ORIGINAL RESEARCH PAPER

Biochemistry

EXPLORING OF SERUM PON 1 ACTIVITY AND ITS ASSOCIATION WITH HDL IN PRE AND POST DIALYSIS CRF PATIENTS : A COMPARATIVE STUDY

KEY WORDS:

Dr. Shilpa N. Chitlange* Assistant Professor, Department of Biochemistry, Govt. Medical College, Akola *Corresponding Author

Dr. Kumud N Harley Associate Professor, Department of Biochemistry, Govt. Medical College, Akola

Dr. Shamali A Jungare Associate Professor, Department of Biochemistry, Govt. Medical College, Akola

ABSTRACT

Background Dyslipidemia is frequently have lipoprotein abnormalities such as low HDL-C concentrations, increased remnant particles and hypertriglyceridemia. HDL-C concentrations are inversely correlated with atherogenic risk. PON-1 activity was lower in patients with renal insufficiency

Aim & Objectives

1) To evaluate activity of PON-1 activity in patients with CRF during pre- and post- hemodialysis sessions
2) To establish interrelationships if any, between PON-1, blood urea and serum creatinine levels.
3) To identify the association of PON -1 with serum HDL levels among CRF patients

Materials And Methods Hospital based case-control study consists of 40 patients who were receiving Hemodialysis (HD) therapy in the dialysis unit. Study designed to evaluate alterations in serum PON -1 activity in patients during pre and post dialysis sessions in CRF. **Result** About 17% increase in the activities of PON-1 have been evident during post-hemodialysis as compared to pre-hemodialysis samples of patients with CRF. The mean activity of PON-1 and HDL were decreased in pre-hemodialysis samples of patients with CRF. PON-1 activity and HDL was increased in CRF patients during post-hemodialysis as compared to pre-hemodialysis sessions. In both, the results were statistically highly significant ($p < 0.001$). There is a positive correaltion between HDL and PON 1. **Conclusion** This study demonstrates that there is an increased risk of cardiovascular complications in patients undergoing hemodialysis. CRF patients undergoing hemodialysis show an increased generation of reactive oxygen species accompanied by an altered lipid profile and paraoxonase-1.

INTRODUCTION

PON-1 (paraoxanase-1) is a 355 amino acid glycoprotein with a molecular weight of 43 kD, synthesized in the liver and secreted into the blood, where it associates with HDL. It is a member of a three gene family consisting of PON-1, PON-2 and PON-3 located on human chromosome 7.[2] PON-1 belongs to the class of hydrolases with one of the broadest known substrate specificities. In circulation it is associated with high density lipoprotein (HDL)[1] . PON1 is involved in the detoxification of organophosphate in some organophosphates (OPs) and its polymorphisms influence enzyme activity and quantity[3] it is serum protein, the activity of which is related to susceptibility to cardiovascular disease and intoxication by organophosphorus (OP) compounds.

Among the non-traditional risk factors, serum paraoxonase (Arylesterase) activity is an important one . Decreased serum arylesterase activity, catalyzed by the high density lipoprotein – associated (PON) 1, is associated with increased oxidant stress and atherosclerosis risk.[7]

Pon-1 Status:

In addition to genetic polymorphisms, PON-1 level can be modified by acquired factors like diet, lifestyle and disease.

Pon-1 And HDL

HDL is the serum vector for PON-1 and is likely to be an important determinant of enzyme concentration as the PON-1 levels are reduced in HDL deficiency syndromes. PON-1 tends to bind to larger sized species of HDL both in vivo and in vitro. Hence in disease like diabetes where HDL size is often reduced, PON-1 secretion is affected.

Mechanism of PON-1 binding to HDL

PON-1 is unusual in retaining its N-terminal hydrophobic signal sequence upon secretion from the cell. An N-terminal PON-1 mutant in which the signal sequence is removed before secretion of the protein was unable to bind to HDL. This shows that PON-1 binds to HDL via its hydrophobic N-terminal signal sequence.

