SERUM PARAOXONASE ACTIVITY IN THE CHRONIC KIDNEY DISEASE

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CERTIFICATE

Certified that, following is the bonafide work done by **Dr. K.GEETHA, M.D,** (Biochemistry), Stanley Medical College, Chennai, on the title **SERUM PARAOXONASE ACTIVITY IN THE CHRONIC KIDNEY DISEASE,** as part of her dissertation during the year **2008-2011.**

DEAN

Professor and Head of
the Department.

DECLARATION

I, **Dr. K.GEETHA,** solemnly declare that the dissertation titled **SERUM PARAOXONASE ACTIVITY IN THE CHRONIC KIDNEY DISEASE,** is a bonafide work done by me at Government Stanley Medical College and Hospital, Chennai during the period from March 2010 to September 2010 under the guidance of **Dr.K. PRAMILA, MD** Professor and H.O.D, Department of Biochemistry, Government Stanley Medical College and Hospital Chennai.

This dissertation is submitted to the Tamil Nadu Dr. MGR Medical University towards partial fulfillment of requirement for the award of M.D. Degree in Biochemistry (Branch XIII).

Dr. K. GEETHA

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CONTENTS

ABBREVIATIONS

1. CKD - CHRONIC KIDNEY DISEASE
2. CVD - CARDIOVASCULAR DISEASE
3. PON - PARAOXONASE
4. HDL - HIGH DENSITY LIPOPROTEIN
5. LDL - LOW DENSITY LIPOPROTEIN
6. VLDL - VERY LOW DENSITY LIPOPROTEIN
7. TAG - TRIACYLGLYCEROL
8. ECM - EXTRACELLULAR MATRIX
9. MCP - MONOCYTE CHEMOATTRACTANT PROTEIN
10. ESRD - END STAGE RENAL DISEASE

INTRODUCTION

Chronic Kidney Disease (CKD) is a global threat to health in general and for developing countries in particular because therapy is expensive and life long.[1]

CKD, encompasses a spectrum of different pathophysiological processes associated with abnormal kidney function and a progressive decline in Glomerular Filtration Rate (GFR).[2]

CKD usually has a gradual onset and in many cases progresses inexorably to a critical state – end stage renal disease (ESRD), in which the patient's survival requires the initiation of renal replacement treatment, either by dialysis or renal transplantation.[3]

The prevalence of CKD is increasing among the different population of the world. According to various studies, the incidence and prevalence of CKD in India is ~ 0.78%.[4] The domiciliary screening programme for CKD in South India has reported the prevalence of CKD (Stage 5) as 870 per million population (pmp)[5].

CKD with its multitude of complications and devastating outcomes leads to a significantly higher risk for Cardiovascular

Disease (CVD) and all cause mortality in an individual [6]. Kidney disease is both a cause and consequence of Cardiovascular Disease. All CKD patients irrespective of the underlying etiology, are at increased risk of CVD including coronary heart disease, cerebrovascular disease, peripheral vascular disease and heart failure. From the mortality stand point, it is not progression to kidney failure is more concerning in CKD patients, but progression to CVD[6].

CVD is the leading cause of morbidity and mortality in patients at every stage of CKD[2]. 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.

Several articles have revealed a fact that decreased levels of High Density Lipoprotein (HDL) & Paraoxonase (PON) activity in CKD patients are being the risk factors for the development of CVD. [7]

PON, an aryl dialkyl phosphatase which reversibly binds & hydrolyzes organophosphates is synthesized in the liver , resides over HDL and has both antioxidant and antiatherogenic functions. The role of PON in the development of CVD has drawn considerable attention in the recent years. [7,8]

PON prevents atherogenesis by protecting the LDL against peroxidation and by inhibiting the monocyte – endothelial interactions in the inflammatory response of vascular endothelial cells. [9,10]

Thus, estimation of PON activity in CKD patients is being valuable in predicting atherosclerosis and of future cardiovascular events. This study aims at establishing the role of PON as an antiatherogenic agent in CKD patients.

AIM & OBJECTIVE

The aim of this study is to determine the Serum PON activity in CKD patients and to compare the results with the healthy control subjects.

The objective of this study is -

to analyze the Serum PON activity and Lipid profile in CKD patients for predicting the atherogenic risk in these patients & to intervene earlier in preventing Cardiovascular complications.

REVIEW OF LITERATURE

The kidneys being the major excretory organs in human with a high degree of structural complexity excrete the waste products of metabolism, precisely regulating the body's concentration of salt and water, maintain the appropriate acid balance of plasma and serve as an endocrine organ, secreting such hormones as erythropoietin, renin and prostaglandin.[11]

Diseases of the kidneys are as complex as its structure. The chronic kidney disease represents the entire spectrum of disease that occurs following the initiation of kidney damage.

DEFINITION OF CKD

According to the National Kidney Foundation :-

CKD is defined as the presence of objective kidney damage and / or the presence of a GFR of 60 ml / min / 1.73 m^2 body surface area or less, for atleast 3 months irrespective of the underlying etiology of the kidney damage. [12]

The severity of the resulting syndrome is denoted by a staging scheme that extends from occult kidney damage, with well-preserved function (Stage 1) down to the level of kidney failure requiring renal replacement therapy (Stage 5). [12]

The stages of CKD are given in the Table - 1

The term ESRD – represents a stage of CKD where the accumulation of toxins, fluid and electrolytes (normally excreted by the kidney) results in uremic syndrome. This expression ESRD is used to represent those subjects receiving or eligible for renal replacement therapy either by some form of dialysis or by transplantation.

EPIDEMIOLOGY

The frequency of CKD continues to increase worldwide[13]. The incidence of CKD has doubled in the last 15 years.[1]

The reported prevalence of CKD Stages 1-4 in the most recent NHANES (National Health and Nutrition Examination Survey) between 1999-2006 was 26 million (13%) out of ~ 200 million United States residents aged 20 and older. Of these 65.3% had CKD Stage 3-4.

The most recent report of the United States Renal Data System estimates that nearly one half million patients in the US were treated for ESRD in the year 2004, and at the end of 2010 ,this figure is expected to increase by ~ 40%.[13]

CKD IN INDIA

In the most representative population based study from North India, using a multistage cluster sampling technique, prevalence of CKD in India is ~785 pmp and the incidence of ESRD is ~ 150 pmp.[4]

The domiciliary screening programme for CKD by a trust in South India has reported the prevalence of CKD Stage 5 to be 0.87 per thousand (870 per million). [5]

ETIOLOGY

CKD may be caused by any condition which destroys the normal structure and function of the kidney. The common causes are

1) Congenital & Inherited
2) Renal artery stenosis
3) Hypertension
4) Glomerular diseases
5) Interstitial diseases
6) Systematic inflammatory diseases
7) Diabetes mellitus.[14]

RISK FACTORS

These factors increase the risk of CKD even in individuals with normal GFR. Among the strongest factors associated with increased risk for CKD are the Diabetes and Hypertension.

Additional Risk Factors are

1) Autoimmune disease
2) Chronic systemic infection
3) Cancer [6]
4) Family History
5) Reduced renal mass
6) Low birth weight
7) Drug exposure
8) Urinary tract infection
9) Structural abnormalities of urinary tract
10) Previous episode of Acute renal failure [2]
11) Older age
12) Ethnicity.

PATHOPHYSIOLOGY OF CKD

The pathophysiology of CKD involves 2 broad sets of mechanisms of damage.

i) **Initiating mechanisms** are specific to the underlying etiology. (eg) Immune complexes and mediators of inflammation in certain types of glomerulonephritis or toxin exposure in certain diseases of renal tubules and interstitium.

ii) A set of **progressive mechanisms**, involving hyper filtration and hypertrophy of the remaining viable nephrons, that are a common consequence following long term reduction of renal mass, irrespective of underlying etiology.

Eventually the short term adaptations of hypertrophy and hyperfiltration become maladaptive as the increased pressure and flow predisposes to sclerosis and drop out of remaining nephrons .[2]

CLINICAL ABNORMALITIES IN CKD

CKD leads to disturbances in the function of virtually every organ system. The disturbances are found in

1. Cardiovascular and pulmonary system.
2. Gastrointestinal system.
3. Haematologic and immunologic functions.
4. Neuromuscular System.
5. Endocrine & metabolic functions.
6. Fluid, Electrolyte & Acid base Balance.
7. Dermatologic manifestation.[15]

Among all these disturbances, the CVD is the leading cause of morbidity and mortality in patients of every stage of CKD.[2]

CARDIOVASCULAR ABNORMALITIES IN CKD

Cardiovascular abnormalities occur almost invariably when renal function deteriorates . Hypertension, congestive heart failure, valvular stenosis or insufficiency and accelerated atheromatosis are among the most frequent epiphenomena. [16]

The early stage of CKD, manifesting as albuminuria and even a minor decrement in GFR, is now recognized as a major risk factor for CVD. Thus most patients with CKD succumb to CVD before reaching stage 5 [2].

