Dissertation on

"Evaluation of Serum Lipoprotein(a) levels and novel Lipid Indices in patients with Chronic Kidney Disease"

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CERTIFICATE

This is to certify that the dissertation titled, *"Evaluation of Serum Lipoprotein(a) levels and novel Lipid Indices in patients with Chronic Kidney Disease"* is a genuine work done by **Dr. Kalaivani. R**, for the partial fulfillment of the requirements for M.D (Biochemistry) Branch XIII Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2017, under the supervision and the guidance of **Dr. R. MAHALAKSHMI,** M.D, D.C.H, during the academic period 2014-2017.

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DECLARATION

I, **Dr.Kalaivani. R**, solemnly declare that the dissertation titled *"Evaluation of Serum Lipoprotein(a) levels and novel Lipid Indices in patients with Chronic Kidney Disease"* is a bonafide work done by me during the period of JANUARY 2016 to JUNE 2016 at Government Stanley Medical College and Hospital, Chennai under the expert guidance of

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This thesis is submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the rules and regulations for the M.D. degree examinations in Biochemistry to be held in April 2017.

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INDEX

ABBREVIATIONS

CKD : Chronic Kidney Disease

NKF-K/DOQI : National Kidney Foundation - Kidney Disease Outcomes Quality Initiative

GFR : Glomerular Filtration Rate

CRF : Chronic Renal Failure

LDL : Low Density Lipoprotein

Lp (a) : Lipoprotein (a)

Apo(a) : Apoprotein (a)

ApoB100 : Apoprotein B100

Apo B48 : Apoprotein B48

ApoA1 : Apoprotein A1

ApoC1 : Apoprotein C1

CM : Chylomicron

VLDL : Very Low Density Lipoprotein

IDL : Intermediate Density Lipoprotein

LDL : Low Density Lipoprotein

TG : Triglyceride / Triacyglycerol

CE : Cholesteryl Ester

LPL : Lipoprotein Lipase

LRP : LDL Receptor related Protein

HL : Hepatic Lipase

ABCA1 : ATP-Binding Cassette Transporter 1

LCAT : Lecithin Cholesteryl Acyl Transferase

SR-B1 : Scavenger Receptor B1

CAD : Coronary Artery Disease

TNF α : Tumor Necrosis Factor α

IL : Interleukin

FGF : Fibroblast Growth Factor

SAA : Serum Amyloid A

AIP : Atherogenic Index of Plasma

LTI : Lipid Tetrad Index(LTI),

CRI-I : Castelli's Risk Index-I (CRI-I),

CRI-II : Castelli's Risk Index-II (CRI-II),

AC : Atherogenic Coefficient (AC)

NCEP ATP III:National Cholesterol Education Program-Adult Treatment Panel III

CRIC : Chronic Renal Insufficiency Cohort study

INTRODUCTION

INTRODUCTION

Chronic kidney disease has emerged a public health problem with worldwide prevalence of 8% to 16%. CKD is the 12th major cause of death and the 17th cause of disability globally.[1] Poor recognition of risk factors in early stages of CKD contributes to significant morbidity and mortality.[2]

About 6% of the adult population in the United States was found to have CKD at stages 1 and 2 and the proportion of this group progressing to advanced stages of CKD is not known. About 4.5% of the U.S. population is estimated to have stages 3 and 4 CKD.[3]

In India, Diabetes and Hypertension account for 40% - 60% cases of CKD.[4] In South India, the main causes of CKD are diabetic nephropathy (29.6%), chronic interstitial nephritis (20.4%), chronic glomerulonephritis(17.4%) and hypertensive nephropathy(11%).[5]

In Indian population, the prevalence of CKD is 13%-15.04%.[6] These individuals are at a higher risk of Cardiovascular disease compared to the general population. Only a small percentage of CKD patients (0.5–1%) reach ESRD, while most of them(19-24%) die mostly of cardiovascular complications, before reaching ESRD. Cardiovascular disease is thus an important cause of mortality and morbidity in CKD. Studies have clearly demonstrated an association between CKD and increased cardiovascular mortality and more so with ESRD.[7,8]

In CKD patients who progress to ESRD, the prevalence of clinical coronary heart disease is 40% and mortality due to CVD is 10 to 30 times higher than in the general population of same gender,age and race. American Heart Association has recommended that patients with chronic impaired renal function should be classified in the highest risk group for developing cardiovascular events.[9,10]

Although there are numerous risk factors that contribute to cardiovascular disease in patients with CKD, one potentially modifiable risk factor is Dyslipidemia. Significant alterations in metabolism of lipoproteins are demonstrated in these patients, which in the later stages result in severe dyslipidemia.[11]

In CKD, Lipid profile varies depending on the stage and associated proteinuria. In the absence of abnormal lipid profile, possibility of CVD cannot be ruled out.[11] Lipoprotein(a) is a widely accepted risk factor for CAD.[12] In developing countries like India, owing to some factors like high cost of standard tests such as Serum Lipoprotein(a), measurement of Apoprotein levels, evaluation of cardiovascular risk assessment can be undermined, in CKD.

Many studies have shown that different combinations of the lipid profile parameters, known as Atherogenic ratios or novel Lipid indices can be used to measure the total burden of dyslipidemia as it waives the need for various cutoff points for individual lipid parameter. These are the calculated fractions which can be used in the clinical setting for assessing the risk of cardiovascular disease, beyond the routinely done lipid profile.[13]

REVIEW OF LITERATURE

CHRONIC KIDNEY DISEASE

Chronic Kidney Disease is defined as a spectrum of continuing pathophysiologic processes characterised by an irreversible and usually progressive decrease in number and function of nephrons, indicated by a reduction in Glomerular filtration rate.[14,15]

The National Kidney Foundation-Kidney Dialysis Outcomes Quality Initiative (K/DOQI) defines CKD as

"Glomerular Filtration Rate (GFR) less than or equal to 60ml/min/1.73m^2 that is present for ≥ 3months with or without evidence of kidney damage or evidence of kidney damage with or without decreased GFR that is present for ≥3months as evidenced by microalbuminuria, proteinuria, glomerular hematuria, pathological abnormalities, anatomical abnormalities" [16]

According to National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) classification system, Chronic Kidney Disease is classified into 6 stages based on eGFR values.[15,16]

STAGE	**DESCRIPTION**	**GFR (ml/min/1.73m^2)**
0	At increased risk	≥90(with CKD risk factors)
1	Kidney damage with normal or ↑GFR	≥90
2	Kidney damage with mild ↓GFR	60-89
3	Moderate ↓GFR	30-59
4	Severe ↓GFR	15-29
5	Kidney failure (end stage kidney disease)	< 15

Major Causes of CKD are :[14]

1. Diabetic nephropathy

2. Glomerulonephritis

3. Hypertension-associated CKD

    a) Primary glomerulopathy with hypertension

    b) Vascular and ischemic renal disease

4. Autosomal dominant polycystic kidney disease

5. Other cystic and tubulointerstitial nephropathy

6. Drugs

Pathophysiology of CKD :[15]

Multiple risk factors interact to instigate a vicious cycle of progressive nephron loss, that leads to a gradual reduction in GFR.

Risk factors are grouped into :

1. Susceptibility factors: Factors such as family history, metabolic syndrome, diabetes mellitus, hypertension, dyslipidemia increase the risk of individual to develop CKD.

2. Initiation factors: Factors such as primary glomerulopathies, nephrotoxins directly cause damage to kidney

3. Progression factors: Factors like hypertension, diabetes mellitus, dyslipidemia, smoking facilitate the progression of damage to kidney once CKD has developed.

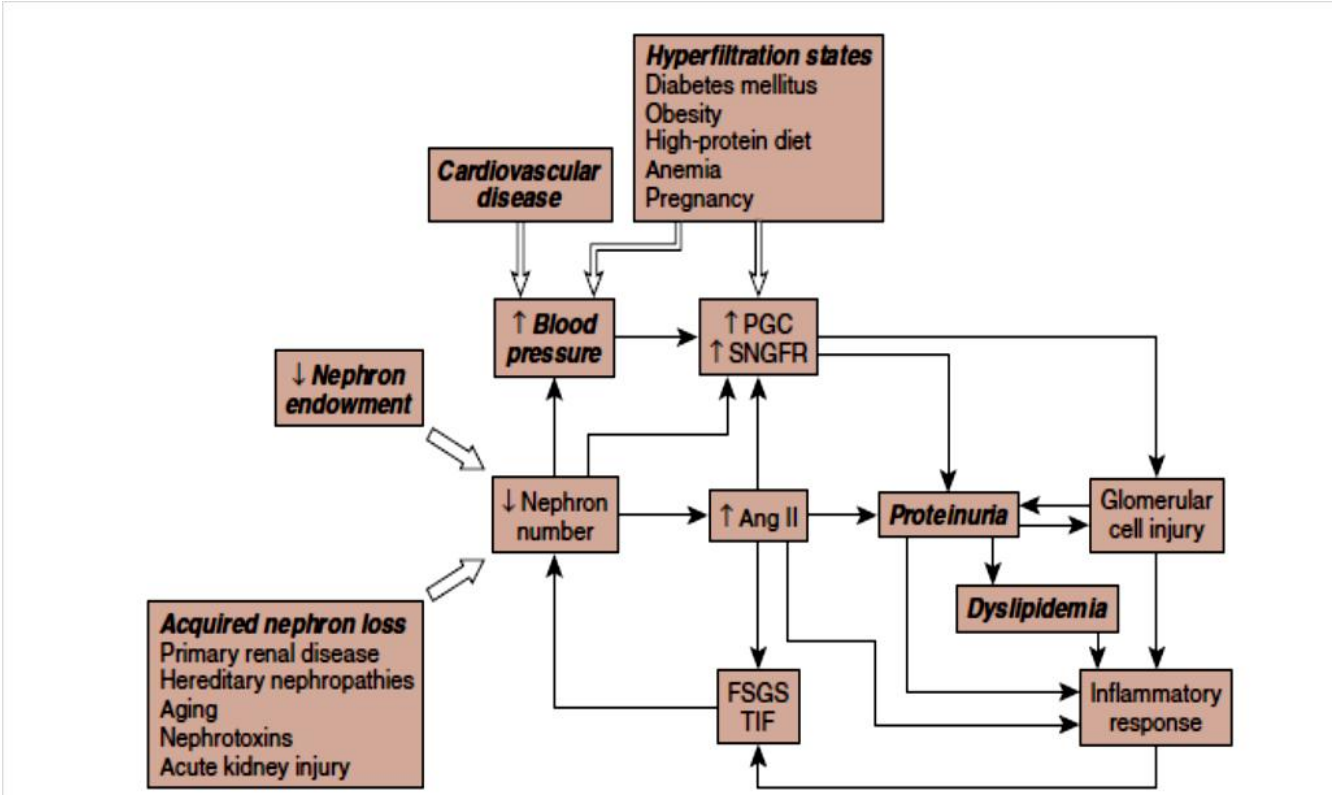


Figure 1: Numerous interrelated mechanisms such as proteinuria, hyperfiltration states, inflammatory responses contribute to the progression of CKD[15]

Cardiovascular Abnormalities : [14]

Cardiovascular disease is the most important cause of morbidity and mortality in patients with CKD at every stage.