A secondary structure prediction of the N-terminal signal sequence shows that the entire region is compatible with a trans-membrane helix. The majority of the N-terminal is disordered and invisible in the tertiary structure, but its hydrophilic part forms a helix. The hydrophobic residues of a second helix, adjacent to the N-terminal helix are oriented towards the solvent as a number of hydrophobic residues in the loops that connect the helix to the main PON-1 structure. These regions provide adjacent hydrophobic areas which would allow PON-1 to bind to membranes or lipoproteins. The potential interface with HDL has an "aromatic belt" rich in tryptophan and tyrosine residues which have been described in a number of membrane binding proteins.[1]

PON-1 may therefore have access to the interstitium and areas of LDL accumulation and oxidative damage where it could protect against adverse effects of oxidation

Paraoxonase-1 in Chronic Renal Failure[4]

Dyslipidemia is frequently have lipoprotein abnormalities such as low HDL-C concentrations, increased remnant particles and hypertriglyceridemia. HDL-C concentrations are inversely correlated with atherogenic risk. PON-1 activity was lower in patients with renal insufficiency (CRF; chronic hemodialysis; chronic peritoneal dialysis) than in control subjects. Renal transplantation seems to restore PON-1 activity.

OBJECTIVES

1) To evaluate activity of PON-1 activity in patients with CRF during pre- and post- hemodialysis sessions and in healthy controls and
2) To establish interrelationships if any, between PON-1,

blood urea and serum creatinine levels.
3) To identify the association of PON -1 with serum HDL levels among CRF patients.

MATERIALS AND METHODS

Study Type: Hospital based case-control study

Study design: Study designed to evaluate alterations in serum PON -1 activity in patients during pre and post dialysis sessions in CRF and their comparision with healthy controls

Study Site: Nephrology Unit, S.S.Institute of Medical Sciences & Research Centre, Davangere

Sample population size: 40 clinically diagnosed CRF patients and 40 healthy controls

Selection criteria:

Inclusion Criteria:

1. Clinically diagnosed CRF cases with age group between 20-60 years
2. An equal number of age and sex matched healthy controls

Exclusion criteria:

The following patients were excluded from the study.
Patients with

1. Liver diseases
2. Infectious diseases
3. Familial hyperlipoproteinemia
4. Malignancies
5. Hypolipidemic drugs.

Sample Collection:

Study commenced after obtaining ethical approval from Institute ethical committe and receiving informed and written consent in regional language from all the study & controls were obtained subjects. Approximately 5 ml of blood sample was collected in a sterile vacutainer from CRF patients during pre- and post- hemodialysis sessions. Serum was separated by centrifugation and used for analysis of Paraoxonase -1 by spectrophotometric method.

Statistical Analysis

Data was entered in SPSS and expressed as mean ± SD and range values. Student's unpaired't'-test was used for comparing the means of two groups (cases & controls) & student's paired 't'test was used for comparing the means of cases (pre-hemodialysis & post-hemodialysis). For all the tests, a p-value of 0.05 or less was considered as statistical significance

Regression analysis was done for exploring association between HDL & PON 1 in CRF and serum HDL concentrated estimated in healthy controls and serum patients respectively.

Following results were observed.

5. RESULTS

In the present study the results of PON-1 were analyzed in a total number of 80 subjects, which included:

1) Healthy controls -40
2) Patients with CRF undergoing hemodialysis (Pre- and Post-hemodialysis sessions) – 40 subjects

Table – 1: Gender wise Distribution of Study Population

Gender	CRF Cases (n=40)	Controls(n=40)
Males	5	16
Females	35	24

Table –2 Shows Age Wise Distribution of Study Population

	CRF Cases(n=40)		Controls (n=40)	
Age (Years)	No.	%	No.	%
22-34	10	25	8	20
35-44	11	27.5	12	30
45-54	8	20	10	25
55-64	9	22.5	7	17.5
65-74	2	5	3	7.5
Total	40		40	
Mean ± SD	43.6 ± 12.4		44.6 ± 11.8	
p* value,sig	0.69 NS			
*Student's unpaired t test				