RISK FACTORS FOR CARDIOVASCULAR DISEASE IN CKD

The increased prevalence of vascular disease in CKD patient derives from both traditional & non-traditional factors (CKD-related risk factors).

Traditional Factors

- Hypertension
- Hypervolemia
- Dyslipidemia
- Sympathetic overactivity
- Hyperhomocysteinemia
- Diabetes Mellitus

CKD related risk Factors

- Anemia
- Hyperphosphatemia
- Hyperparathyroidism
- Dyslipidemia
- Uremic toxins
- Proteinuria
- Oxidative stress
- Elevated homocysteine
- Generalized inflammation.[17,18]

The inflammatory state associated with a reduction in kidney function is reflected in increased circulating acute phase reactants, and proinflammatory agents such as the cytokines, oxidation products, C-reactive protein and glycation products. These are considered as the main culprits in CVD [16]. This inflammatory state appears to accelerate vascular occlusive disease.

Atheromatosis has been classified as an inflammatory rather than a degenerative disease. In CKD, inflammation and CVD have

been linked to each other. Uremia induced atherogenic alterations probably start to take place much earlier in the course of CKD.[16, 18]

ATHEROSCLEROSIS

Atherosclerosis, the leading contributor to coronary artery disease and cerebrovascular disease is a form of arteriosclerosis in which soft deposits of intra arterial fat and fibrin on the vessel walls harden over time.

Atherosclerosis is not a single disease entity. It can take several forms, depending on the anatomic location, age, genetic and physiologic status and risk factors to which the individual is exposed.[19]

PATHOGENESIS

Atherosclerosis is a complex disease that involves lipoprotein influx and modification, increased pro-oxidant stress and inflammatory angiogenic and fibroproliferative responses intermingled with Extracellular Matrix (ECM) and smooth muscle cells proliferation in the vessel wall resulting in the formation of

atherosclerotic plaque which causes obstruction of the blood vessels . [20]

The arterial smooth muscle cells, endothelial injury, blood monocytes and dyslipidemia are the four key factors involved in the origin and progression of the lesion of atherosclerosis.

Currently pathogenesis of atherosclerosis is explained on the basis of 2 theories.

1) **Reaction to Injury Hypothesis**

The modified response to injury hypothesis was described by Ross in 1993. It implicates lipid entry into the intima of the vessel walls as the initial event , followed by lipid accumulation in the macrophages (foam cells), which according to the modified theory, are believed to be the dominant cells in early lesions.

2) **Monoclonal Hypothesis**

This hypothesis is based on the postulate that proliferation of smooth muscle cells is the primary event in atherogenesis and this

proliferation is monoclonal in origin. This proliferation may be initiated by mutation caused by;

1. Exogenous chemical	(eg) Cigarette smoking.
2. Endogenous metabolites	(eg) Lipoproteins.
3. Some viruses	(eg) Marek's disease virus in chicken herpes virus. [21]

Development of Atherosclerosis:

Chronic endothelial injury due to toxins from smoking, hypertension, hyperlipidemia & hemodynamic disturbances lead to endothelial dysfunction causing increased permeability and accumulation of lipoproteins in the intima of the vessel walls.[22, 23]

The oxidative modification of the accumulated plasma lipoproteins- Low Density Lipoprotein (LDL) and association with the proteoglycans occurs within the intima of the arteries forming fatty streak.[2] The endothelial cells begin to express a Vascular Cell Adhesion Molecule (VCAM-1) on their surface, which binds precisely the monocyte and T-lymphocyte.

After monocytes adhere to the endothelium, they

i) Migrate between ECM to localize in the intima, largely stimulated by chemokines .

ii) Transform into macrophages and avidly engulf lipoprotein, largely oxidized LDL via scavenger receptors forming foam cells. [25]

Interleukin-I (IL-I),`Tumour Necrosis Factor (TNF) and Monocyte Chemoattractant Protein–I (MCP-1) are produced by macrophages and they increase the adhesion of leukocytes and recruit more of them into the plaque. Macrophages produce toxic oxygen species that also cause oxidation of the LDL in the lesions and they elaborate growth factors that may contribute to smooth muscle cell proliferation. Moreover the activated leukocytes and vascular wall cells also release growth factors [(26, 27)] (like Platelet Derived Growth Factor, Fibroblast Growth Factor and Transforming Growth Factor), that promote smooth muscle cell proliferation and ECM synthesis converting it to a mature atheroma and contribute to the progressive growth of atheromatous plaques, forming lesions.[28,29]

The Role of Lipids in Atherosclerosis

- **Hypercholesterolemia** is by for the most important risk factor for atherosclerosis. It can cause plaque formation and its growth, in the absence of other known risk factors.[30]

Genetic and acquired disorders that cause hypercholesterolemia lead to premature and severe atherosclerosis.[21]

The evidences implicating hypercholesterolemia in the genesis of atherosclerosis includes the following.

- The major lipids in the atheromatous plaques are plasma derived Cholesterol and Cholesterol Esters.
- Oxidized LDL is observed in macrophages in arteries at sites of fatty streaks.
- Genetic defects in lipoprotein metabolism causing hyperlipoproteinemia are associated with accelerated atherosclerosis.
- Lowering levels of serum Cholesterol by diet or drugs slows the rate of progression of atherosclerosis, causes

regression of some plaques and reduces the risk of cardiovascular events.

- **Dyslipoproteinemia** resulting either from mutation that yield defective apolipoproteins or some other underlying disorders (for example CKD, Hypothyroidism or Diabetes Mellitus),[25] can lead to atherosclerosis.

LIPID ABNORMALITIES IN CKD

Patients with CKD suffer from a secondary form of **complex dyslipidemia** consisting of both qualitative and quantitative abnormalities in serum lipoproteins resulting from alteration in lipoprotein metabolism and composition [31].

The prominent features of uremic dyslipidemia are ;

1) A profound defect in postprandial lipid disposal, which exposes the vasculature to high Chylomicron remnant concentration.[15]

2) Increase in Serum Triglycerides (TGL) [due to increased Very Low Density Lipoprotein (VLDL) remnants & Intermediate Density Lipoprotein (IDL)] and low HDL. [31]

3) Increased heterogeneity of LDL and HDL apoproteins. [15]

4) Serum LDL is mostly normal but the Cholesterol may originate from the atherogenic small and dense LDL subclass(sdLDL) .[31]

5) Increased LDL susceptibility to oxidation.

6) Altered cell surface LDL epitope and recognition.

7) The apolipoprotein-B (apo B) containing part of lipoprotein may undergo modifications. [Enzymatic and Advanced Glycation End Product (AGE) peptide modification, oxidation or glycosylation]. These modifications contribute to the impaired LDL-receptor mediated clearance from plasma and promote prolonged circulation.

8) HDL maturation is impaired and its composition is altered. The antioxidant and anti-inflammatory functions of HDL are depressed.[32]The defect in HDL maturation is largely caused by acquired Lecithin Cholesterol Acyl Transferase (LCAT) deficiency while its TGL enrichment is due to Hepatic Lipase deficiency.[33]

ACCELERATED ATHEROGENESIS IN CKD

In CKD there is an impaired clearance and accumulation of oxidation prone VLDL and Chylomicron remnants and abnormal LDL composition leading to oxidative stress and inflammation favouring their uptake by macrophages and resident cells in the artery wall. [33]

While LDL particles undergo vicious cycle of accumulation and modification, reverse cholesterol transport is also impaired due to low LCAT and PON activity.[31]

Therefore, the discoid HDL particles are structurally altered and hepatic cholesterol clearance is limited. Thus in CKD, there is an accelerated atherogenesis 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]

ATTENUATION OF ATHEROSCLEROSIS

Protection of LDL against oxidation by antioxidants can contribute to the attenuation of atherosclerosis. [34,35,36,37]

They can act ;

1. Directly on the LDL
2. Indirectly on the cellular oxidative machinery
3. By converting oxidized LDL to a non-atherogenic particle by HDL-associated PON I

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 **PON I** which reduces the inflammation associated with atherosclerosis.[38]

PARAOXONASE (PON)

PON is an aryl dialkyl phosphatase . E.C. 3.1.8.1.

PON is a multifunctional antioxidant enzyme component of HDL, which can protect LDL against oxidation.[39] PON is a serum arylesterase that was initially identified by its hydrolysis of aromatic carboxylic esters and organophosphorus insecticides and nerve

agents.[40,41]Its name reflects its ability to hydrolyze paraoxon, a metabolite of insecticide parathion.

Serum PON I and perhaps other mammalian Paraoxanases, act as important guardian against cellular damage from toxic agents, such as organophosphates, oxidized lipids in the plasma LDL and bacterial endotoxins [(42, 43).] There are three members in the PON gene family.