1. The increased prevalence of ischemic vascular disease in CKD is attributed to both traditional risk factors and non-traditional CKD related risk factors (anaemia, hyperparathyroidism, CKD induced dyslipidemia, increased FGF-23, generalized inflammation). The inflammatory state accelerates the vascular occlusive disease.

2. Salt and water retention that occurs in CKD, in conjunction with impaired cardiac function secondary to myocardial ischemia, left ventricular hypertrophy leads to heart failure and pulmonary edema.

3. Hypertension is a common complication of CKD. It starts to develop in the earlier stages of CKD. Long standing hypertension and extracellular fluid volume overload lead to the development of left ventricular hypertrophy and dilated cardiomyopathy.

Fluid and electrolyte disturbance :[14]

- Volume expansion & Hyponatremia - glomerulotubular imbalance in sodium homeostasis i.e. dietary sodium >> urinary excretion resulting in

sodium retention and consequent expansion of extracellular fluid volume leading to edema, hypertension and congestive heart failure.

- Fluid depletion in prerenal fluid losses like gastrointestinal loss because of inability of the kidneys to conserve water.
- Hyperkalemia is common due to defective secretion of potassium in the distal nephron leading to fatal arrhythmias.
- Hypokalemia due to reduced intake, gastrointestinal losses and overuse of diuretics is very rare.

Other features are

Neuromuscular, Endocrine, Pulmonary, Dermatologic, Gastrointestinal, Hematologic and Immunological disturbances.

Clinical presentation :[14]

May be

- Asymptomatic - high creatinine levels in routine screening as an incidental finding - Association with diabetes, hypertension.
- Symptomatic – non-specific features of CRF - anorexia, lethargy, dyspnea and 5% of cases landing up in uremia requiring emergency dialysis.

Lipoprotein (a)

Lipoprotein(a) is an inherited cholesterol rich particle; resembles LDL in lipid composition. Unlike LDL, the protein component of Lp(a) is composed of highly glycosylated Apoprotein(a) covalently bound to Apo B 100 through disulfide bridges.[17] Lipoprotein(a) seems to be directly secreted from the liver,and there are no lipoprotein precursors for Lp(a).[18]

Human Apo(a) contains 2 domains:

1. Serine protease domain
2. Multiple kringle repeats : repeat sequences of 80 to 90 aminoacids arranged in triple loop tertiary structure and tandemly arrayed. [19]

   The number of kringle IV type 2 repeats determines the size heterogeneity of Apo(a). The size of apo(a) isoform is genetically determined.[20]

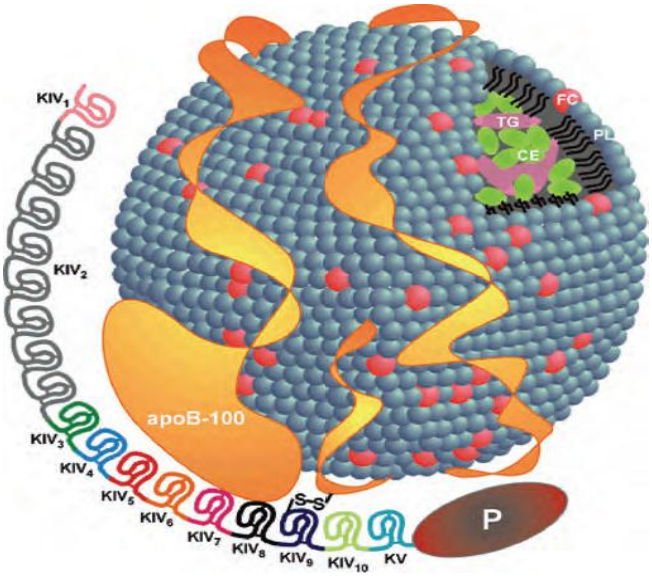


Figure 2: Structure of Lipoprotein(a) particle[27,28]

Role of Lipoprotein(a) in atherosclerosis :

Lp(a) is a 'sticky' particle, which binds to glycosaminoglycans, proteoglycans, collagen and other connective tissue structures.[21] It is immunochemically related to plasminogen. Plasminogen is a zymogen found in plasma. Upon activation, it forms plasmin which causes fibrinolysis. Apo(a) moiety [kringle IV & V repeats] of Lp(a) has about 70% aminoacid sequence homology to plasminogen.[22]

By different ways, Lp(a) interferes with fibrinolytic activity of plasminogen.

1. Because of the structural homology, Lp(a) inhibits the binding of plasminogen to it's receptor on endothelial cells. Thus, the activation of plasminogen to plasmin is prevented.
2. Lp(a) competes with plasminogen for binding sites on fibrin, interfering with the action of plasminogen in causing fribrinolysis.
3. Lp(a) attaches to lysine residues on fibrin in atherosclerotic plaques inhibiting fibrinolysis.

Lp(a) levels of about 30 mg/dL has traditionally been used as an atherogenic cutoff, above which elevated levels of Lp(a) are associated with an increased risk of cardiovascular disease.[20] The European Atherosclerosis Society recommended screening for Lp(a) in a consensus report, in which the desirable cut off was set at less than 50 mg/dL.[26]

Lipoprotein(a) and Cardiovascular disease risk:

Lipoprotein(a) is a major independent genetic risk factor for cardiovascular disease.[27]

Elevated Lp(a) levels associate robustly and specifically with increased risk of cardiovascular disease. This association is continuous and does not depend on the presence of other CVD risk factors. Lp(a) levels, like elevated LDL, is causally related to premature development of atherosclerosis and CVD.[26]

In healthy individuals, there exists a negative association between serum Lp(a) levels and apo(a) isoform size.[9] Studies have shown that subjects with small apo(a) phenotypes have a two fold risk of CVD and stroke compared with those with larger isoforms of apo(a).[9,11]

METABOLISM OF LIPOPROTEINS- AN OVERVIEW

1. Liver and Intestinal metabolism of Triglyceride rich ApoB containing Lipoproteins :[11,20]

ApoB-48 Chylomicron (CM) particles assembled in the enterocytes (exogenous pathway) and ApoB-100 Very low density lipoprotein (VLDL) particles produced in the hepatocytes (endogenous pathway) are released into the circulation.These nascent CM and VLDL particles acquire ApoE and ApoC from HDL particles in circulation and become functionally mature. ApoC II activates the enzyme Lipoprotein Lipase (LPL),while the ApoC I and ApoCIII have inhibitory roles. LPL hydrolyzes triglycerides in mature CM and VLDL particles in the capillaries of perfused skeletal muscle and adipose tissue. This leads to the release of free fatty acids which are taken up by adipocytes/myocytes and CM remnants and VLDL remnants(Intermediate density lipoproteins [IDL]) are formed.

CM remnants are removed by the liver via LDL receptor related protein(LRP), after triglyceride and phospholipids are hydrolyzed by Hepatic Lipase (HL).

Most of the IDL particles are subjected to further hydrolysis by HL to becomes Low Density Lipoproteins (LDL). LDL particles are taken up by

the LDL receptor. Rest of the IDL particles are taken up by the LRP. VLDL are also removed via the VLDL receptor in adipocytes/myocytes. [11,20]

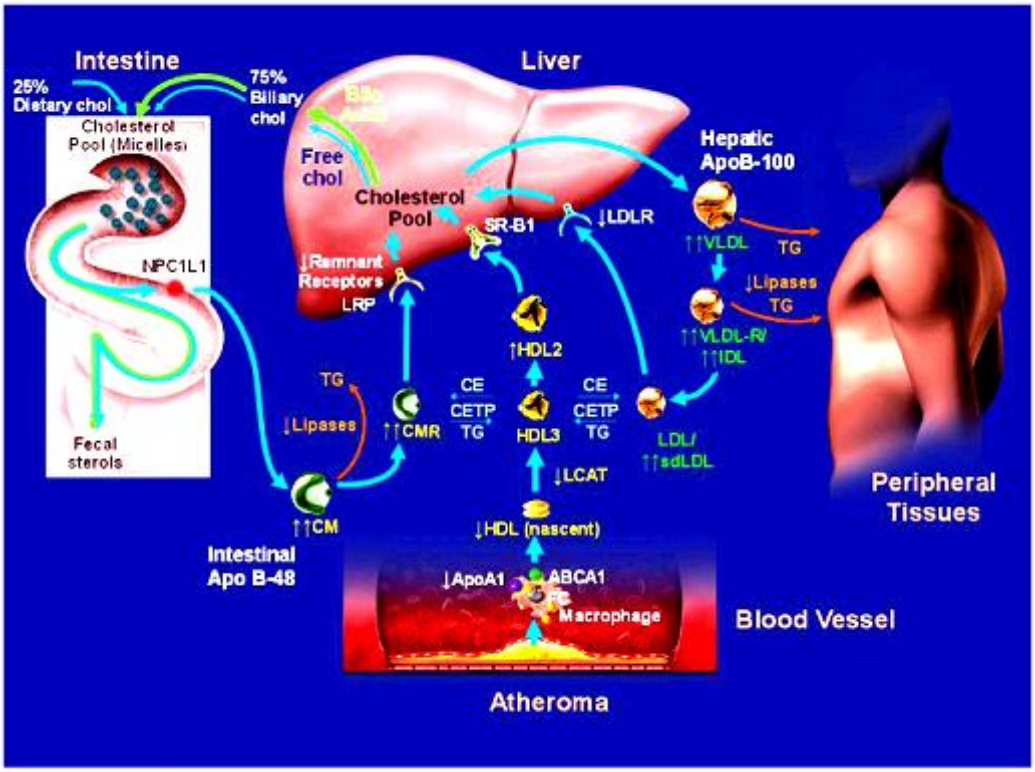


Figure 3: Overview of Lipoprotein metabolism & Effect of CKD on ApoB, ApoA1 and HDL metabolism[11]

2. <u>Metabolism of ApoA1 and High Density Lipoprotein</u> :[11,20]

The Reverse Cholesterol Transport process operates to remove the excess cholesterol from peripheral tissues via the High density lipoproteins (HDL), to be disposed in the liver. The free cholesterol (FC) effluxes from the cell to the surface of lipid-poor ApoA1 via the ATP-binding cassette

transporter-1(ABCA1). Nascent ApoA1 particles becomes mature HDL particles through the esterification of free cholesterol to cholesteryl-esters (CE) by the enzyme Lecithin-Cholesteryl Acyl Transferase (LCAT). CE-rich HDL particles enter the circulation. These are then, taken up directly by the liver through Hepatic Scavenger Receptor (SR-B1) or indirectly after exchange of Cholesteryl Esters in HDL for Triglycerides in ApoB containing lipoproteins (LDL, VLDL, CM, IDL) by Cholesteryl Ester Transfer Protein (CETP).[11,20]

DYSLIPIDEMIA IN CHRONIC KIDNEY DISEASE

Dyslipidemia can be defined as any or a combination of the following: Total cholesterol (TC) >200mg/dl, HDL < 40 mg/dl, LDL > 135 mg/dl and TG >150mg/dl.[29] In Chronic kidney disease, Dyslipidemia is prevalent. Both qualitative and quantitative derangements occur in all lipoprotein classes depending on the stage of renal impairment, etiologies, presence of nephritic syndrome, and the method of dialysis.[9] With impaired renal function, abnormal removal of lipoproteins is an important contributor of lipid abnormalities.[30] The pattern is unique in CKD and is characterized by hypertriglyceridemia, with accumulation of VLDL, both CM and VLDL remnants, reduced concentration

