SERUM PARAOXONASE ACTIVITY IN CHRONIC KIDNEY DISEASE

K. Geetha¹, K. Pramila², K. Chelladurai³

ABSTRACT

Background: Kidney disease is both a cause and consequence of cardiovascular vascular disease (CVD). All CKD patients irrespective of the underlying etiology are at increased risk of CVD. From the mortality standpoint, it is not progression to kidney failure is more concerning in CKD patients, but progression to CVD.¹ Thus a focus of patient care in CKD should be directed to the prevention of cardiovascular complications, in which atherosclerotic narrowing of vessels plays a major role.

Uremia induced atherogenetic alterations probably start to take place much earlier in the course of CKD³,⁴ and they suffer from a secondary form of the complex dyslipidemia consisting of both qualitative and quantitative abnormalities in serum lipoproteins resulting from alteration in lipoprotein metabolism and composition.⁵

In the uremic dyslipidemia, there is an increase in serum triglycerides, altered cell surface low-density lipoprotein (LDL) epitope and recognition, increased LDL susceptibility to oxidation, and impaired LDL-receptor mediated clearance from plasma. But high-density lipoprotein (HDL) levels decreases with depression in its antioxidant and anti-inflammatory functions due to impaired maturation.⁶ Thus, there is an impaired clearance and accumulation of oxidation prone LDL and abnormal LDL composition leading to oxidative stress and inflammation favoring their uptake by macrophages and resident cells in the artery wall.⁷ While LDL particles undergo vicious cycle of accumulation and modification, reverse cholesterol transport is also impaired due to low lecithin-cholesterol acyl transferase and paraoxonase (PON) activity.⁸ Thus in CKD, there is an accelerated atherogenesis, also due to the effect of heightened influx of lipids compounded by impaired HDL-mediated reverse cholesterol transport leading to foam cell formation which is the central event in atherosclerosis plaque formation 33. The ability of HDL to inhibit the oxidation of LDL and promote macrophage cholesterol efflux is also through the action of several of its associated proteins, particularly

INTRODUCTION

Chronic kidney disease (CKD) is a global threat to the health in general and for developing countries in particular because therapy is expensive and life long.⁹ CKD with a gradual onset, progresses inexorably to a critical state-end stage renal disease. Kidney disease is both a cause and consequence of cardiovascular

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PON I which reduces the inflammation associated with atherosclerosis.\[8\]

The role of PON in the development of CVD has drawn considerable attention in recent years.\[9,10\] PON, an aryl dialkyl phosphatase (E.C. 3.1.8.1), is a multifunctional antioxidant enzyme component of HDL, can protect LDL against oxidation\[11\] and inhibits monocyte endothelial interactions in the inflammatory response of vascular endothelial cells.\[12,13\] PON is a serum aryl esterase that was initially identified by its hydrolysis of aromatic carboxylic esters and organophosphorus insecticides and nerve agents.\[14,15\] Its name reflects its ability to hydrolyze paraaxon, a metabolite of the insecticide parathion.

There are three members in the PON gene family. PON I is the most studied of all the members of PON family. It is a glycoprotein with a molecular weight of 38 kDa and 354 amino acids encoded by the PON I gene, which maps to the human chromosome 7q, 21-22.50. It is synthesized in the liver and transported along with HDL in the plasma. PON I is transported primarily on apolipoprotein A-I containing HDL.\[15\] Its serum concentration is influenced by the inflammatory changes.\[16\]

PON I hydrolyzes the toxic oxon metabolites of a number of organophosphorus insecticides and degrades bioactive phospholipids such as platelet activating factor, thereby preventing intravascular coagulation.\[17\]

PON protects lipids in lipoprotein, in macrophages and in erythrocytes from oxidation\[18\] and increases the HDL binding to macrophages which in turn stimulates HDL's ability to promote cholesterol efflux and also inhibits cholesterol biosynthesis.\[18,17,19\] PON was shown to increase the breakdown of specific oxidized lipids in oxidized LDL (ox-LDL) and decrease the macrophage uptake of ox-LDL.\[18\] It attenuates the ox-LDL induced monocyte chemoattractant protein-I production by endothelial cells.\[20\]

The inactivation of PON I reduces the ability of HDL to inhibit LDL modifications and monocyte endothelial interactions. Since both these mechanisms are important in the inflammatory response of arterial wall cells, atherogenesis is favored.\[21,22\]

**MATERIALS AND METHODS**

This case control study was done after obtaining the approval from institutional ethical committee.

Cases: 50 patients with CKD.

Controls: 50 age and sex matched healthy subjects with normal clinical and biochemical parameters.

Inclusion criteria: Patients with CKD.

Exclusion criteria: Patients with liver disease, chronic infections and diabetes mellitus.

Fasting venous blood sample was collected with strict aseptic precautions.

PON activity, total cholesterol, HDL cholesterol, triacylglycerol (TAG), creatinine, urea, glucose and albumin were estimated. LDL cholesterol and very low-density lipoprotein (VLDL) cholesterol levels were calculated.

**Estimation of serum PON activity**

PON activity was estimated spectrophotometrically using paraaxon (0,0-diethyl-0-4-nitro phenylphosphate) as the substrate for hydrolysis. Serum PON, hydrolyzes paraaxon in the presence of calcium at pH 8.0 at 25°C and releases para-nitrophenol (p-NP). The liberated p-NP is measured, and the activity of PON can be calculated using the molar absorption of p-NP in a kinetic assay. The absorbance was monitored at 405 nm. One unit of PON activity is defined as 1 μmol of p-NP formed per min per litre at 25°C, and the activity was expressed as U/L of serum.\[13\]

The Student's t-test was used to compare the serum PON activity between the two groups.