1) **PON 1**

- It is synthesized in the liver & transported along with HDL in the plasma.
- Its serum concentration is influenced by the inflammatory changes. [(44, 45, 46, 47)]

2) **PON 2**

It is a ubiquitously expressed intracellular protein, which can protect the cells from oxidative damage (such as cells of arterial wall & macrophages) and may not be associated with lipoprotein. [(48, 49)]

3) <u>PON 3</u>

- It is similar to PON I, but differs from its substrate specificity.
- It lacks paraoxon and phenyl acetate hydrolyzing activity.
- It is not regulated by inflammation & by the levels of oxidized lipids [44].

PON 1, 2&3, genes are aligned next to each other on the long arm of chromosome 7 (7q 21.3-22.1) [50]All the members of the PON gene family share ~65% similarity at the amino acid level.[51]

PON I

PON I is the most studied of all the members of PON family. It is a glycoprotein with a MW of 38 kDa and 354 Amino acids encoded by the PON-1 gene, which maps to the human chromosome 7q, 21-22. [51,52]

There are 2 common PON 1 gene coding polymorphisms

1)PON 1 Q 192 R

2)PON 1 L 55 M

The first polymorphism differ in the aminoacid at position 192. (glutamine & arginine)The second polymorphism differ in the aminoacid at position 55 (methionine and leucine) [53,54,55]

PON I is synthesized in the liver and transported primarily on apolipoprotein A-1(apoA-1) containing HDL[56]. The secreted protein retains its hydrophobic leader sequence, which is a structural requirement for PON's association with HDL [46,57]

In addition to the presence of PON 1 mainly on HDL, postprandial Chylomicrons also contain minor, but significant amount of PON 1.PON 1 was also detected in VLDL but not in LDL.[58]

PON & HDL

PON activity is highest in the more dense HDL 3 fraction, where PON I was not dissociated from these particles even during centrifugation.[59]

Using the immuno affinity chromatography, a subclass of HDL containing apoA-I apo J and PON was obtained. Thus PON has been isolated from human plasma in association with apoA-I and with apo J. [60, 61]

Structure of PON I

The first crystal structure of PON I was obtained by direct evolution, at a resolution of 2.2A^{o}

PON I is a six-bladed Beta-propeller, with an unique active site lid that is also involved in HDL binding.[62,63]

REGULATION OF PON-ACTIVITY

Genetic Factors

PON 1 gene is regulated by SP I and Protein kinase C, whereas PON 2 gene in macrophages is regulated by NADPH oxidase. [64,65]

Non-genetic factors

A variety of non-genetic factors have been shown to influence PON 1 levels such as diet, smoking, acute phase reactants and hormones. Nutritional antioxidants such as polyphenols, increase PON I mRNA expression and activity by an aryl hydrocarbon receptor dependent mechanism.[49]PON 1 levels are influenced by a variety of environmental factors including statins & cytokines[66]In human, consumption of degraded cooking oil [67] lowered the PON1 level, while alcohol [68] and Vitamin C& E elevated it.[69]

FUNCTIONS OF PON

1) Paraoxonases(PONS) metabolize 5- hydroxy eicosatetraenoic acid and 1,5 – lactone, & 4-hydroxy docosa hexaenoic acid, which are the products of both enzymatic and non-enzymatic

oxidation of arachidonic acid and docosa hexaenoic acid respectively and may represent PON's endogenous substrates.

2) PON I hydrolyzes the toxic oxon metabolites of a number of organophosphorus insecticides such as parathion, diazinon and chlorpyrifos and even nerve agents such as sarin and soman.

3) The bulky drug substrates like lovastatin and spironolactone are hydrolyzed only by PON 3 [70]

4) The PONS catalyze the hydrolysis of variety of aromatic and aliphatic lactones as well as the reverse reaction, lactonization of γ and δ -hydroxy carboxylic acid .[71,72,73]

5) PON I degrades bioactive phospholipids such as platelet activating factor, thereby preventing intravascular coagulation.[74]

6) PON protects lipids in lipoprotein, in macrophages and in erythrocytes from oxidation [65.]

7) PON increases the HDL binding to macrophages which in turn stimulates HDL's ability to promote cholesterol efflux and also inhibits Cholesterol biosynthesis [(74, 75, 38)]

8) PON was shown to increase the breakdown of specific oxidized lipids in oxidized LDL(ox-LDL) and decrease the macrophage uptake of ox-LDL [65]

9) PON attenuates the ox-LDL induced MCP-I production by endothelial cells. [76]

PON & ATHEROSCLEROSIS

PON 1 is inactivated under oxidative stress[75]. PON activity may also be altered as a part of inflammatory response[77] and it is evident by the fact that the HDL became proinflammatory during the acute phase response, possibly due to the loss of PON I activity from HDL.

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 favoured. [78, 79]

The PON is involved in 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. [38,80]

PON involvement in the pathogenesis of atherosclerosis is suggested by the increasing PON I immunoreactivity in the arterial wall as the atheroma advances.[54]

Thus, PON I confers protection against coronary artery disease by inactivating and removing the pro-inflammatory lipid oxidation products from the carotid and coronary plaques. [81, 82]

PON IN CKD

Decreased PON activity in CKD can be due to decreased HDL concentration & also due to the uremic environment. In uremic environment, the PON activity is inhibited through the post-translational modification of PON as a result of reactions 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.

MATERIALS AND METHODS

The study was done after obtaining the approval from the Ethical Committee of Stanley Medical College & Hospital.

This is an age and sex matched study. Hundred subjects were chosen for the study. Both males and females in the age group of 15-75 yrs were included and an informed consent was obtained from all of them. The study population includes 2 groups:

Group I : Consists of 50 healthy subjects with normal clinical & biochemical parameters. They were selected from the Master Health check up ,Outpatients department of Stanley Medical College & Hospital.

Group II: Consists of 50 patients with Chronic Kidney Disease. They were selected from the Department of Nephrology, Stanley Medical College & Hospital.

Inclusion Criteria

Patients with CKD.

Exclusion Criteria

1) Patients with liver disease
2) Patients with chronic infections
3) Patients with Diabetes Mellitus
4) Patients who are not willing to give informed consent.

Sample Collection

Fasting venous blood sample of 5ml was collected with strict aseptic precautions. 2 ml of the collected blood was transferred to the test tube containing anticoagulant for glucose estimation.3ml of the blood sample was transferred to a plain test tube. The samples were centrifuged, plasma & serum were separated respectively. The following parameters were estimated.

1) Paraoxonase activity
2) Total cholesterol
3) HDL cholesterol
4) TAG
5) Creatinine
6) Urea

7) Glucose

8) Albumin

LDL cholesterol and VLDL cholesterol levels were calculated.

ESTIMATION OF SERUM PON ACTIVITY

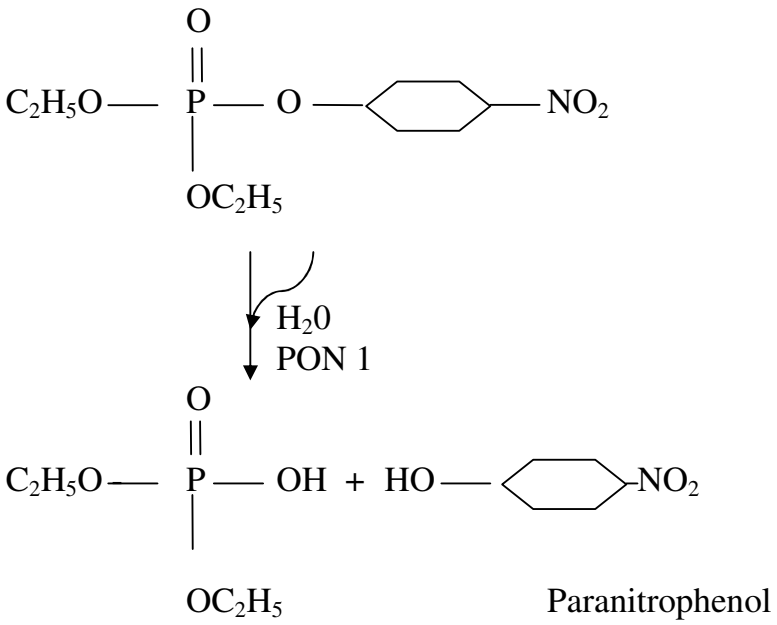
PON activity was estimated spectrophotometrically using Paraoxon (0,0-diethyl-0-4-nitro phenylphosphate) as the substrate for hydrolysis.

The chemicals used were of analytical reagent grade from SIGMA chemicals.

Principle

Serum PON, hydrolyzes paraoxon in the presence of calcium at pH 8.0 at 25^0C 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 (1U) of PON activity is defined as 1 µmol of p-NP formed per min per litre at 25^0C and the activity was expressed as U/L of serum.

Paraoxon



Diethyl phosphate

Reagents :

The assay mixture consisted of 2.2mM paraoxon substrate in 0.1m Tris HCL buffer, pH 8.0 containing 2mm $CaCl_2$.

Reagent Preparation

Buffer :

O.1M Tris

12.114 gm of Tris is dissolved in 1000ml of Distilled water (DW).

O.1M HCL

1 ml of 10 molar solution of HCL in 99ml of DW.