HDL, and predominance of atherogenic small-dense LDL (sd LDL). Total cholesterol and LDL cholesterol are generally not elevated. Lp(a) levels are reported to be raised in CKD population.[32,36]

Standard Lipid Panel

	Predialysis CKD (Stages 3-4)	Nephrotic syndrome (Stages 3-4)	Hemodialysis (Stage 5)	Peritoneal dialysis (Stage 5)
Total Cholesterol[1]	↔	↑	↔	↔ or ↑
LDL Cholesterol[2]	↔ or ↓	↑	↔ or ↓	↑
Triglycerides[2]	↑	↔ or ↑	↑	↑
HDL Cholesterol[2]	↓	↓ or ↔ or ↑	↓	↓

Additional Lipid Parameters

	Predialysis CKD (Stages 3-4)	Nephrotic syndrome (Stages 3-4)	Hemodialysis (Stage 5)	Peritoneal dialysis (Stage 5)
Small dense LDL[2]	↑	↑	↑	↑
Lipoprotein(a)[2]	↑*	↑	↑	↑
Lipase activity[1]	↓	↓	↓	↓
Apolipoprotein B[1]	↑	↑	↑	↑
Apolipoprotein AI[1]	↔ or ↓	↑	↔ or ↓	↔ or ↓

↑ = Increase; ↓ = decrease; ↔ = no change; *Mainly in individuals with high molecular weight apolipoprotein(a) phenotypes
1. Kwiterovich O. *The Johns Hopkins Textbook of Dyslipidemia.* (New York, NY: Wolters Kluwers/Lippincott Williams & Wilkins,2010), 134; 2. Tsimihodimos V et al. *Am J Nephrol.* 2008;28(6):958–973.

Figure 4: Serum levels of lipids in patients with chronic kidney disease varies across stages[11]

Metabolic disorders of Triglyceride rich Lipoproteins in CKD :

Hypertriglyceridemia is the most prevalent lipid abnormality in CKD. The predominant mechanism contributing to raised triglyceride rich lipoproteins in predialysis stage, is delay in the catabolism of ApoB containing lipoproteins.[9,11] Clearance of TG from VLDL , CM and their remnants is impaired.

This is attributed to :

- Diminished Lipoprotein Lipase (LPL) activity in CKD[46]:

- Increased levels of ApoCI occur in Uremic plasma which inhibit LPL[47]
- Elevateds levels of ApoCIII is seen, which is an inhibitor of LPL. Also, it suppresses the binding of lipoprotein remnants to (LRP)LDL receptor-related protein[48].
- Reduced ApoCII and ApoE in the composition of VLDL and CM is also responsible for decreased LPL activity[32].

- Decreased activity of Hepatic Lipase and diminished gene expression of the enzyme:
  - This is attributed partly to secondary hyperparathyroidism and dysregulation of cytosolic calcium observed in CKD[49].
- Moreover, decreased activity of Hepatic LRP[48], Downregulation of mRNA for VLDL receptor[50] and it's protein expression have been implicated to contribute to decreased removal of VLDL,CM and their remnants from circulation.
- Other factors which lead to increased concentrations of Triglycerides and VLDL are:
  - Insulin resistance develops during the progression of CKD, which drives the overproduction of VLDL.

- Hypoalbuminemia and proteinuria decrease the efficiency of LPL-induced lipolysis of Triglyceride rich lipoproteins by interfering with the endothelial binding of the enzyme LPL[11,9].

Abnormalities of ApoA containing Lipoproteins in CKD :

HDL removes cholesterol from peripheral tissues, decreases inflammatory processes, limits oxidative stress, inhibits blood clotting mechanism[11].

- In Chronic kidney disease, there prevails an environment of increased oxidative stress and pro-inflammatory state[11].
- High degree of oxidative modification of apoproteins occurs, which leads to low production of HDL particles.
- Hepatic ApoA-I synthesis is decreased, and it's catabolism increased[54].
- Synthesis of LCAT is reduced which results in limited maturation of HDL because of decreased esterification of free cholesterol[11].
- High levels of inflammatory markers are found in circulation in CKD, namely hsCRP, TNF α, IL-1, IL-6, FGF-23.These interact with HDL and make it dysfunctional[55].
- Serum Amyloid A(SAA) is increased in HDL, which is accompanied by a reduction in the anti-inflamatory capacity of HDL. SAA stimulates the production of inflammatory cytokines[56].

As a consequence of the above events, poorly functional HDL is unable to modulate inflammatory systems and fails to check the oxidative stress.

- Because of diminished LCAT activity and decreased ApoA-I, HDL cannot clear the oxidized phospholipids, lipoperoxides, and endotoxins. HDL, in turn, becomes pro-oxidant and pro-inflammatory[57].

LIPOPROTEIN(a) IN CKD

Raised Serum Lp(a) levels predicts the risk of Cardiovascular disease, although a well defined direct causal relationship has not been established[27,28]. Increased Lp(a) levels are observed in CKD subjects, independent of the isoform of Apo(a) inherited; in particular, the concentrations of High molecular weight (HMW) isoforms of Lp(a) are increased[51]. Raised Lp(a) levels is attributed to increased synthesis in subjects with nephrotic syndrome, while in dialysis patients, the predominant mechanism is believed to be impaired removal due to the diminished catabolism. Although HMW isoforms of Lp(a) are increased in CKD subjects, risk of CVD is primarily associated with high levels of the (LMW) Low molecular weight isoform in these subjects[52,53].

"In predialysis CKD, an isoform-specific increase in Lp(a) is observed. CKD subjects with HMW apo(a) isoforms have much higher Lp(a) values than

apo(a) phenotype-matched healthy controls, whereas CKD subjects with LMW apo(a) isoforms have similar Lp(a) concentrations with phenotype-matched healthy individuals, who already have high Lp(a) levels". [11,53]

NOVEL LIPID INDICES

Many Indices had been derived from lipid profile to predict the risk of CAD. Several markers have come into vogue such as apolipoprotein levels, plasminogen activator inhibitor, inflammatory markers in evaluation of CAD risk. However, it still remains controversial whether these markers contribute to CAD risk independently of traditional risk factors and lipid variables.

NCEP guidelines recommend LDL concentration be regarded the primary therapeutic target (raised serum LDL levels are strongly proven to be atherogenic), and HDL levels may also be used in assessment of CAD risk.[58] Based on this, the ratio LDL:HDL was contemplated in risk evaluation. This is referred to as Castelli's Risk Index-II (CRI-II). It indicates the two way transport of cholesterol into and out of arterial intima, indirectly.[59]

Prospective studies have demonstrated that high LDL:HDL ratio accompanied by raised serum triglyceride levels is associated with high risk of cardiovascular disease. This lipid triad has been referred to as atherogenic dyslipidemia.[60]

In individuals with raised serum triglycerides, there is more content of cholesterol in VLDL than in LDL; in this setting, LDL:HDL ratio is liable to underevaluate the impact of dyslipidemia. Hinged on this, it was proposed that the ratio TC:HDL can be used in prediction of CAD risk.[61] This is referred to as Castelli's Risk Index-I (CRI-I). Framingham study [62] showed that the CAD risk increased parallel to increase in TC:HDL ratio. It depicts both the deleterious effects of Non HDL and the protective effects of HDL.

Non HDL cholesterol reflects the sum total of cholesterol found in all lipoproteins, except HDL, namely: VLDL, chylomicrons and their remnants, LDL, IDL. Non HDL correlates with Apo B concentration which reverberates the atherogenic potential of all LDL particles, including small dense LDL.[63] According to NCEP ATP III Guidelines, Non HDL goal in the coronary heart disease risk equivalent group should be less than 130 mg/dL.[70,71]

Atherogenic Coefficient is an estimate of cholesterol content in VLDL, LDL fractions relative to protective HDL cholesterol. Individuals with AC values <3.25 carry low risk of CAD. It expresses the atherogenic potential of the spectrum of lipoprotein fractions intoto.[64]

Castelli et al. explored how well summary estimates of cholesterol (LDL:HDL and TC:HDL ratios) predict the development of coronary heart disease when considered alone or in the presence of joint configuration of individual levels of cholesterol values. The study demonstrated that both these ratios had

strong associations with CAD; thus risk of disease be evaluated. Low risk values in individuals free of coronary artery disease: CRI-I : <5.1, CRI-II: <3.3. However, neither ratio by itself can be as informative about the CAD risk as the information expressed in the configuration of specific cholesterol values. [65]

Gaziano et al. contemplated the ratio TG:HDL as an index proven to be an extremely significant prognosticator of myocardial infarction, superior to TC:HDL and LDL:HDL ratios.[41]

Atherogenic Index of Plasma theoretically reflects the balance between risk and protective lipoprotein forces; calculated as AIP=Log_{10} {[TG]/[HDL]}, where both are expressed in molar concentrations. AIP is a surrogate measure of small dense LDL particles. Studies have shown that AIP values of : -0.3 to 0.1 are associated with low, >0.1 to 0.24 with medium, and above 0.24 with high cardiovascular disease risk.[42,43] Optimal value should be less than 0.1 .[42] CVD risk is a function of the size of LDL and HDL particles. Cholesterol esterification rate in HDL (FER_{HDL}) strongly correlates with lipoprotein particle sizes; thus regarded as a functional risk marker. AIP, the logarithmically transformed ratio of TG:HDL is the best determinant of FER_{HDL}.[43]

Lipid Tetrad Index (LTI) described by Enas et al., relates the product of atheogenic particles, TC, TG and Lp(a) to non atherogenic HDL particle. It reflects the overall lipid profile of individuals.[66] Low risk values in general population for LTI is <20000. [66,69]

A typical lipid panel in CRF encompasses a combination of qualitative and quantitative abnormalities with a predominance of small dense LDL particles. Accumulation of modified lipoprotein particles occurs even in the early, predialysis stage of CKD. Plasma lipids and apoproteins may thus be inadequate predictors of CVD risk in CRF induced dylipidemia, especially in the early stages of kidney disease. Novel Lipid Indices have been shown to reflect the atherogenic lipoprotein phenotype seen in CKD involving the delicate metabolic interrelations within the whole lipoprotein system.[36]

AIM & OBJECTIVES

AIM :

To evaluate Serum Lipoprotein(a) levels and assess the significance of novel Lipid Indices in predialysis stage of patients with Chronic kidney disease.

OBJECTIVES :

1. To estimate Fasting Lipid Profile in Controls and Chronic Kidney disease subjects
2. To calculate Lipid Indices:

   Atherogenic Index of Plasma (AIP),

   Lipid Tetrad Index(LTI),

   Castelli's Risk Index-I (CRI-I),

   Castelli's Risk Index-II (CRI-II),

   Atherogenic Coefficient (AC)
3. To estimate Serum Lipoprotein (a) levels
4. To correlate between Serum Lipoprotein(a) levels and novel Lipid indices across Stages 1 to 5 of Chronic Kidney disease (predialysis stage)

MATERIALS & METHODS

MATERIALS AND METHODS

Study centre :

Nephrology clinic, Govt Stanley Hospital &

Department of Biochemistry, Stanley Medical College, Chennai-1.