Table 2 shows age wise distribution of study population. (n=40) healthy controls and 40 CRF cases on hemodialysis. Among the cases, the incidence of hemodialysis was higher in the age group of 35-44 years. Average mean age was 44.6 ± 11.8 (years) in controls and 43.6 ±12.4 years in cases respectively which was not significant (NS)

Table-3 Comparison Of Biochemical Parameters Among Crf Predialysis Cases And Controls

	Healthy Controls	Pre-Hemodialysis		
Biochemical Parameter	Mean ± SD	Mean ± SD	Mean Difference	p* Value, sig
Blood Urea mg/dL	28.50 ± 7.60	69.50 ±16.50	40.90	0.001 HS
Serum Creatinine mg/dL	1.31 ± 0.30	7.60 ± 2.80	6.37	0.001 HS
PON -1 nmol/mL/ min	63.0 0 ±5.80	24.60 ± 4.70	36.24	0.001 HS
HDL-C mg/dL	44.50 ± 3.20	41.20 ± 0.50	0.93	0.268 NS

Students unpaired't' test; p <0.001HS- highly significant

Table 3 shows significantly low PON1 as compared to pre hemodialysis (P0.001 HS) With mean ±SD 24.60±4.70 in CRF cases and controls mean ±SD 63.0 0 ± 5.80 respectively. The mean concentrations of blood urea, serum creatinine, serum PON-1 and HDL-C were in the range of 28.50 ± 7.60,1.31 ± 0.30, 63.0 ± 5.80 ,44.50±3.20 respectively in controls and 69.50 ± 16.50,7.60 ± 2.80, 24.60 ± 4.70, 41.20±0.50 respectively in cases. Statistical analysis by unpaired 't'- test showed that the mean values of blood urea, serum creatinine, were increased in cases and were statistically highly significant (p<0.001) when compared to controls. PON-1 levels were decreased in case compared to controls. HDL-C was nothing significant.

Table 4 Comparison Of Biochemical Parameters Among Crf Post Dialysis Cases And Controls

	Healthy Controls	Post-Hemodialysis		
Parameter	Mean ± SD	Mean ± SD	Mean Difference	p* Value, sig
blood urea mg/dL	28.50 ± 7.60	29.75 ± 9.71	1.19	0.54 NS
serum creatinine mg/ dL	1.31 ± 0.30	3.68 ± 1.30	3.16	0.002 S
PON -1 nmol/mL/min	63.00 ± 5.80	29.50 ± 4.90	33.50	0.001 HS
HDL-C mg/dL	44.50 ± 3.20	48.40 ± 3.20	4.47	0.001 HS

Student's unpaired't' test; p < 0.002S- significant; p >0.54NS- not significant; p <0.001 HS-Highly significant

Table 4 shows comparison of blood urea, serum creatinine, serum PON-1 activity & HDL-C levels between controls and in cases of CRF during post-hemodialysis session. The mean concentrations of blood urea, serum creatinine, serum PON-1

and HDL-C were in the range of 28.50 ± 7.60, 1.31 ± 0.30, 63.0 ± 5.80, 44.50 ± 3.20 respectively in controls and 29.75 ± 9.71, 3.68 ± 1.30, 29.50 ± 4.90, 48.40 ± 3.20 respectively in cases. Statistical analysis by unpaired 't'- test showed that the mean values of PON-1 levels were increased in cases and were statistically highly significant (p<0.001) when compared to controls.