2 mM $CaC1_2$

294.04 mg of $CaCl_2$ is dissolved along with Tris.

To Prepare the Buffer:

90ml of Tris solution with $CaCl_2$ is taken and 0.1M HCl is added till the pH 8.0 is obtained. The buffer solution is refrigerated.

Substrate Preparation

2.2mM paraoxon is needed. To achieve this, 3 ml of the paraoxon is added to 6.355 ml of the buffer. This is freshly prepared.

MOLAR ABSORPTIVITY OF PARA NITRO PHENOL

The micromolar absorptivity of p-NP was measured as follows;

Molecular weight of p-NP = 139.11g /mole

1 micromole = 139.1 µg/L

The amount is weighed and dissolved in the buffer and the absorption is read at 405 nm in the spectrometer.

Absorbance = 0.026

As per Beer's law

$A = \Sigma bc$ A – Absorption

Σ - absorptivity

$\Sigma = \frac{A}{bc}$ b – pathlength

c – concentration of the substance

Micromolar absorptivity

$= \frac{0.026}{1x1}$ b = 1 cm

c = 1 µmol

= 0.026

Enzyme activity U/L =

$$\frac{\text{(Delta absorbance / min)x Total Volume}}{\text{Sample Volume x Micromolar Absorptivity x Pathlength}}$$

Factor has been calculated from the formula:

$$\frac{\text{Total Volume}}{\text{Sample Volume}\times\text{Micromolar Absorptivity}\times\text{Pathlength}}$$

$$=\frac{550}{50\times0.026\times1}$$

=423

Thus, Enzyme activity (U/L)= Δ abs/min x 423.

PROCEDURE

Assay Parameters

Mode	-	Kinetic
Wavelength	-	405nm
Sample volume	-	50 µl
Reagent volume	-	500 µl

Lag Time	-	60 sec
Kinetic Interval	-	180 sec
Number of readings	-	3
Factor	-	423
Reaction temperature	-	25 ^{0}C
Reaction direction	-	Increasing
Units	-	U/L

Assay Procedure:

500µl of the reagent and 50µl of the sample were taken, mixed well & read at 405nm using the semi auto analyzer MICROLAB 300.

Reference Range : 90 – 150 U/L .

QUANTITATIVE ESTIMATION OF BLOOD UREA NITROGEN

(UV KINETIC METHOD)

Principle

Urea was hydrolyzed in the presence of water and urease to produce ammonia and CO_2. The ammonia produced combines with α-Ketoglutarate(α-KG) and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate & NAD. The amount of urea nitrogen may be calculated by determining the absorbance decrease per minute relative to urea nitrogen standard at 340nm.

Reagents	TRIS BUFFER	pH 7.55	– 75 m mol / L
		GLDH	≥ 1000 U/L
		Urease	≥ 30,000 U/L
		NADH	– 0.32 mmol / L
		α-KG	– 9 mmol

Standard-50mg/dl

Procedure :

10µl of sample was added to1 ml of reagent at 37^0C, mixed and read at 340nm.

	Blank	Std	Test
Reagent	1ml	1ml	1ml
Std		10µl	
Sample			10µl

Reference values : 6-20 mg/dl.

QUANTITATIVE ESTIMATION OF SERUM CREATININE (BY MODIFIED JAFFE'S METHOD)

Principle

Creatinine present in the sample reacts with picric acid in alkaline medium and forms creatinine picrate (red colored complex) which is measured at 490 nm.The intensity of the coloured complex is directly proportional to the concentration of the creatinine in the sample.

Reagents

Saturated picric acid 10 ml

0.75N Sodium hydroxide 10 ml

Standard 2mg/ dl.

Procedure

100µl of sample was added to 1 ml of reagent at 37^0C, mixed and read at 490 nm .

	Blank	**Std**	**Test**
Reagent	1ml	1ml	1ml
Std	-	100µl	-
Sample	-	-	100µl

Reference values : 0.8 – 1.2 mg/dl

ESTIMATION OF TOTAL CHOLESTEROL

The tests were performed in the reagent kit by ENZYMATIC CHOLESTEROL ESTERASE METHOD.

Principle

The free cholesterol liberated from the cholesterol esters by cholesterol esterase is oxidized by cholesterol oxidase to cholestenone with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4 aminoantipyrine and a phenolic compound in the presence of peroxidase to yield a red coloured complex.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholestenone + H_2O_2

H_2O_2 + 4 amino antipyrine + Phenolic compound

$\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + H_2O

The concentration of cholesterol in the sample is directly proportional to the intensity of the red coloured complex which is measured at 500nm.

Reagent :

Reagent 1 (Enzyme / chromogen)

Reagent 1A (Buffer)

Cholesterol Standard -200mg/dl

Reconstituted Reagent :

Dissolve the contents of one bottle of reagent I with one bottle of reagent 1A.

Procedure

	Blank	**Std**	**Test**
Reagent	1ml	1ml	1ml
Std	-	100µl	-
Sample	-	-	100µl

Incubate for 5 min at 37^0C , absorbance is read at 500nm.

Reference Values**:** 150 – 220mg /dl

ESTIMATION OF TRIACYLGLYCEROL

The tests are performed in the reagent kit by ENZYMATIC COLORIMETRIC METHOD.

Principle :

Lipoprotein lipase catalyzed hydrolysis of triacylglycerol yield glycerol which is phosphorylated by glycerol kinase using ATP to glycerol -3 -phosphate which upon oxidation yields dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with phenolic compound and 4 aminoantipyrine to form a coloured complex.

Triacylglycerol + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + Free Fatty Acids.

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol 3 phosphate + ADP

Glycerol 3 phosphate + O_2 $\xrightarrow{\text{Glycerol phosphate Oxidase}}$ DAP+ H_2O_2

H_2O_2 + 4 – Aminoantipyrine + DHBS $\xrightarrow{\text{Peroxidase}}$

Quinoneimine dye +H_2O

(DAP – Dihydroxy acetone phosphate

DHBS – 3, 5 Dichloro 2 – hydroxyl benzene sulfonate)

The intensity of the purple coloured complex formed during the reaction is directly proportional to the triacylglycerol concentration in the sample and it is measured at 500 nm.

Reagent :

Reagent 1 (enzymes / chromogen)

Reagent 1A (Buffer)

Standard-200mg/dl

Reconstituted Reagent :

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A.

Procedure

	Blank	**Std**	**Test**
Reagent	1ml	1ml	1ml
Std	-	10µl	-
Sample	-	-	10µl

Mix well and incubate for 5minutes at 37C and read at 500nm.

Reference values : Male ; 60 - 150 mg/dl

Female ; 40 - 140 mg/dl

ESTIMATION OF HDL CHOLESTEROL

The tests are performed in the reagent kit by PHOSPHOTUNGSTATE METHOD.

Principle :

Chylomicrons, VLDL & LDL fractions in serum or plasma are separated from HDL by precipitating with phsophotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, is assayed with enzymatic cholesterol esterase method.

Reagents :

Reagent – 1 (enzymes / Chromogen)

Reagent 1 A (Buffer)

Reagent 2 (Precipitating Reagent) Phosphotungstic acid 50 mmol /L Magnesium chloride 39 mmol/L

Standard-50mg/dl

Procedure

1) **Precipitation**

Dispense into centrifuge tubes.

Sample	200µl
Precipitating reagent	200µl

Mix well centrifuge at 3500 – 4500 rpm for 10 minutes, separate the clear supernatant immediately and determine the cholesterol content.

Procedure

	Blank	**Std**	**Test**
Reagent	1ml	1ml	1ml
Std	-	50µl	-
Sample	-	-	50µl

Mix well and incubate for 5minutes at 37^0C and read at 500nm.

Reference values : 40 -70 mg / dl

CALCULATED PARAMETERS

Friedwald's Formula

If TAG is < 400mg/dl

$$\text{VLDL} = \frac{\text{TAG}}{5} \text{ mg/dl}$$

If TAG is > 400mg/dl

$$\text{VLDL} = \frac{\text{TAG}}{7} \text{ mg/dl}$$

LDL = Total Cholesterol – (HDL+VLDL) mg/dl

QUANTITATIVE DETERMINATION OF ALBUMIN

(BCG DYE BINDING METHOD)

Principle

Albumin in a buffered solution reacts with the anionic bromo cresol green with a dye binding reaction to give a green colour which is measured at 630 nm (600 – 650 nm).The intensity of the colour is directly proportional to the concentration of the albumin in the sample.

Reagents: **Reagent I** **-Bromocresolgreen**

Succinic Acid	-	94m mol/L
Sodium hydroxide	-	10.2 m mol / L
BCG	-	0.149 m mol/L

Procedure

	Blank	**Standard**	**Test**
Reagent	1 ml	1 ml	1 ml
Std	-	10µl	-
Sample	-	-	10µl

Add 10 µl of sample to1 ml of reagent, mix , incubate for1 minute at room temperature and read at 630 nm.