Duration of the study :

6 months (January 2016 to June 2016)

Study Design :

Analytical Study

Study population:

Sample size: 140

70 cases & 70 controls

Cases:

Inclusion criteria:

Patients of both sexes, aged between 20 to 60yrs diagnosed as a case of Chronic Kidney Disease-in the predialysis stage were included in this study.

Exclusion criteria:

- ✓ History of cigarette smoking,
- ✓ History of Ishemic heart disease and vascular diseases,
- ✓ History of taking lipid lowering drugs,
- ✓ History of taking drugs causing dyslipidemia,
- ✓ History of chronic liver disease

The cases are selected on the basis of simple random sampling method.

Controls:

For each Case, healthy age and sex matched Control was selected.

METHODS:

Sample Collection and Preparation:

After getting informed consent from the patients, morning fasting blood sample was collected under strict aseptic precautions in plain red topped venipuncture tubes without any additives or gel barrier. The blood was allowed to clot. After centrifugation at 2000-2500 rpm for 15 minutes, Serum was separated immediately from the samples. Serum samples were stored at -20ºC in deep freezer upto 30days.

Study population was examined and the following tests were done in the serum,

(i) Renal Function Tests:

- **Serum urea**
- **Serum Creatinine**
- **Estimated GFR**

Estimated GFR (eGFR) was calculated using Modification of Diet in Renal Disease(MDRD)formula and CKD subjects were classified into 6 stages.

According to NKF-K/DOQI classification system, Staging of Chronic Kidney Disease was done as follows

Stages	GFR ml/min
0	> 90[a]
I	≥ 90[b]
II	60-89
III	30-59
IV	15-29
V	< 15

a - With risk factors for CKD.

b - With demonstrated kidney damage

(ii) Estimation of Serum Lp(a)

(iii) Lipid Profile:

- **Serum Total Cholesterol**
- **Serum Triglycerides**

- **Serum LDL:** calculated using Friedewald equation:

  LDL = Total Cholesterol - HDL-(Triglyceride/5)

  Reference Interval : Adults

  Desirable: <130mg/dL [20,45]

- **Serum HDL**
- **Non HDL =** Total Cholesterol – HDL
- **Serum VLDL =** TGL/5

  Reference Interval : Adults

  Desirable : <30 mg/dL[20,45]

(iv) LIPID INDICES:

1. Atherogenic Index of Plasma (AIP) = $\log_{10}$ [TG/HDL], (Milada, 2004)[67]

   In which concentrations of TG and HDL are expressed in molar concentrations

2. Lipid Tetrad Index = [Total Cholesterol * Triglycerides

   * Lipoprotein (a)] / HDL (Rajappa et al., 2006)[69]

3. Castelli's Risk Index-I (CRI-I) = TC/HDL (Castelli et al., 1983)[65]

4. Castelli's Risk Index-II (CRI-II) = LDL/HDL (Castelli et al., 1983)[65]

5. Atherogenic Coefficient (AC) = (TC– HDL)/HDL (Brehm et al., 2004)[68]

ESTIMATION OF BLOOD UREA :

Method:

Urease coupled with glutamate dehydrogenase method.

Test principle

Urea is hydrolyzed by Urease to form ammonium and carbonate ions.

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{urease}} 2\ NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium ion in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate.

In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + NADH \rightarrow \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically at 340 nm.

REAGENTS COMPOSITION:

R1 : TRIS buffer: 50 mmol/L, pH 8.0; 2-oxoglutarate: 15 mmol/L;

Urease (jack bean): ≥1000 IU/L

GLDH (bovine liver): ≥ 6000 IU/L

R2 : NADH: 0.18mmol/L

SYSTEM PARAMETERS

Mode of reaction	Fixed time kinetic
Slope of reaction	Decreasing
Wavelength I	340nm

Temperature	37ºC
Standard concentration	50 mg/dL
Linearity	300 mg/dL
Blank Absorbance	>0.8
Delay/lag Time	30 seconds
Interval time	60 seconds
No. of readings	1
Sample volume	10 µL
Reagent volume	1000 µL
cuvette	1cm light path
Low normal at 37ºC	15mg/dL
High normal at 37ºC	50mg/dL

LABORATORY PROCEDURE:

	Standard	Sample
Standard	10 µL	-
Sample	-	10 µL
Reagent	1000 µL	1000 µL

Mix well and read after 30secs (initial absorbance of sample A_{1s} and standard A_{1std}) and read again after 60secs (A_{2s} and A_{2std}).

Tests were assayed on Beckman Coulter AU 480 autoanalyser after calibration.

CALCULATION:

$$Urea\ mg/dL = \frac{A_{2s} - A_{1s}}{A_{2std} - A_{1std}} \times 50 mg/dL$$

Reference intervals: [31]

- Plasma or serum

Healthy adults : 6 to 20 mg/dL

Adults > 60 years of age : 8 to 23 mg/dL

Untreated ESRD : 108 to 135 mg/dL

Urine, 24hrs : 12–20 g/day

ESTIMATION OF SERUM CREATININE :

Method: Modified Jaffe's kinetic method

Principle:

Creatinine in Serum reacts with alkaline picrate to form a colored complex. The rate of formation of colored complex is directly proportional to creatinine concentration. The rate of reaction is measured photometrically at 510 nm and compared with that of the standard.

Creatinine + Alkaline picrate $\longrightarrow$ Creatinine picrate complex (orange red)

REAGENT COMPOSITION:

Picric acid : 10 mmol/L

Sodium hydroxide : 100 mmol/L

Creatinine Standard : 2 mg/dL

SYSTEM PARAMETERS:

Mode of Reaction	Fixed Time kinetic
Wavelength	510 nm
Cuvette temperature	30 °C
Lag time	20 seconds
Interval	60 seconds
Measuring time	80 seconds
Sample volume	100 µL
Standard concentration	2 mg/dL
Reagent volume	1 mL
Light path	1 cm
Zero setting with	Distilled water
Linearity	12 mg/dL

LABORATORY PROCEDURE:

	Standard	Sample
Reagent (mL)	1000 µL	1000 µL
Standard (mL)	100 µL	-
Sample (mL)	-	100 µL

Mix and aspirate. Record the absorbane at 20secs (initial absorbance of sample A_{1s} and standard A_{1std}) and read again at 80secs (A_{2s} and A_{2std}).

Tests were assayed on Beckman Coulter AU 480 autoanalyser after calibration.

CALCULATION:

$$\text{Concentration of Creatinine } (mg/dL) = \frac{A_{2s} - A_{1s}}{A_{2std} - A_{1std}} \times 2\ mg/dL$$

Reference Intervals[20,45]:

- Plasma or serum

Adults: Male : 0.9 -1.3 mg/dl

Female : 0.6 - 1.1 mg/dl

- Urine, 24hrs

Adults: Male : 800 - 2,000 mg/day

Female : 600 - 1,800 mg/day

CALCULATION OF eGFR

Kidney function was calculated by using,

Modification of Diet in Renal Disease Formula (MDRD)

Estimated GFR

eGFR(mL/min per 1.73 m^2) =1.86 ×(PCr) $^{-1.154}$×(age)$^{-0.203}$

Multiply by 0.742 for women

ESTIMATION OF SERUM TOTAL CHOLESTEROL:

Method : Enzymatic Colorimetric: Cholesterol oxidase

CHOD – PAP (based on Trinder's methodology)

Principle :

Step 1: Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty Acids

Step 2: Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3-one + H_2O_2

Step 3: $2H_2O_2$ + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$

Quinoneimine dye+4 H_2O

The intensity of the pink colour due to quinoneimine dye formed, is directly proportional to Cholesterol concentration.

REAGENT COMPOSITION:

Goods Buffer (pH – 6.4) : 100 mmol/L

Cholesterol oxidase : > 100 U/L

Cholesterol esterase : > 200 U/L

Peroxidase : > 3000 U/L

4-Aminoantipyrine : 0.3 mmol/L

Phenol : 5 mmol/L

SYSTEM PARAMETERS:

Mode	End Point
Wavelength 1	505 nm
Wavelength 2	670 nm

Sample Volume	10 µL
Reagent Volume	1000 µL
Incubation time	5 minutes
Incubation Temperature	37 °C
Normal Low	50 mg/dL
Normal High	230 mg/dL
Linearity	750 mg/dL
Standard concentration	200 mg/dL
Absorbance limit	0.4
Blank with	Reagent

ASSAY PROCEDURE:

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix well and incubate for 5 minutes at 37°C. Read the absorbance of the test sample and standard against reagent blank.

Tests were assayed on Beckman Coulter AU 480 autoanalyser after calibration

CALCULATION:

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{Absorbance of sample} \times \text{Std conc.mg/dl}}{\text{Absorbance of standard}}$$

Reference intervals[20,45]:

- Plasma or serum

Adults

Serum Total Cholesterol: Desirable : <200 mg/dL

Borderline High : 200-239 mg/dL

High : > 239 mg/dL

ESTIMATION OF SERUM TRIGLYCERIDES :

Method: Enzymatic Colorimetric : Glycero-3-phosphate oxidase method

GPO-TOPS

Principle:

Step 1: Lipase catalyzed hydrolysis of triglycerides to glycerol and free fatty acids

$$\text{Triglyceride} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\text{Fatty acids}$$

Step 2: Glycerokinase phosphorylates the glycerol in an ATP-requiring reaction

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

Step 3: Glycerophosphate oxidase (GPO) oxidizes Glycerol-3-phosphate to dihydroxyacetone phosphate and H_2O_2

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H_2O_2

Step 4: H_2O_2 formed is measured in a peroxidase catalyzed reaction that forms a colored dye

$2H_2O_2$ + 4-Aminoantipyrine + TOPS $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye+ $4H_2O$

The intensity of the colour due to quinoneimine dye formed, is directly proportional to Triglyceride concentration.

REAGENT COMPOSITION:

Pipes Buffer (pH – 7.0) : 5 mmol/L

TOPS : 5.3 mmol/L

Potassium Ferrocyanate : 10 mmol/L

Magnesium salt : 17 mmol/L

4-Aminoantipyrine : 0.9 mmol/L

ATP : 3.15 mmol/L

Lipoprotein lipase : > 1800 U/L

Glycerol kinase : > 450 U/L

Glycerol-3-phosphate oxidase : > 3500 U/L

Peroxidase : > 450 U/L

* TOPS: N-Ethyl-N-sulfopropyl-m-toluidine

SYSTEM PARAMETERS :

Mode of reaction	End Point
Slope of reaction	Increasing
Wavelength 1	546 nm
Wavelength 2	630 nm
Temperature	37 °C
Standard concentration	200 mg/dL
Linearity	1000 mg/dL
Blank with	Reagent
Incubation time	5 minutes
Sample volume	10 µL
Reagent volume	1000 µL
Cuvette	1 cm light path

ASSAY PROCEDURE:

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix well and incubate for 5 minutes at 37°C. Read the absorbance of the test sample and standard against reagent blank.