**RESULTS**

The mean value of the serum PON activity in the study group (46.47 ± 24.45 U/L) showed a significant fall of 60.4% compared with the mean value of the control group (117.38 ± 30.62 U/L); P ≤ 0.001 [Table 1]. The mean values of serum PON activity between the control and study groups in different age groups (15-35, 36-55 and 56-75 years) were compared. All of them showed a significant decrease in PON activity in the study group (P ≤ 0.001), [Table 2]. Sex distribution among the cases and controls are shown in the Table 3. The sex matched comparison of PON activity between the two groups also showed a significant decrease in PON activity in the study group [Table 4].
Table 1: Comparison of test parameters between study group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Paraoxonase U/L</td>
<td>117.38</td>
<td>30.62</td>
<td>46.47</td>
</tr>
<tr>
<td>Urea mg/dL</td>
<td>29.34</td>
<td>6.74</td>
<td>94.32</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>0.80</td>
<td>0.15</td>
<td>7.49</td>
</tr>
<tr>
<td>Total cholesterol mg/dL</td>
<td>193.68</td>
<td>40.84</td>
<td>197.46</td>
</tr>
<tr>
<td>HDL mg/dL</td>
<td>45.30</td>
<td>16.52</td>
<td>30.80</td>
</tr>
<tr>
<td>LDL mg/dL</td>
<td>119.06</td>
<td>33.83</td>
<td>126.36</td>
</tr>
<tr>
<td>VLDL mg/dL</td>
<td>26.42</td>
<td>6.76</td>
<td>41.20</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>132.28</td>
<td>33.69</td>
<td>207.44</td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td>3.85</td>
<td>0.55</td>
<td>3.49</td>
</tr>
</tbody>
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*Significant, NS: Not significant, HDL: High density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, TAG: Triglyceride, SD: Standard deviation

There is a highly significant difference between the mean values of HDL cholesterol in the study group (30.80 ± 6.96 mg/dL) and the control group (45.30 ± 16.52 mg/dL). The HDL level is significantly decreased in the study group. P ≤ 0.001. The comparison of the mean values of TAG and VLDL cholesterol showed a significant difference, with increased levels in the study group (P ≤ 0.001), while the values of LDL cholesterol and total cholesterol in the study group and the control group showed no significant difference. The levels of these parameters between the two groups are associated with the secondary dyslipidemic changes in CKD.

Among the other parameters measured, urea and creatinine levels are significantly increased with the P < 0.001, while albumin levels are decreased in the study group. The levels of urea, creatinine, total cholesterol, VLDL and TAG are negatively correlating with the PON activity [Table 5].

The receiver operating characteristic curve of PON activity [Figure 2 and Table 6] revealed an area under the curve of 0.955 and a standard error of 0.018, showing as asymptotic significance of 0.001, proving it as a good early predictor in diagnosing cardiovascular events in CKD.

**DISCUSSION**

This study shows the involvement of PON in the LDL peroxidation and presents a potential explanation of accelerated atherosclerosis in CKD. A significant
decrease in PON activity and its antiatherogenic properties in CKD could be an important factor in premature vascular aging.

PON 1 is inactivated under oxidative stress and its activity may also be altered as part of the inflammatory response, which is evident by the fact that the HDL became proinflammatory during the acute phase response, possibly due to the loss of PON 1 activity from HDL. The inactivation of PON 1 reduces the ability of HDL to inhibit LDL modifications and monocyte endothelial interactions. PON is also involved in the hydrolysis of homocysteine thiolactone into homocysteine (thiolactonase activity). Thus, decrease in PON activity may initiate a positive feedback mechanism causing further accumulation of homocysteine thiolactone, which can damage protein by homocysteinylation of the lysine residues, which are auto-immunogenic and prothrombotic leading to atherosclerosis. Thus, PON 1 confers protection against coronary artery disease by inactivating and removing the pro-inflammatory lipid oxidation products from the carotid and coronary plaques.

Uremic environment in CKD, depresses PON activity, which may be due to decreased HDL concentration and also through the post-translational modification of PON, as a result of the interaction with advanced glycation end products and urea derived cyanate. Due to the increased production of toxic free radicals, the efficiency to hydrolyze lipid peroxides by PON is decreased, which further decreases its specific activity. The decreased levels of PON, and thus reduction of its antiatherogenic activity in CKD could be an essential factor of premature vascular aging leading to cardiovascular complications.

Nutritional antioxidants such as polyphenols, increase PON 1 messenger RNA expression and activity by an aryl hydrocarbon receptor dependent mechanism. PON 1 levels are influenced by a variety of environmental factors. In human, consumption of degraded cooking oil lowered the PON 1 level, while alcohol and vitamin C and E elevated it. Thus by determining the PON activity earlier in CKD patients, interventions can be made to increase the PONs by dietary or pharmacological means.

CONCLUSION
The introduction of PON is a welcome event and based on the results obtained, the present study supports the previous studies that PON is a useful marker for the early diagnosis of atherosclerosis in CKD patients. Thus the estimation of PON activity in CKD patients is being valuable in predicting atherosclerosis and of future cardiovascular events.

Limitations of the study
- The study should have included the CKD patients on conservative management, hemodialysis and those who had undergone renal transplantation
- Dietary determinants of serum PON activity in human had not been taken into account
- The study can be done in a large population and could have been better if combined with PON polymorphisms.
REFERENCES