Blood urea was not statistically significant between controls and cases and serum creatinine was statistically significant between controls and cases (p<0.002)

Table -5 Comparison Of Biochemical Parameters Among Crf Pre Dialysis Post Dialysis

	Pre-hemodialysis	Post-hemodialysis		
Parameter	Mean ± SD	Mean ± SD	Mean Difference	p* Value, sig
blood urea mg/dL	69.50 ± 16.50	29.75 ± 9.71	39.75	0.001 HS
serum creatinine mg/dL	7.60 ± 2.80	3.68 ± 1.30	3.92	0.001HS
PON -1 nmol/mL/min	24.60 ± 4.70	29.50 ± 4.90	4.9	0.001HS
HDL-C mg/dL	41.20 ± 0.50	48.40 ± 3.20	7.20	0.001 HS

Student's paired't' test ; p<0.001 HS-Highly significant; p>0.009S-Significant

Table 5 shows comparison of concentrations of blood urea, serum creatinine, PON-1 activity levels among the cases of CRF during pre- hemodialysis and post- hemodialysis sessions. The mean concentrations of blood urea, serum creatinine, serum PON-1& HDL-C were in the range of 69.50 ± 16.50, 7.60 ± 2.80, 24.60 ± 4.70, 41.20 ± 0.50 respectively during pre- hemodialysis and 29.75 ± 9.71, 3.68 ± 1.30, 29.50 ± 4.90, 48.40 ± 3.20, respectively during post-hemodialysis session. Statistical analysis by paired 't'-test showed that the mean values of blood urea, serum creatinine decreases during post- hemodialysis session which was statistically highly significant (p<0.001).

Serum PON-1activity concentrations were increased during post- hemodialysis and were statistically significant when compared to pre- hemodialysis session.

Table -6 Shows Karl Pearson's Coefficient (r) Of Correlation Of Pon-1 With Hdl-c, Blood Urea, Serum Creatinine In Cases Of Crf During Pre- Hemodialysis Session.

Correlation with PON-1	Correlation coefficient*	p value
HDL-C	0.965	0.01S
Blood Urea	-0.683	0.01S
Serum Creatinine	-0.780	0.01S
*Karl pearson's coefficient of correlation		

Table 6 shows the Karl pearson's correlation of PON-1with HDL-C, blood urea, serum creatinine in cases of CRF during pre- hemodialysis session.

It is evident from the table that there is a positive correlation between HDL and PON-1, and negative correlation between PON-1and blood urea and serum creatinine. As the activity of PON-1 decreases, concentration of HDL-C also decreases and that of blood urea and serum creatinine increases.

Table 7 Shows Karl Pearson's Coefficient Of Correlation Of Pon-1 With Hdl, Blood Urea, Serum Creatinine In Cases Of Crf During Post-hemodailysis Session.

Correlation with PON-1	Correlation coefficient*	p value
HDL	0.464	0.01S
Blood urea	-0.855	0.01S
Serum creatinine	-0.911	0.01S

* Karl pearson's coefficient of correlation

Table 7 shows the Karl pearson's Coefficient of correlation of PON-1with HDL-C, blood urea, serum creatinine in cases of CRF during post- hemodialysis session.

It is evident from the table that there is a positive correlation between HDL-C and PON-1 and negative correlation between PON-1and blood urea and serum creatinine. As the activity of PON-1 increases, the HDL-C concentration increases and that of blood urea and serum creatinine decreases during post-hemodialysis session.

DISCUSSION

PON-1 is an esterase associated with apolipoprotein AI and clusterin (apolipoprotein J) in HDL. PON-1 displays paraoxonase and arylesterase activities since it hydrolyzes organophosphate compounds such as paraoxon and aromatic carboxylic acid esters such as phenylacetate.[4]

It has been shown to prevent atherosclerosis by inhibiting oxidation of LDL. Cardiovascular disease is the main cause of mortality and morbidity in patients with CRF and undergoing HD. In this study, the mean activity of PON-1 was lower in both during pre and post dialysis session as compared to apparently healthy controls.

The mean activity of paraoxonase-1 in controls and in patients with CRF during pre- and post-hemodialysis sessions were in the range of 63 ± 5.80 nmol/mL/min, 24.60 ± 4.7 nmol/mL/min and 29.50 ± 4.90 nmol/mL/min, respectively. About 17% increase in the activities of PON-1 have been evident during post-hemodialysis as compared to pre-hemodialysis samples of patients with CRF. The mean activity of PON-1were decreased in pre-hemodialysis samples of patients with CRF as compared to healthy controls. PON-1 activity was increased in CRF patients during post-hemodialysis as compared to pre-hemodialysis sessions. In both, the results were statistically highly significant (p< 0.001). This is in accordance with the studies of Tadashi suehiro, et al[6] and Thierry F Dantoine, et al[7].