Tests were assayed on Beckman Coulter AU 480 autoanalyser after calibration

CALCULATION:

$$\text{Triglyceride concentration (mg/dL)} = \frac{\text{Absorbance of sample} \times \text{Std conc.mg/dl}}{\text{Absorbance of standard}}$$

Reference intervals[20,45]:

- Plasma or serum

Adults

Serum Triglycerides: Desirable : <150 mg/dL

Male : 60-165 mg/dL

Female : 40-140 mg/dL

ESTIMATION OF SERUM HDL CHOLESTEROL:

Method : Direct Homogenous Assay

Modified Polyvinyl Sulfonic acid (PVS-PEGME 5th Gen.)Method

Principle:

Polyvinyl Sulfonic Acid (PVS) and polyethylene glycol-methyl ether (PEGME) coupled classic precipitation method. LDL, VLDL, CM react with PVS and PEGME. This reaction results in inaccessibility of LDL, VLDL, CM by cholesterol oxidase(CHOD) and cholesterol esterase(CHER). These enzymes,then,

selectively react with HDL to produce H_2O_2 which is detected by Trinder's reaction.

Step 1 : HDL+LDL+VLDL+CM $\xrightarrow{\text{PVS}}$ HDL+ (LDL+VLDL+CM)**.**PVS/PEGME

Step 2 : HDL $\xrightarrow{\text{CHOD/CHER}}$ Fatty acid + H_2O_2

Step 3 : $2H_2O_2$ + 4-Aminoantipyrine + TODB $\xrightarrow{\text{Peroxidase}}$ Quinone+$4H_2O$ (λ_{max}=560nm)

REAGENT COMPOSITION:

R1 Reagent : MES Buffer(pH-6.5), TODB N,N-Bis(4-sulfobutyl)-3-methylaniline, polyvinyl sulfonic acid, polyethylene glycol-methyl ether, $MgCl_2$, detergent, EDTA

R2 Reagent : MES Buffer(pH-6.5), cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine, detergent

ASSAY PROCEDURE:

4 µL of sample + 300 µL of Reagent 1

↓ Incubate for 5 minutes at 37°C

Measure absorbance 1 bichromatically at 660/546 nm

↓

Add 100 µL of Reagent 2

↓ Incubate for 5 minutes at 37°C

Measure Absorbance 2 bichromatically at 660/546 nm

↓

Calculate HDL concentration by using ΔAbsorbance(=Absorbance1 ~ Absorbance 2)

Other Assay Parameters:

Standard concentration : 49mg/dL

Linearity : 200 mg/dL

Blank with : Reagent

Reagent blank absorbance : <0.20

Cuvette : 1cm light path

Tests were assayed on Beckman Coulter AU 480 autoanalyser after calibration

CALCULATION:

$$\text{HDL concentration(mg/dL)} = \frac{\Delta\text{Absorbance of sample}}{\Delta\text{Absorbance of standard}} \times \text{Std conc.mg/dl}$$

Reference intervals[20,45]:

- Plasma or serum

Adults

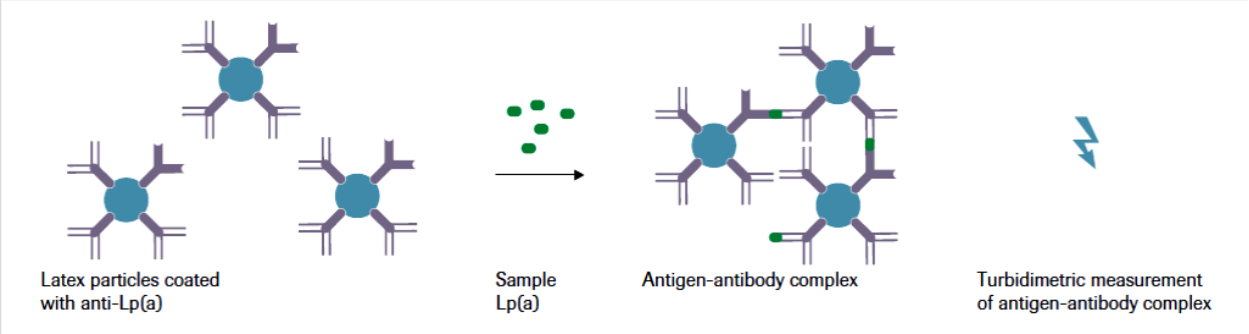
Serum HDL: 40- 60 mg/dL

DETERMINATION OF SERUM LIPOPROTEIN (a):

Method : Immunoturbidimetry

Principle :

Latex particles coated with anti-Lp(a) antibodies agglutinate with Lp(a) in samples. This agglutination causes a change in absorbance, depending on the Lp(a) content in the sample, which is quantified by comparing with a calibrator of known Lp(a) concentration.



REAGENTS:

Diluent (R1) : Glycine buffer (pH-9.0) : 50 mmol/L

Sodium azide : 0.95 g/L

Reagent R2 : Latex particles coated with mouse monoclonal anti-human Lp(a), pH-8.2

Sodium azide : 0.95 g/L

SYSTEM PARAMETERS:

Mode	Multi-Point Cal.

Reaction type	Ascending
Wavelength	570 nm (540-600nm)
Blank with	Distilled water
Sample volume	7 µL
Reagent volume	500 µL
Delay time	10 seconds
Read time	240 seconds
Linearity	90 mg/dL

CALIBRATOR :

The Calibrator is a lyophilized serum of human origin containing Lp(a) of known concentration of 96.6 mg/dL, after reconstitution with 1mL NaCl 9g/L.

The following Lp(a) calibrator dilutions in NaCl 9g/L were prepared. The concentration of each of the Lp(a) calibrators was obtained by multiplying the Lp(a) concentration – 96.6 mg/dL by the corresponding factor.

Calibrator dilution	1	2	3	4	5
Lp(a) Calibrator (µL)	-	25	50	75	100
NaCl 9 g/L (µL)	100	75	50	25	-
Factor	0	0.25	0.5	0.75	1.0
Concentration of Lp(a) Calibrator dilution (mg/dL)	0	24.15	48.30	72.45	96.60

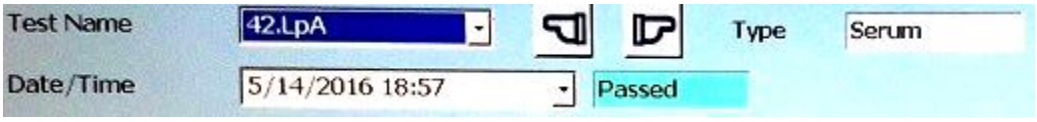
ASSAY PROCEDURE:

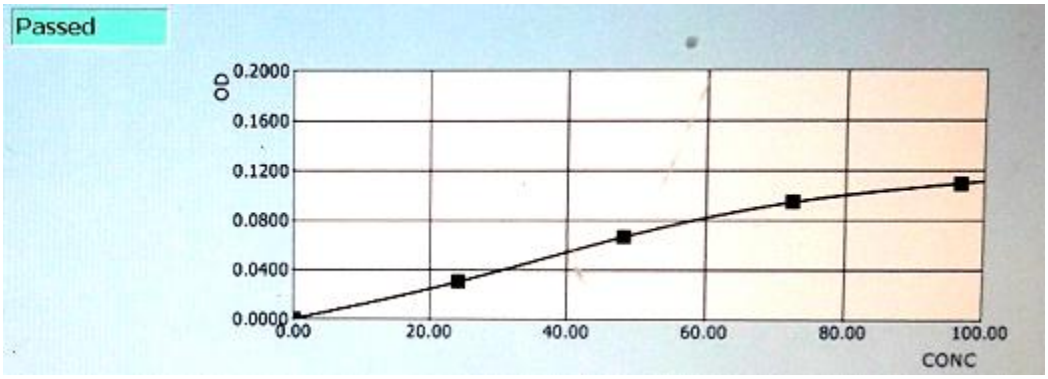
The reagents are brought to temperature of 37°C.

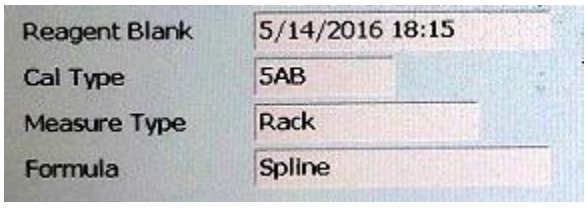
	Blank	Calibrator	Sample
Distilled water	500 µL	-	-
Reagent R1		400 µL	400 µL
Reagent R2		100 µL	100 µL
Calibrator		7 µL	-
Sample		-	7 µL

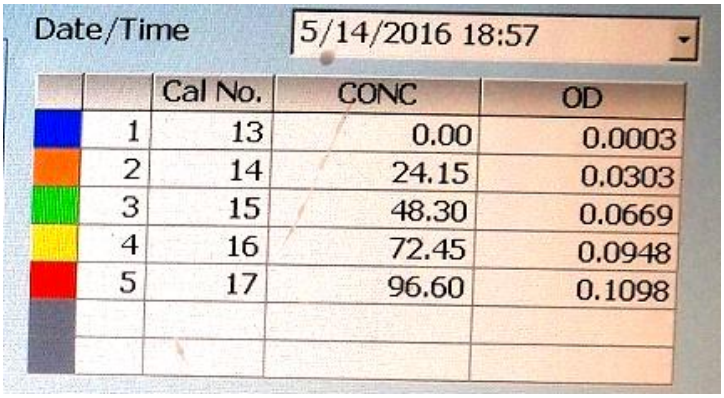
Mix and record the absorbance immediately (A_1) and after 4 minutes (A_2) of the sample dilution.

CALIBRATION REPORT:









Date/Time 5/14/2016 18:57

	Cal No.	CONC	OD
1	13	0.00	0.0003
2	14	24.15	0.0303
3	15	48.30	0.0669
4	16	72.45	0.0948
5	17	96.60	0.1098

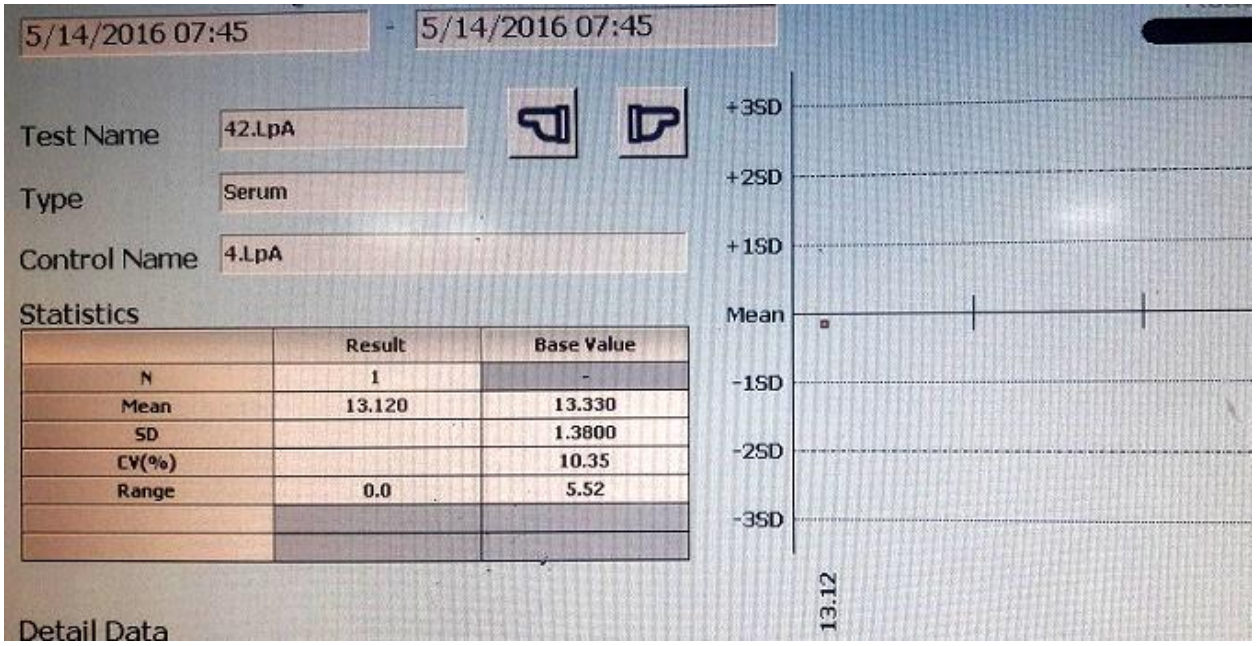
QUALITY CONTROL:

Quality control pool values are within the established ranges.