Reference values – 3.5-5 g/dl

ESTIMATION OF GLUCOSE

GLUCOSE OXIDASE PEROXIDASE METHOD

Principle:

Glucose is oxidized by glucose oxidase to gluconic acid and H_2O_2 with gluconolactone as intermediate.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine (4AAP) with phenol to yield a coloured quinoneimine complex, with absorbance proportional to the concentration of glucose in sample.

$$H_2O_2 + \text{phenol} + 4AAP \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 2H_2O$$

Reagent

Reagent I : Enzyme Reagent

Glucose oxidase	-	$\geq$ 20000 U/L
Peroxidase	-	$\geq$ 2000 U/L
Phenol	-	10 mmol/L
Phosphate buffer	-	200 mmol/L

Glucose Standard : 100mg / dl

Procedure

	Blank	Std	Test
Reagent	1ml	1ml	1ml
Std	-	10µl	-
Sample	-	-	10µl

Mix & incubate for 15 minutes at 37^0 C. Read at 520nm.

Reference values : Glucose (Fasting) - 65 – 110 mg/dl

RESULTS AND STATISTICAL ANALYSIS

A total of 100 subjects were included in the present study. Out of this 100, 50 were under the study group (i.e. patients with CKD) and the other 50 were under the control group (i.e. apparently healthy individuals).

Age Distribution among the study and control group :-

Male and female subjects in the age group of 15 to 75 years were included in the study. Both the study and control group were age matched.

Quantitative variable (age) is given as frequency and their percentage with the cross – tabulation and Pearson chi-square test in the **Table-I.**

The cross tabulation and Pearson Chi Square test for sex distribution in the study is given in the **Table - II**

The PON activity and the levels of Total Cholesterol, TAG, HDL, Glucose, Urea, Creatinine & Albumin were estimated for all the

samples collected for the study. VLDL and LDL values were calculated.

The values obtained in both the control and study groups are presented in the Master Chart I & II

The values of serum PON activity of the control & study groups are plotted in the Scatter Diagram.

The values were analyzed and the results are presented in Table III-V.

Correlation between PON and other variables were analyzed using Pearson's Correlation Analysis. The results are presented in the Table VI.

The Receiver Operating Characteristic curve is plotted for PON and presented in Figure I and area under the curve was analyzed & presented in the Table VII.

Table –I

AGE GROUP IN YEARS - CROSS TABULATION

Age in years		Group		Total	P value
		Control	Test		
15-35	Count	20	19	39	0.917 (NS)
	% with in age group in yrs.	(57.3%)	(48.7%)	100%	
	% within group	[40.0%]	[38.0%]	39.0%	
36-55	Count	19	21	40	
	% with in age group in yrs.	(47.5%)	(52.5%)	100%	
	% within group	[38.0%]	[42.0%]	40%	
36-55	Count	11	10	21	
	% with in age group in yrs.	(52.4 %)	(47.6%)	100%	
	% within group	[22.0%]	[20.0%]	21%	
Total	Count	50	50	100	

Note :

1) The value with in ( ) refers to row %

2) The value with in [ ] refers to column %

Table –II

SEX DISTRIBUTION -CROSS TABULATION

Sex		Group		Total	P value
		Control	Test		
Male	Count	33	34	67	0.832 (NS)
	% with in sex	(49.3%)	(50.7%)		
	% within group	[66.0%]	[68.0%]		
Female	Count	17	16	33	
	% with in sex	(51.5%)	(48.5%)		
	% within group	[34.0%]	[32.0%)]		
Total	Count	50	50	100	

Note :

1) The value with in ( ) refers to Row %

2) The value with in [ ] refers to Column %

Table –III

STUDENT- T TEST FOR DIFFERENCE OF TWO MEANS

PARAMETERS	GROUP				t value	P value
	CONTROL		TEST			
	MEAN	SD	MEAN	SD		
PARAOXONASE U/L	117.38	30.62	46.47	24.45	12.798	<0.001 **S**
UREA mg/dL	29.34	6.74	94.32	39.41	11.491	<0.001 **S**
CREATININE mg/dL	0.80	0.15	7.49	3.77	12.521	<0.001 **S**
TOTAL CHOLESTEROL mg/dL	193.68	40.84	197.46	36.64	0.487	0.627 **NS**
HDL mg/dL	45.30	16.52	30.80	6.96	5.720	<0.001 **S**
LDL mg/dL	119.06	33.83	126.36	25.76	1.214	0.228 **NS**
VLDL mg/dL	26.42	6.76	41.20	15,82	6.076	<0.001 **S**
TAG mg/dL	132.28	33.69	207.44	78.84	6.198	<0.001 **S**
ALBUMIN g/dL	3.89	0.55	3.49	0.84	2.835	0.006 **S**

S -SIGNIFICANT ; NS- NON SIGNIFICANT.

P value < 0.05 is Significant

Table –IV

AGE MATCHED COMPARISON OF PON ACTIVITY IN THE STUDY AND CONTROL GROUP.

AGE Group	Group	Mean U/L	SD	t value	'P' value
15-35	CONTROL STUDY	138.38 52.39	19.49 29.71	10.627	<0.001 **SIGNIFICANT**
36-55	CONTROL STUDY	110.83 47.68	24.76 25.98	7.956	<0.001 **SIGNIFICANT**
56-75	CONTROL STUDY	88.46 42.62	27.58 17.99	4.459	<0.001 **SIGNIFICANT**

Table –V

SEX MATCHED COMPARISON OF PON ACTIVITY IN THE STUDY AND CONTROL GROUP

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SEX	Group	Mean U/L	SD	t value	'P' value
MALE	CONTROL STUDY	117.95 46.09	30.60 24.69	10.59	<0.001 **SIGNIFICANT**
FEMALE	CONTROL STUDY	110.39 47.26	38.97 24.71	5.517	<0.001 **SIGNIFICANT**

Table –VI

PEARSON CORRELATION ANALYSIS OF PON ACTIVITY WITH OTHER VARIABLES.

PARAMETERS	CORRELATION CO-EFFICIENT	P VALUE
AGE	- 0.233	0.020 **S**
UREA	-0.679	<0.001 **S**
CREATININE	-0.659	<0.001 **S**
TOTAL CHOLESTEROL	-0.070	0.491 **NS**
HDL	0.508	<0.001 **S**
LDL	-0.179	0.075 **NS**
VLDL	-0.489	<0.001 **S**
TAG	-0.254	0.011 **S**
ALBUMIN	0.256	0.010 **S**

P value < 0.05 is Significant (S); Non Significant(NS)

RECEIVER OPERATING CHARACTERISTIC CURVE FOR PARAOXONASE

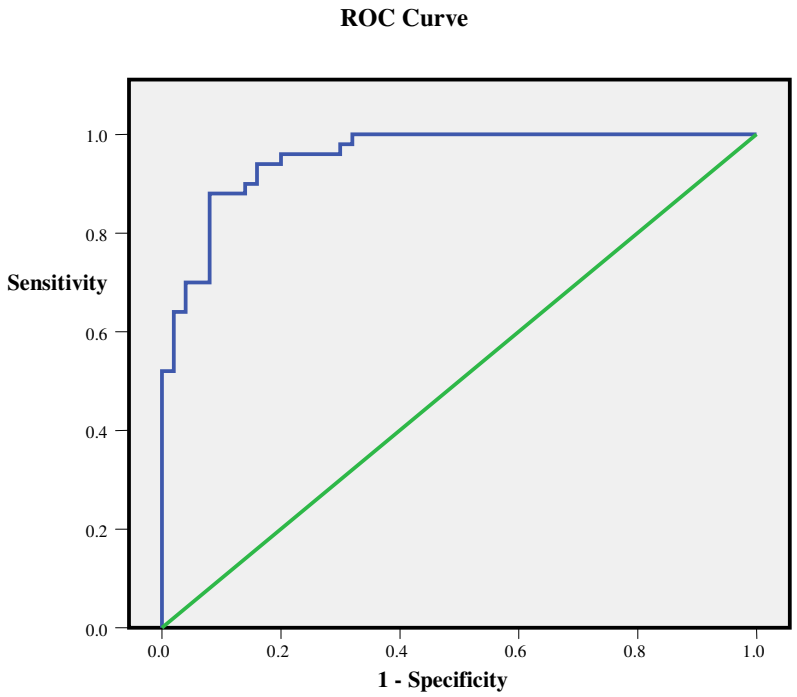


Table –VII

AREA UNDER THE CURVE

Area	Std Error (a)	P Value	Asymptotic 95% Confidence Interval	
			Lower Limit	Upper Limit
0.955	0.018	<0.001	0.920	0.990

DISCUSSION

The present study establishes the characterization of the PON activity for its association in the early prediction of atherosclerosis in CKD patients.

The ability to detect atherogenesis earlier, is necessary for taking preventive measures against cardiovascular disease in CKD. This study supports the fact that the measurement of PON activity is useful in detecting the risk for cardiovascular disease in CKD patients.

