Introduction
Paraoxonase (aryl dialkyl phosphatase, EC 3.1.8.1) is a calcium dependent polymorphic enzyme with three activities, paraoxonase, arylesterase and diazoxonase. 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 hydrophobic N-terminal domain to apo A1 of HDL-C. Biochemical studies have shown that antioxidant activity of HDL-C is shown to reside in its enzymes, particularly paraoxonase and hence a major contributor to its antiatherogenic role.

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). Accelerated atherosclerosis and altered lipoprotein metabolism is responsible for increased cardiovascular events and cardiac death in chronic renal failure. 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.

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. The phenotypic distribution of PON can be used to subclass the population into three groups AA represents low, AB intermediate and BB high enzyme activity. Arylesterase activity is a measure of PON activity, in which p-nitrophenyl 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
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 [60-89 mL/min/1.73m²] 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 [30-59 mL/min/1.73m²]

**Stage 4 CKD**

Severe reduction in GFR [15-29 mL/min/1.73m²]

**Stage 5 CKD**

Kidney failure (GFR <15mL/min/1.73m² 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. 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 mol/ 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

\[
\text{SALT STIMULATED PON ACTIVITY - BASAL PON ACTIVITY} \times 100\%
\]

Individuals were classified for PON phenotype using the antimode of PON as proposed by Eckerson et al. 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’s 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, arylerase activity was standardized for HDL cholesterol concentrations. It was found that HDL Standardized arylerase activity was significantly reduced in the CRF patients than in the control subjects.

Table-I

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

* Student t test, ** Significant

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. 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.

Our current findings have shown that serum arylerase 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.

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. 

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. 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.

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