BIO-RAD Internal Quality Control for Immunological assays

Mean: 13.33 Standard deviation : 1.38

Coefficient of Variation : 10.35 % Range : 10.57 to 16.09



CALCULATIONS:

The absorbance differences (A_2-A_1) of each Lp(a) calibrator was calculated and the values obtained against the corresponding Lp(a) concentration were plotted in a calibration curve.

Lp(a) concentration in the sample was calculated by interpolation of it's (A_2-A_1) in the calibration curve.

Normal Value[20,45]:

Serum Lp(a) : Desirable cut-off : <30 mg/dL

RESULTS

STATISTICAL ANALYSIS AND RESULTS

Results of clinical and biochemical profile obtained in patients with chronic kidney disease were compared with those of the control group by statistical analysis using Excel software. Student's unpaired 't' test was used to compare the means between two independent groups. F test was applied between the study variables to know whether 't' test can be applied to study the parameters and also which type of 't' test –either equal variance or separate variance unpaired 't' test can be applied in this study. Pearson coefficient of correlation was used to estimate the degree of association between two quantitative variables. A p-value of <0.001 will be considered as *statistically significant.*

Table.1 Baseline Characteristics of the Study Population

Parameters	Mean ± SD		**'t' value**	**p value**
	Controls (n=70)	**Cases (n=70)**		
Age	44.23±9.97	48.19±9.24	2.44	<0.001,statistically significant
Glucose	108.39±51.33	140.34±72.36	3.01	<0.001,statistically significant
Urea	24.96±6.65	53.74±27.00	8.66	<0.001,statistically significant
Creatinine	0.78±0.13	2.46±1.69	8.29	<0.001,statistically significant
eGFR	105.80±17.06	34.72±16.70	24.91	<0.001,statistically significant

Graph 1: Comparison of Blood Glucose, Urea, Creatinine and eGFR values between Controls and CKD subjects

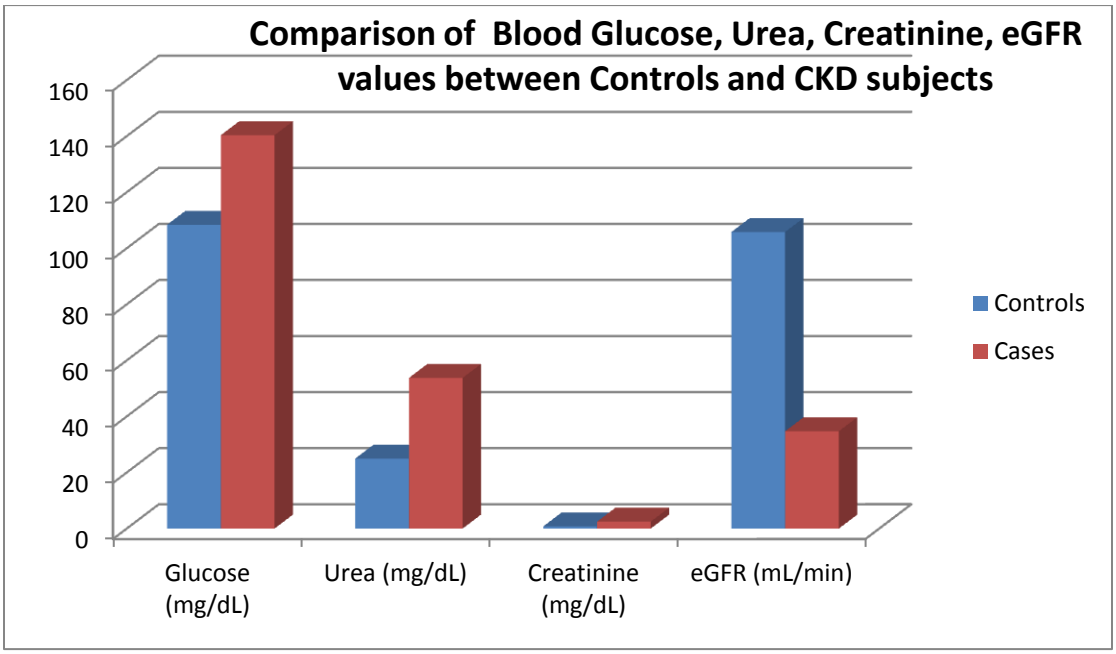


Table 1 and Graph 1 shows the comparison of baseline characteristics between controls and CKD subjects. The data suggests that there exists significant difference in Blood Glucose, Urea, Creatinine, eGFR values between controls and CKD subjects .

Table 2: Gender Distribution among Controls and CKD subjects

Gender	Controls	Cases
Males	42 (60%)	31 (44.3%)
Females	28 (40%)	39 (55.7%)
Total	70 (100%)	70 (100%)

Graph 2:

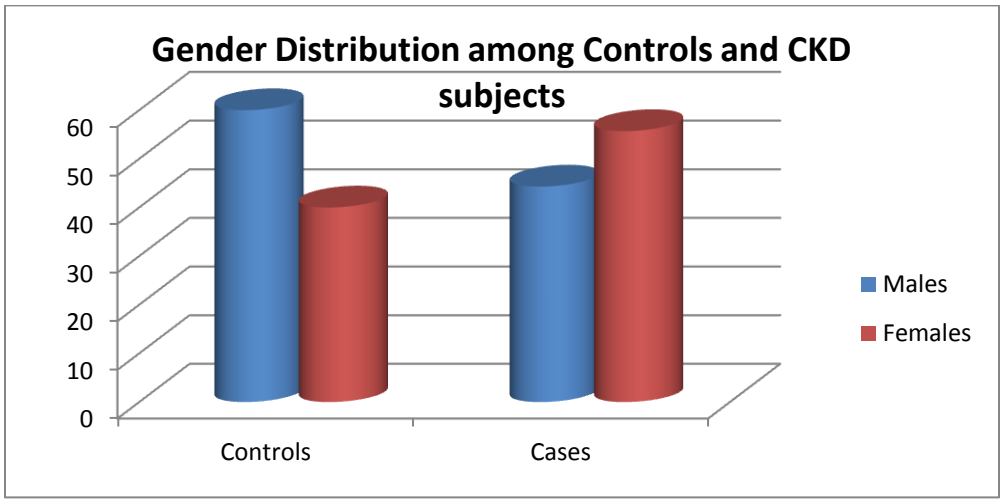


Table 2 & Graph 2 show the Gender Distribution among Controls and CKD subjects. More or less the distribution of both sexes is equal within CKD subjects.

Table 3: Comparison of Prevalence of Diabetes mellitus and Hypertension between controls and CKD subjects

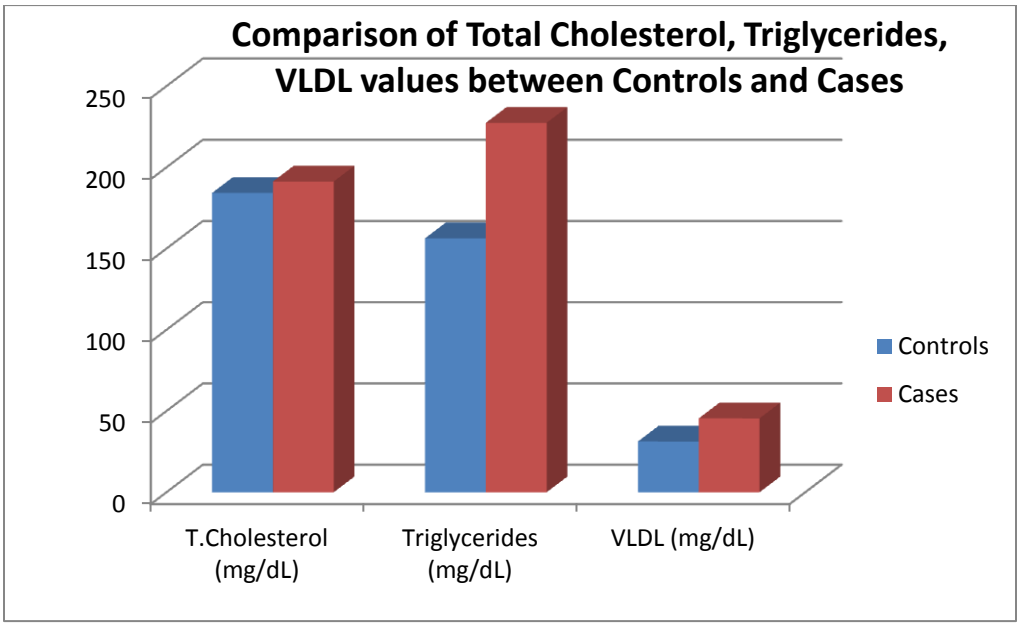
Major etiology	Controls (n=70)	Cases (n=70)
Diabetes Mellitus	12 (17.1%)	36 (51.4%)
Hypertension	15 (21.4%)	47 (67.1%)

Table 3 shows that 67.1% of CKD subjects are hypertensives and 51.4% are diabetics which are significantly higher compared to controls in whom diabetes and hypertension are prevalent in 17.1% and 21.4% of individuals, respectively.