In the present study, the mean age of the control group was **41.6±13.1yrs** and for the study group the mean age was **41.4±13.8 yrs**. The two groups were found to be age-matched using the cross – tabulation and Chi-Square tests (p=0.917) ,**Table I.**

Similarly, Chi-Square test was performed to find out any difference in the male and female composition among the two groups. It was found out that the two groups were sex matched (p=0.832) **Table II.**

The Student 't' – test was used to compare the Serum PON activity between the two groups.

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 to the mean value of the control group **(117.38 ± 30.62 U/L);** p= <0.001. This study also correlates with the data given in the previous studies on Serum PON activity in CKD and there is a significant decrease in PON activity in CKD patients [83].

The mean values of Serum PON activity between the control and study groups in different age groups (15-35 , 36-55 & 56-75 yrs) were compared. All of them showed significant decrease in PON activity in the study group(p=<0.001), **Table IV.**

Similarly the sex matched comparison of PON activity between the two groups also showed a significant decrease in PON activity in the study group Table V.

The parameters, Total Cholesterol, TAG, HDL, VLDL & LDL (the traditional risk factors used in the prediction of atherosclerosis) were also analyzed using Student 't' – test.

The comparison of the mean values of **Total cholesterol** in the study group (**197.46 ± 36.64 mg/dl**) and the control group **(193.68 ± 40.84 mg/dl)** showed no significant difference. p=0.627.

The comparison of the mean values of **TAG** in the study group **(207.44 ±78.85 mg/dl)** and the control group **(132.28±33.69mg/dl)** showed a significant increase in the study group. p= <0.001.

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 mean values of **LDL Cholesterol** between the study group **(126.36 ± 25.76 mg/dl)** and the control group **(119.06 + 33.83 mg/dl)** showed no significant difference. P = 0.228.

The comparison of the mean values of **VLDL Cholesterol** in the study group **(41.20 ± 15.82 mg/dl)** and the control group **(26.42 + 6.76mg/dl)** showed a significant difference ,with increased levels in the study group. P=<0.001.

The differences in 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 in the study group with the p value of <0.001, while Albumin levels are decreased in the study group.

In the Pearson's Correlation Analysis (Table VII), the PON activity is negatively correlating with age in years (P= 0.020).

The negative correlation of PON activity with age and the comparison study in the age groups shows a possible relation between PON activity and age. As age increases PON activity decreases. Considering the suspected protective role of PON in atherosclerosis, it is interesting to note that aging is accompanied by this reduction in enzyme activity. PON activity diminishing with time could be a non-negligible factor of increased atherosclerosis development in elderly people [10].

The levels of Urea, Creatinine, Total Cholesterol, VLDL and TAG are negatively correlating with the PON activity.

The HDL Cholesterol levels had significant positive correlation with the PON activity with the p value <0.001. This correlation proves the association of PON with HDL.

The ROC curve of PON activity 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 patients.

In CKD patients, an increased effect of oxidative stress on LDL lipoprotein in vitro has been reported as it had been [84,85,86,] noticed in patients with Familial hypercholesterolemia, Diabetes Mellitus or Coronary heart disease. This work sustains the relationship between low PON activity and diseases with low anti-oxidant defense and excessive lipid peroxidation.

PON plays 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 in uremia, loss of that protection could represent a very important factor in atherosclerosis.

In renal failure, the accumulation of Nitrogen derived products within the environment of the arterial wall, could decrease PON activity by directly inhibiting the enzyme or by modifying its synthesis and secretion.

The intake of nutritional antioxidants such as Vitamin C, Carotenoids, (lycopene and beta-carotene) and mainly Polyphenols (such as those present in red wine, licorice root ethanolic extract, or in pomegranate)[48] by atherosclerotic animals and also by CVS patients, leads to a reduction in oxidative stress and to the attenuation of atherosclerosis development. These latter phenomena could be related to the nutritional anti-oxidants induced increase in HDL PON 1 activity. (effects on gene expression, or preventing enzyme inactivation and on increasing PON 1 stability through its binding to HDL)[87].

Thus by determining the PON activity earlier in CKD patients, interventions can be made to increase the PONS by dietary or pharmacological means. Thereby we can reduce the macrophage foam cell formation and attenuate the development of atherosclerosis [75].

CONCLUSION

This present study, shows the involvement of PON in the excessive LDL peroxidation noticed in uremia. This study presents a potential explanation of accelerated atherosclerosis in CKD.

There is a significant decrease in PON activity in the CKD patients and thus the decrease of its anti atherogenic properties in renal failure could be an important factor in premature vascular aging.

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.

SCOPE FOR FURTHER STUDY

- Further studies can be done for comparison of PON activity in CKD patients on conservative management, hemodialysis and those who had undergone renal transplantation.

- Studies on determination of PON polymorphisms are needed to evaluate the discordance in human PON 1 gene between phenotypes and genotypes in CKD.

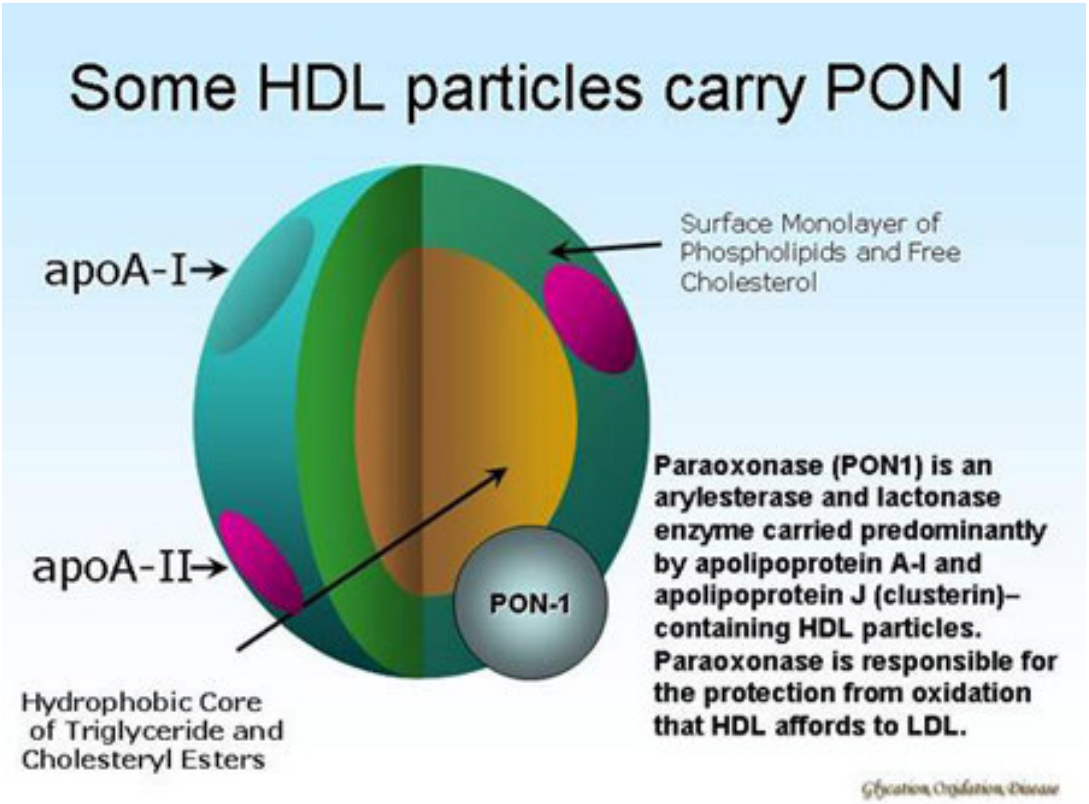
- Dietary determinants of Serum PON activity in human can be studied for the timely intervention.

TABLE - 1

CLASSIFICATION OF CKD

Stage	GFR, mL/min per 1.73 m^2
0	>90 with risk factors for CKD
1	≥ 90 with demonstrated Kidney damage e.g. persistant proteinuria, abnormal blood and urine chemistry.
2	60 – 89
3	30 – 59
4	15 – 29
5	< 15

Source : Modified from National Kidney foundation. K/DOQI clinical Practice guidelines for CKD : Evaluation, Classification and Stratification. AmJ Kidney Dis 39: Supply 1, 2002.