PON-1 possesses peroxidase- like activity that can contribute to its protective effect against lipoprotein oxidation.[4]

As we noticed, paraoxonase activity decreased with advancing age in (table 1) control subjects. Considering the suspected protective role of paraoxonase in atherosclerosis it is noted that aging is accompanied by reduction in enzyme activity.

In CRF patients, an increased effect of oxidative stress on LDL lipoproteins in vitro has been reported and it has been noticed in patients with familial hypercholesterolemia, diabetes mellitus, or coronary heart disease. This work sustains the relationship between low paraoxonase activity and diseases with low antioxidant defense and excessive lipid peroxidation. Paraoxonase may play its protective role in atherogenesis by hydrolyzing some products of lipid peroxidation and consequently by limiting LDL oxidation and foam cell synthesis. This allows us to hypothesize that during uremia, loss of that protection could represents a very important factor in atherosclerosis.[7]

The decrease in PON-1 activity observed in this study could be the result of lower HDL-C concentrations in CRF given that HDL is the main serum carrier of PON-1.

PON-1 inhibit the oxidative modifications of LDL during

copper oxidation in vitro, possibly by destroying active phospholipids in minimally oxidized LDL. Serum PON-1 activity is inversely related to the risk of developing atherosclerotic lesion which contains cholesterol loaded macrophage foam cells. Hemodialysis seems to be effective also in raising serum PON-1 activity of the patients. PON-1 present in serum is located on HDL, being tightly bound to a HDL subfraction containing apo A-I and clusterin.

In CRF, the concentration of middle size and low molecular weight plasma Advanced Glycation End Products (AGE) are highly elevated. These AGE residues are formed on long and short lived proteins. Due to low molecular weight, AGE free adducts are easily excreted through the urine. Hence, it acts a good renal clearance tool which distinctly declines in CRF adduct. Retention of AGE free adducts could play a role in decreasing PON-1 activity.

During hemodialysis procedure, AGE free adducts may be removed, along with the uremic toxins urea and creatinine. Thus, the inhibition of PON 1 may be removed. This may result in elevation of PON-1 activity after successful hemodialysis process.

Acrolein and α- β unsaturated aldehyde is highly elevated in CRF. PON-1 contains 2 critical cysteine residues in its catalytic hydrophobic pocket. Acrolein may be partially removed by hemodialysis and due to this the level of PON-1 may be elevated after dialysis process.[8]

In present study, we have observed the inverse correlation between PON1 and HDL-C as seen in table 4 and 5.

CONCLUSION

This study demonstrates that there is an increased risk of cardiovascular complications in patients undergoing hemodialysis. CRF patients undergoing hemodialysis show an increased generation of reactive oxygen species accompanied by an altered blood urea and serum creatinine & Paraoxonase-1. PON1 activity was significantly decreased in CRF patients before HD and significantly increased after HD but remained lower than control. Hence, this study suggests the need for assessing the malondialdehyde as a biomarker of degree of oxidative stress which can be controlled by intiating the antioxidant therapy in patients with CRF undergoing hemodialysis.

Strength And Further Scope Of The Study:

In this study, the patient's condition was improved after the dialysis session and it can be further improved by supplementation of antioxidants which can reduce oxidative stress. Hence, the dialysis session is highly beneficial as it reduces the complications and helps in improving the deteriorating conditions in CRF patients.

Limitations Of Study :

1. This study has not included the antioxidant levels to know about their antioxidant status in patients with CRF.
2. Further, this study has not taken into consideration the effects of use of two membranes such as cuprophane and polysulfone membrane as they result in different altered antioxidant status in these patients.
3. This study could not categorize the patients on the basis of number of times of dialysis sessions they have undergone.

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