Table 4: Comparison of Lipid Profile parameters between Controls and CKD subjects

Lipid Profile Parameters	Mean±SD		't' value	p value
	Controls (n=70)	**Cases (n=70)**		
Total Cholesterol (mg/dL)	184.26±30.7	191.28±53.62	0.951	0.34,(>0.001) statistically **not significant**
Triglycerides (mg/dL)	156.31±28.28	227.41±109.95	5.239	<0.001,statistically significant
VLDL (mg/dL)	31.26±5.66	45.48±21.99	5.239	<0.001,statistically significant
LDL (mg/dL)	111.61±25.11	105.62±39.76	1.066	0.29, (>0.001) statistically **not significant**
HDL (mg/dL)	41.39±6.18	40.18±7.57	1.036	0.30, (>0.001)statistically **not significant**
Lp(a) (mg/dL)	17.88±14.5	53.26±39.56	7.023	<0.001,statistically significant
Non HDL (mg/dL)	142.87±26.68	151.10±48.64	1.241	0.2,(>0.001)statistically **not significant**

Graph 3: Comparison of Total Cholesterol, Triglycerides, VLDL values between Controls and CKD subjects



Graph 4: Comparison of LDL, HDL, Lp(a) values between Controls and CKD subjects

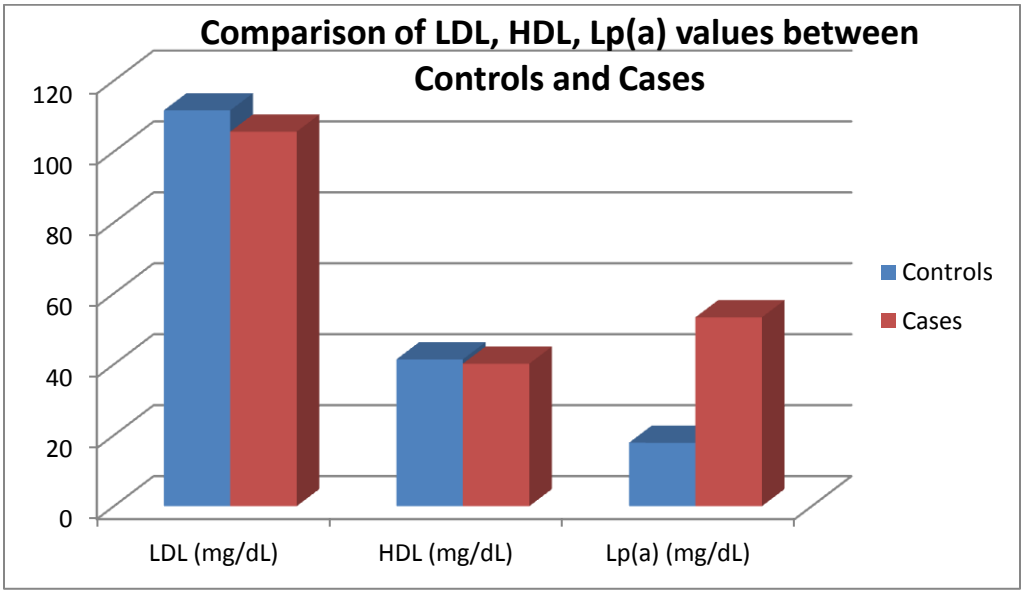


Table 4 , Graphs 3 & 4 show the comparison of lipid profile variables between Controls and CKD subjects. The data indicates that there exists statistically significant differences in Triglyceride, VLDL and Lp(a) values. There is no statistically significant difference between Total Cholesterol, LDL, HDL values between controls and CKD subjects.

Table 5: Prevalence of Dyslipidemia in Controls and CKD subjects

Lipid Profile parameters	**Controls (n=70)**	**Cases (n=70)**
Total Cholesterol (>200 mg/dL)	21 (30%)	27 *(38.6%)*
Triglycerides (>150 mg/dL)	43 (61.4%)	52 **(74.3%)**
LDL (>130 mg/dL)	17 (24.3%)	16 *(22.9%)*
LP(a) (>30 mg/dL)	25 (35.7%)	34 **(48.6%)**
HDL (<40 mg/dL)	10 (14.3%)	46 **(65.7%)**

Graph-5 : Prevalence of Dyslipidemia in Controls and CKD subjects

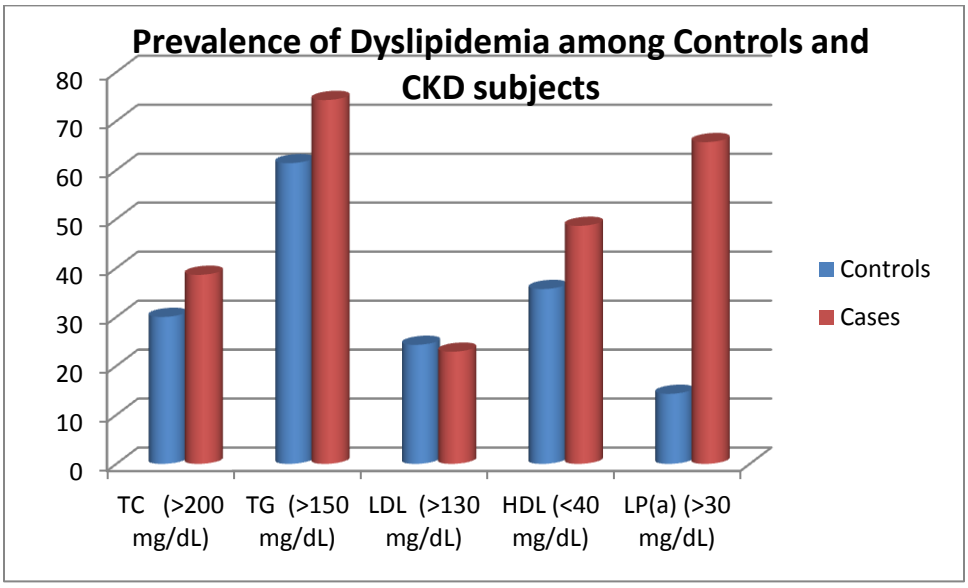


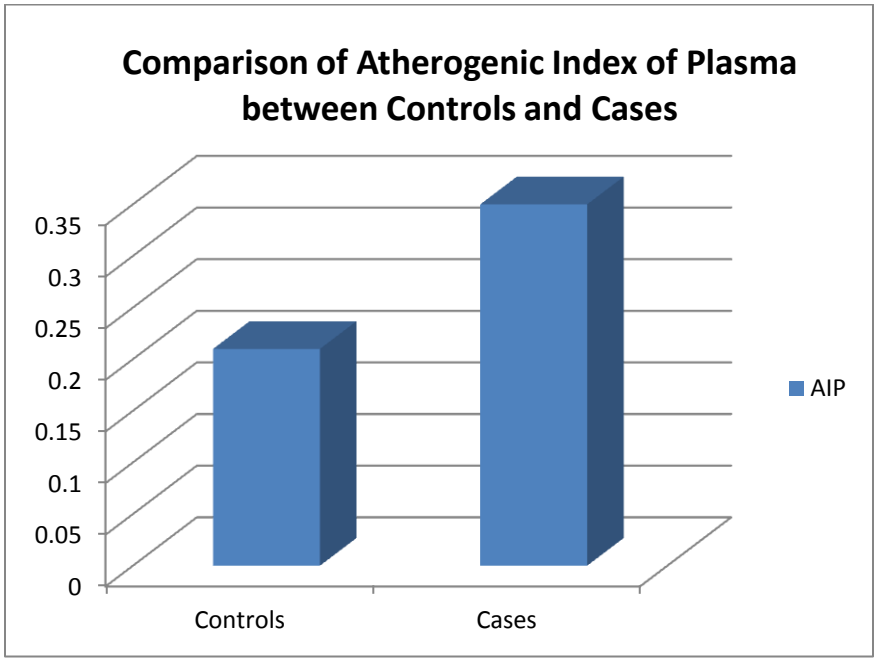
Table 5 & Graph 5 show prevalence of Dyslipidemia among controls and CKD subjects. The prevalence rates are higher for Hypertriglyceridemia (74.3%) in CKD population compared to Controls. There is no significant difference in the prevalence rates of increased Total Cholesterol and increased LDL values between Cases and Controls. The prevalence rates of increased Serum Lp(a) levels and decreased HDL are 48.6% and 65.7% respectively in Cases , which are higher than in Controls.

Table 6:Comparison of Novel Lipid Indices between Controls and CKD subjects

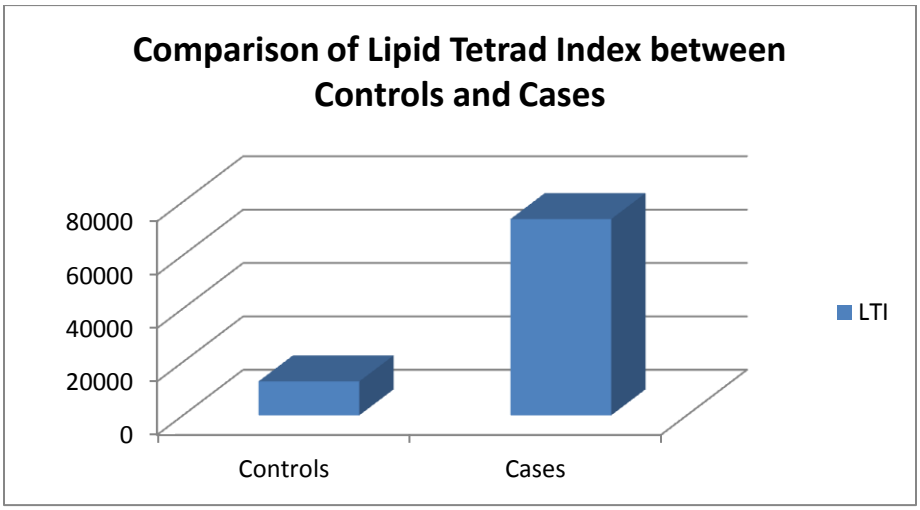
Lipid Indices	Mean ± SD		**'t' value**	**p value**
	Controls	**Cases**		
Atherogenic Index of Plasma (AIP)	0.21±0.09	0.35±0.19	5.571	<0.001, statistically significant

Lipid Tetrad Index (LTI)	12712.65±10790.82	73532.92±98208.05	5.150	<0.001, statistically significant
Atherogenic Coefficient (AC)	3.48±0.64	3.77±1.15	1.843	0.06, (>0.001) statistically **not significant**
Castelli's Risk Index-I (CRI-I)	4.48±0.64	4.77±1016	1.831	0.07, (>0.001) statistically **not significant**
Castelli's Risk Index-II (CRI-II)	2.72±0.60	2.64±1.02	0.565	0.57, (>0.001) statistically **not significant**

Graph 6 : Comparison of Atherogenic Index of Plasma (AIP) between Controls and CKD subjects



Graph 7: Comparison of Lipid Tetrad Index (LTI) between Controls and CKD subjects



Graph 8: Comparison of Lipid Indices- Atherogenic Coefficient, Castelli's Risk Index -I & Castelli's Risk Index -II between Controls and CKD subjects

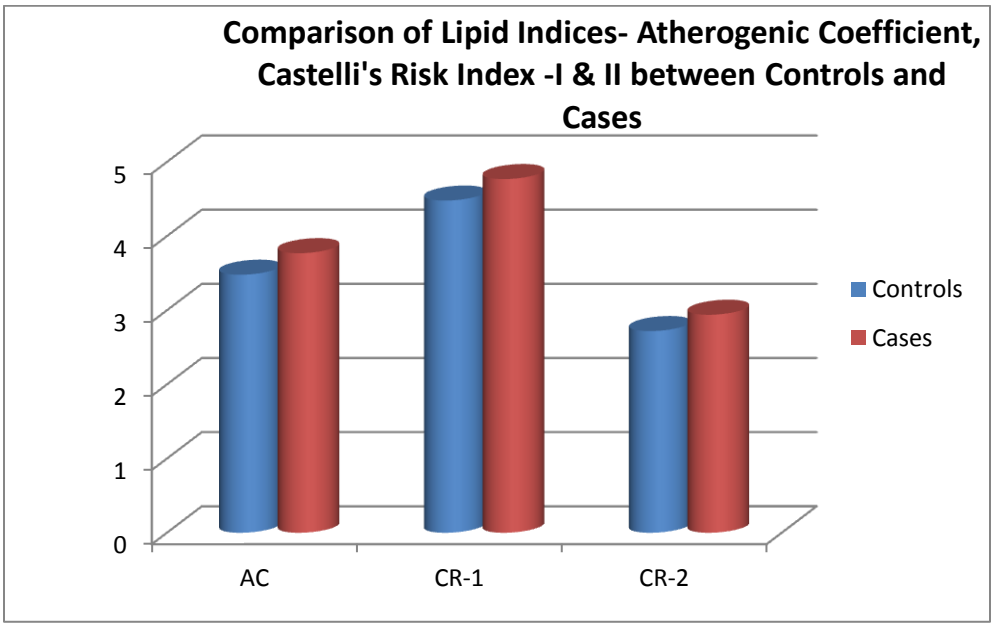


Table 6 & Graphs 6, 7 & 8 show the comparison of Lipid Indices between controls and CKD subjects. The data demonstrate that extremely significant differences exist in the values of AIP, LTI between controls and CKD subjects. Whereas, difference in the values of AC, CRI-I and CRI-II between controls and CKD subjects is not significant.

