkiran, Nefsal Mar, Bilge Aygen, Ferit Gursu, Aziz Karaoglu ,Suleyman Koca . Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC Cancer 2007; 7: 48

14. Aviram M, Rosenblat M, Bisgaier CL, Newton RS,Primo-Parmo SL, La Du BN: Paraoxonase inhibits highdensity lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101: 1581-1590

15. Morena M, Cristol JP, Dantoine T, Carbonneau MA, 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

16. Suguru Yamamoto ,Valentina Kon**.** Mechanisms for increased cardiovascular disease in chronic kidney dysfunction. Curr Opin Nephrol Hypertens. 2009; 18(3): 181–188