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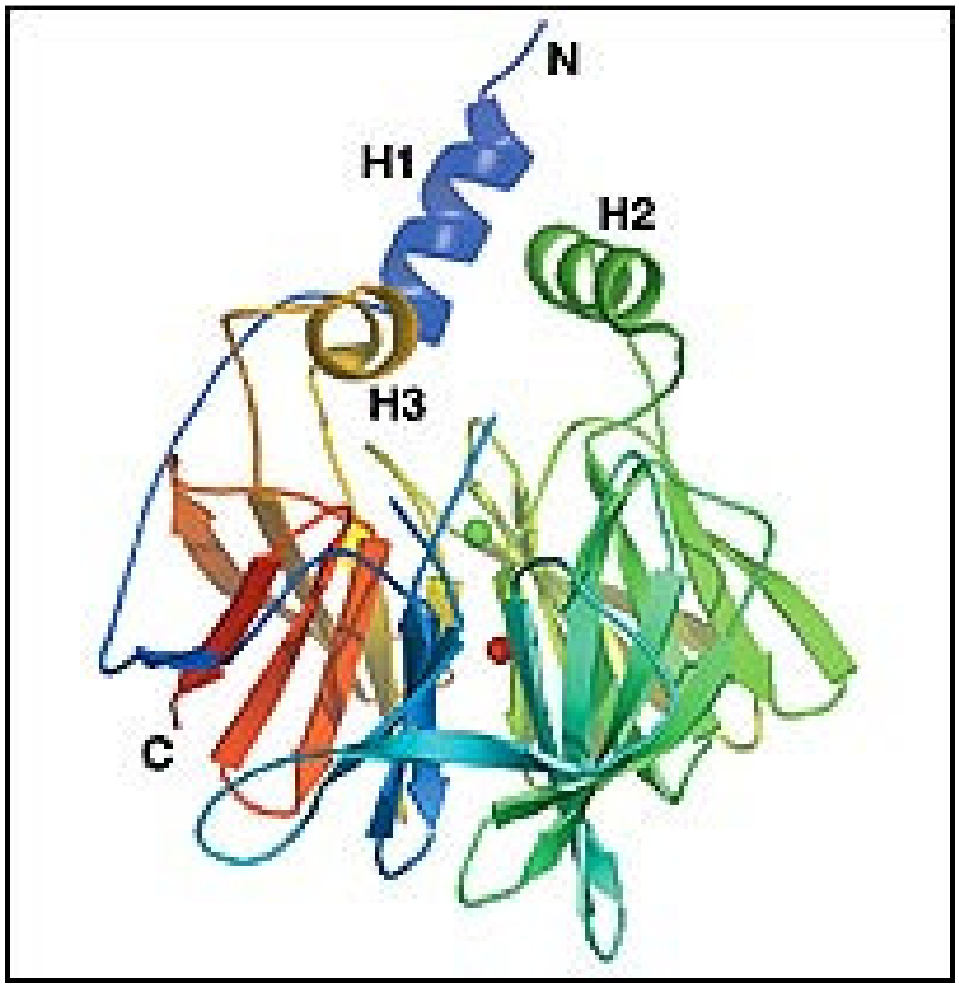
PARAOXONASE



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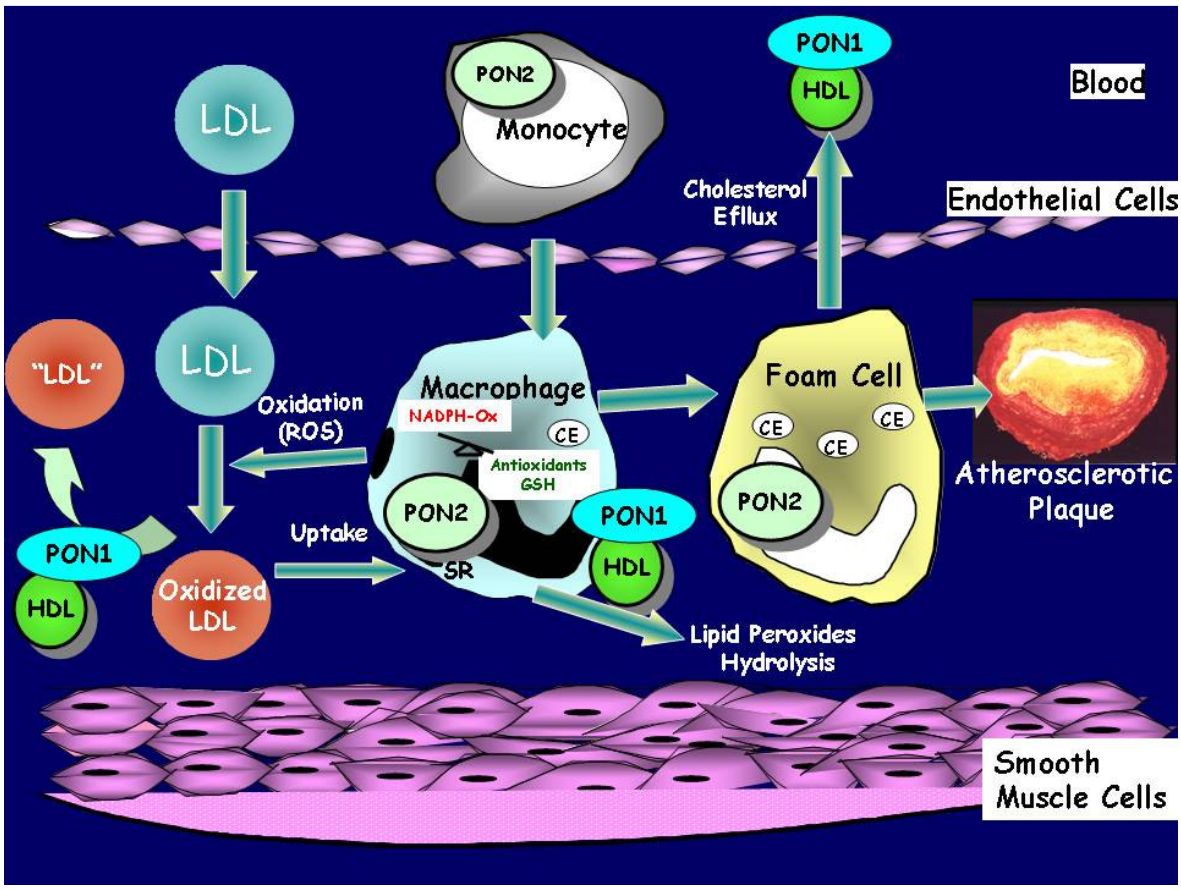
PARAOXONASE & ATHEROSCLEROSIS



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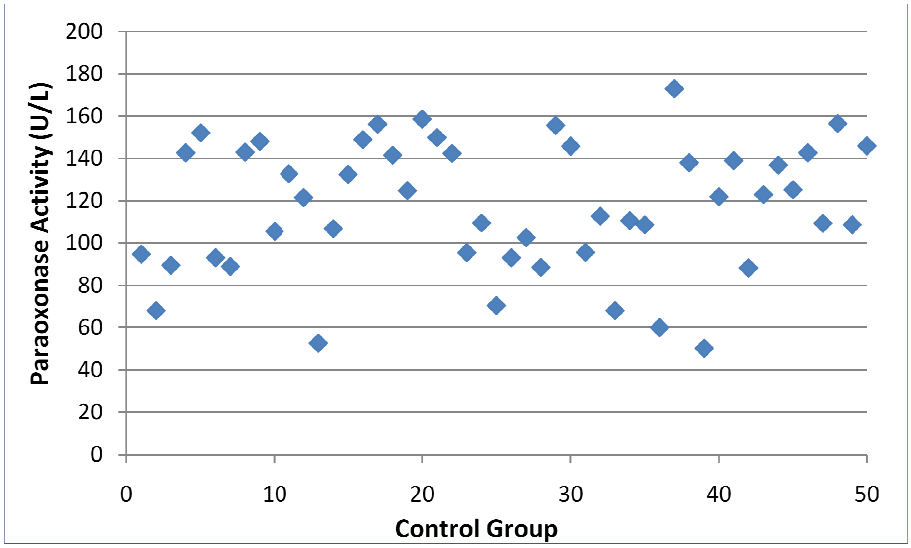
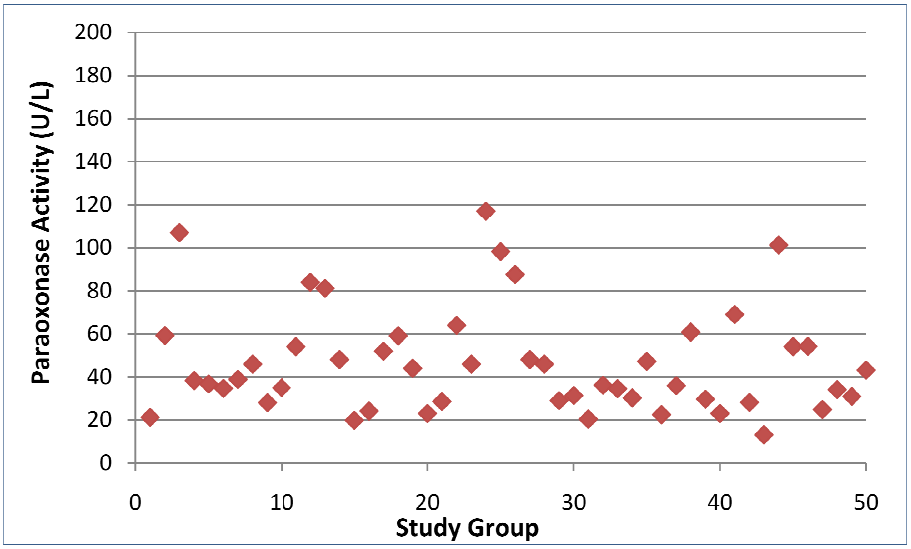
Scatter Diagram





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PROFORMA

Govt. Stanley Medical College & Hospital

Name : OP/IP. No ; Date

Age / Sex :

Occupation :

Address :

Presenting Complaints (PC)

History of PC

Past History

Family History

O/E

PR BP RR Wt.

CVS

RS

ABD

CNS

Investigations

Serum Paraoxonase activity

Lipid Profile

Blood Urea

Seum Creatinine

Serum Albumin

Plasma Glucose

ஒப்புதல் படிவம்

_____ என்னும் நான், மருத்துவர் செய்யும் ஆய்வுக்காக எனது இரத்தத்தை பரிசோதனை செய்துகொள்ள என் முழு மனதுடன் சம்மதிக்கிறேன்.

பரிசோதனை பற்றிய விவரங்களை மருத்துவர் கூறக்கேட்டு அறிந்து கொண்டேன்.

நோயாளியின் உறவினர் இப்படிக்கு,

MASTER CHART CONTROL GROUP

S.No	Age	Sex	Paraoxonase U/L	Urea mg/dl	Creatinine mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	TGL mg/dl	Albumin gm/dl	Glucose mg/dl
1	56	F	94.8	40	1.1	253	45	186	22	110	3.5	112
2	37	F	68.1	30	0.6	186	53	98	35	177	4.3	126
3	56	F	89.5	26	0.9	167	46	106	15	78	4.7	100
4	38	F	142.6	30	0.6	237	49	167	21	105	4	112
5	33	F	152.1	28	0.7	210	57	108	45	227	5.5	114
6	41	F	93	35	0.6	233	40	169	24	121	4.4	127
7	51	F	89	24	0.7	180	45	110	24	121	3.4	98
8	38	F	142.9	21	0.9	210	52	132	26	130	3.5	75
9	24	F	148	18	0.6	160	48	90	22	110	3.8	90
10	43	F	105.5	36	0.9	194	42	27	25	124	4.3	72
11	32	F	132.7	18	0.6	170	46	95	29	143	3.6	84
12	52	F	121.5	27	0.6	199	48	131	20	100	4.2	89
13	60	F	52.7	24	0.8	200	38	143	18	92	4.5	87
14	65	F	106.8	40	0.8	160	44	90	26	128	3.4	92
15	36	F	132.4	23	0.7	169	47	92	30	150	3	109
16	30	F	148.9	26	0.6	164	47	93	24	120	3	83
17	26	F	156.2	30	0.8	180	48	108	24	120	3.6	90
18	45	M	141.5	26	0.7	380	57	209	34	169	4.2	140
19	57	M	124.8	25	0.8	307	47	209	51	255	4	128
20	20	M	158.6	25	0.6	147	49	84	14	70	4.8	106
21	35	M	149.9	38	0.9	220	60	140	16	82	4.3	74
22	20	M	142.4	28	0.8	156	5	83	22	108	4	106
23	35	M	95.4	26	0.7	200	145	125	30	150	4	110
24	50	M	109.5	30	1	220	40	146	34	168	4.5	116
25	42	M	70.5	34	0.8	175	35	112	28	142	3.6	80
26	47	M	93	23	0.9	194	42	128	24	120	3.5	110
27	42	M	102.6	18	1	200	43	135	22	109	3.6	104
28	40	M	88.5	32	0.9	220	38	155	26	132	4	110
29	36	M	155.7	20	0.9	174	50	96	30	148	4	100
30	29	M	145.7	22	1	190	41	127	22	110	4.6	62
31	54	M	95.6	40	1.2	230	44	156	30	150	3.9	70
32	56	M	112.8	40	0.6	192	48	118	36	180	3.5	74
33	62	M	68.1	30	0.7	210	34	135	31	153	3.5	78
34	45	M	110.6	21	0.7	204	40	140	24	120	3.8	87
35	45	M	108.7	38	0.8	189	45	114	30	149	3.2	100
36	70	M	60	49	0.8	200	39	113	34	172	3.3	106
37	30	M	173	33	0.9	191	45	124	22	110	3.5	92
38	32	M	138	34	1	169	38	104	27	136	3.8	69
39	64	M	50.2	26	1	162	32	108	22	110	4	104
40	28	M	122	35	0.7	149	35	91	23	115	4.2	91
41	29	M	139	28	0.9	170	40	105	25	126	4.3	108
42	56	M	88.2	29	0.7	180	37	115	28	140	3.5	116
43	32	M	123	37	0.8	168	42	96	30	148	3	96
44	50	M	136.8	29	0.7	159	49	91	19	95	3.1	82
45	58	M	125.2	35	0.8	184	40	120	24	123	3	120
46	28	M	142.6	26	0.9	196	41	129	26	132	4.2	119
47	33	M	109.4	32	0.7	156	39	87	30	152	3.8	112
48	19	M	156.5	24	0.9	132	48	61	23	116	5	98
49	49	M	108.6	32	1	220	38	151	31	155	3.8	116
50	25	M	145.9	26	0.9	168	44	101	23	113	4.5	106

MASTER CHART STUDY GROUP

S.No	Age	Sex	Paraoxonase U/L	Urea mg/dl	Creatinine mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	TGL mg/dl	Albumin gm/dl	Glucose mg/dl
1	38	F	21.2	160	7.8	219	28	123	68	348	2.6	116
2	40	F	59.2	76	4	166	30	105	31	158	4.2	78
3	22	F	106.9	56	1.9	170	42	101	27	136	3.5	128
4	72	F	38.3	60	3	220	32	140	48	242	6	120
5	45	F	36.8	56	12	180	35	119	26	134	2.6	132
6	60	F	34.7	70	8.4	131	38	64	29	146	3.1	106
7	35	F	38.9	96	2.8	220	30	133	57	285	2.5	126
8	62	F	46	106	9	178	32	118	28	140	4	94
9	32	F	28	84	6.6	200	24	131	45	225	3.2	100
10	29	F	35	92	6.8	186	31	129	26	128	2.7	116
11	26	F	54	87	12.4	196	32	127	37	188	3	132
12	56	F	84	104	9.2	222	22	130	70	350	3	136
13	52	F	81.2	69	7.6	188	30	121	29	142	3.5	106
14	56	F	48	71	6.2	192	31	106	37	188	4	88
15	42	F	19.8	112	12.8	250	25	166	59	296	2.7	110
16	60	F	24.1	78	14.2	201	26	123	52	262	4	82
17	36	M	52	155	13.9	170	30	110	30	150	3	78
18	55	M	59	84	5.6	186	32	128	26	133	5	64
19	29	M	44	71	7.6	179	27	119	33	167	4	62
20	40	M	23	86	6.5	220	20	159	41	204	2.7	120
21	32	M	28.6	158	11.8	220	21	167	33	168	2.8	128
22	36	M	64	60	6.2	190	42	116	32	160	5	120
23	46	M	46	66	6.2	259	30	168	61	306	3.2	140
24	30	M	117	101	11.1	240	45	159	32	182	2.2	136
25	42	M	98.2	93	9.8	198	47	113	38	190	2.6	142
26	50	M	87.6	57	5.5	205	40	105	60	299	4	118
27	34	M	48	60	6.3	108	29	105	46	230	4	78
28	60	M	46	98	8.4	228	30	146	52	264	5.5	68
29	47	M	29	90	12.2	198	21	122	55	276	3	150
30	49	M	31.4	85	13	204	27	143	34	174	2.8	112
31	24	M	20.3	161	7	139	26	98	15	78	3.7	60
32	48	M	36.3	124	4.8	130	24	72	34	170	3.7	68
33	45	M	34.6	82	3.8	235	31	147	57	285	3.2	133
34	43	M	30.2	53	2.1	238	32	152	54	273	4	130
35	27	M	47.3	64	2.6	171	38	113	20	101	3.6	120
36	20	M	22.4	49	1.6	170	39	115	16	82	5.1	110
37	16	M	36	192	13.6	168	19	119	30	150	3.3	141
38	20	M	60.7	76	2.5	144	25	99	20	102	2.4	130
39	32	M	29.7	54	2.8	160	30	92	38	190	2.8	90
40	60	M	23	140	12	250	29	144	77	384	3.5	142
41	32	M	69	126	2.4	190	36	124	30	150	3.5	118
42	62	M	28.1	46	5.6	240	26	143	71	356	4	106
43	38	M	13.1	106	9.8	190	19	115	56	282	2.9	120
44	55	M	101.3	62	6	214	48	135	31	156	3.9	96
45	65	M	54	56	5.8	281	35	173	73	364	3.4	114
46	15	M	54.2	68	5.5	194	36	134	24	120	3.2	127
47	35	M	24.8	192	11.8	218	28	155	35	174	2.9	119
48	40	M	34.2	198	14.5	165	25	91	49	244	3.2	151
49	35	M	31	112	3.6	272	29	195	48	240	2.8	100
50	46	M	43.2	114	7.8	180	36	106	40	200	5	72