Table 7: Staging of Chronic Kidney Disease in Cases

STAGES	eGFR(ml/min)	No. of cases	Percentage of Cases(%)
Stage I	>90	0	**0**
Stage II	60-89	7	10
Stage III	30-59	30	42.86
Stage IV	15-29	25	35.71
Stage V	<15	8	11.43

Graph 9: Staging of Chronic Kidney Disease in Cases (Pie Chart)

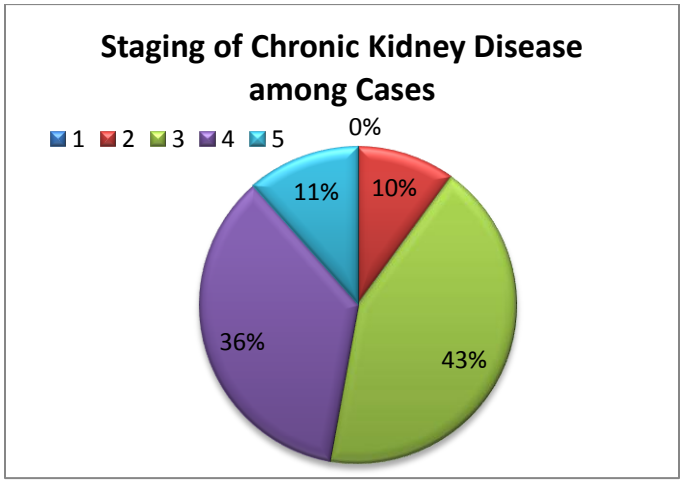


Table 7 & Graph 9 explain the classification of Chronic Kidney Disease subjects into 5 stages based on their estimated GFR (MDRD formula). 43% patients are in stage III and no patients in stage I. Stages II, IV & V have 10%, 36% and 11% patients respectively.

Table 8: Prevalence of Dyslipidemia across different stages of CKD subjects

Lipid Profile parameters	**Stage 2 (n=7)**	**Stage 3 (n=30)**	**Stage 4 (n=25)**	**Stage 5 (n=8)**
Total Cholesterol (>200 mg/dL)	4 *(57.1%)*	11 (36.7%)	12 (48%)	0
Triglycerides (>150 mg/dL)	6 **(85.7%)**	21 **(70%)**	21 **(84%)**	4 (50%)
LDL (>130 mg/dL)	4 *(57.1%)*	6 (20%)	6 (24%)	0
LP(a) (>30 mg/dL)	5 (71.4%)	19 **(63.3%)**	16 (64%)	6 **(75%)**
HDL (<40 mg/dL)	4 (57.1%)	15 (50%)	9 (36%)	6 (75%)

Graph 10: Prevalence of Dyslipidemia across various stages of CKD subjects

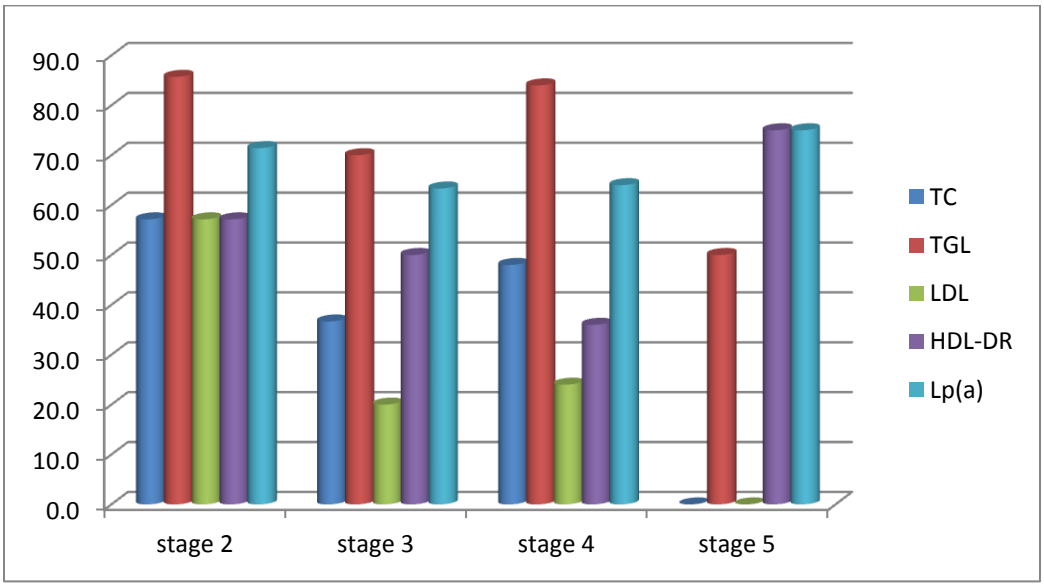
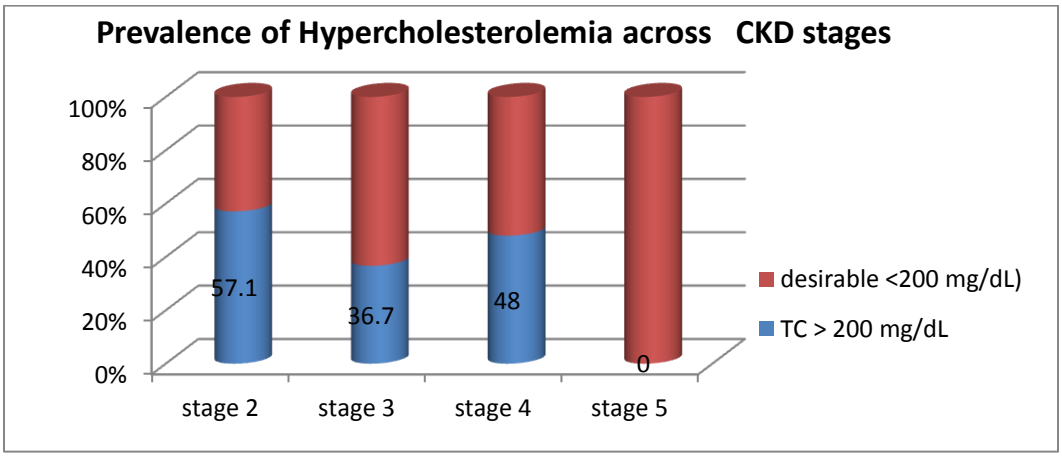


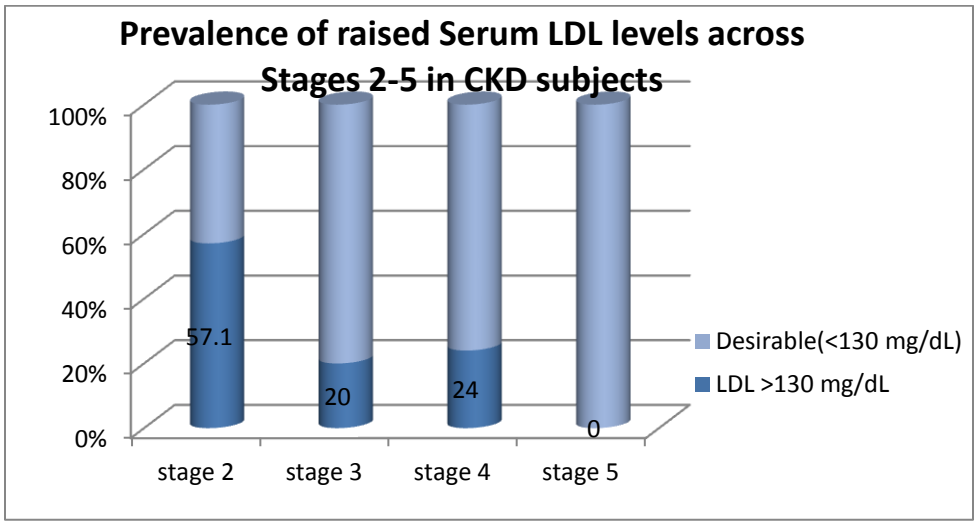
Table 8 & Graph 10 show the most prevalent quantitiative lipid abnormality is Hypertriglyceridemia (85.7% in Stage 2 & 84% in Stage 4) followed by Increased Serum Lp(a) levels (75% in Stage 5 and 71% in Stage 2), reduced HDL levels (75% in Stage 5 and 57.1% in Stage 2)

Graph 11:Prevalence of Hypercholesterolemia across Stages 2-5 in CKD subjects



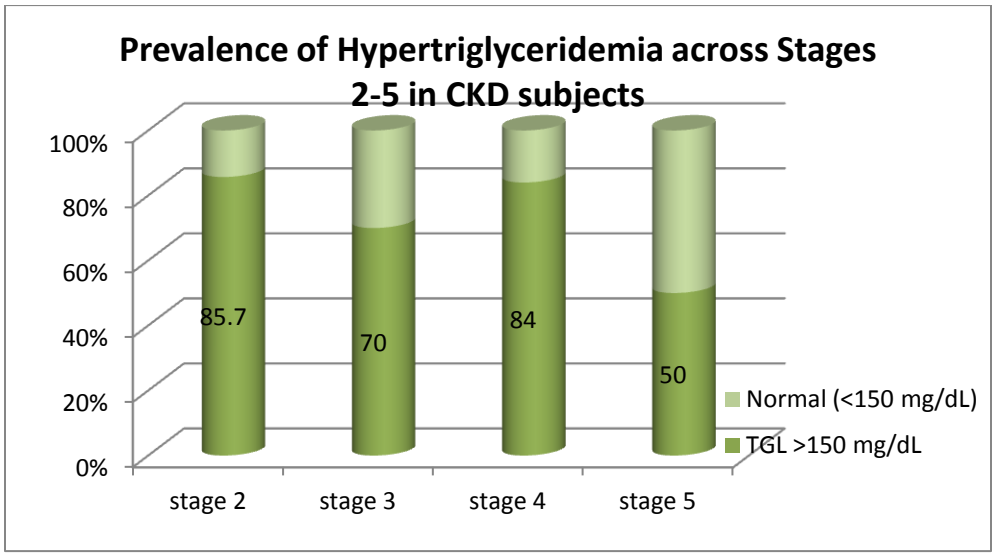
Graph 11 shows Hypercholesterolemia is more prevalent in Stage 2 CKD and relatively lower in Stages 3&4. CKD subjects in Stage 5 have reduced Total Cholesterol values.

Graph 12: Prevalence of raised Serum LDL levels across Stages 2-5 in CKD subjects



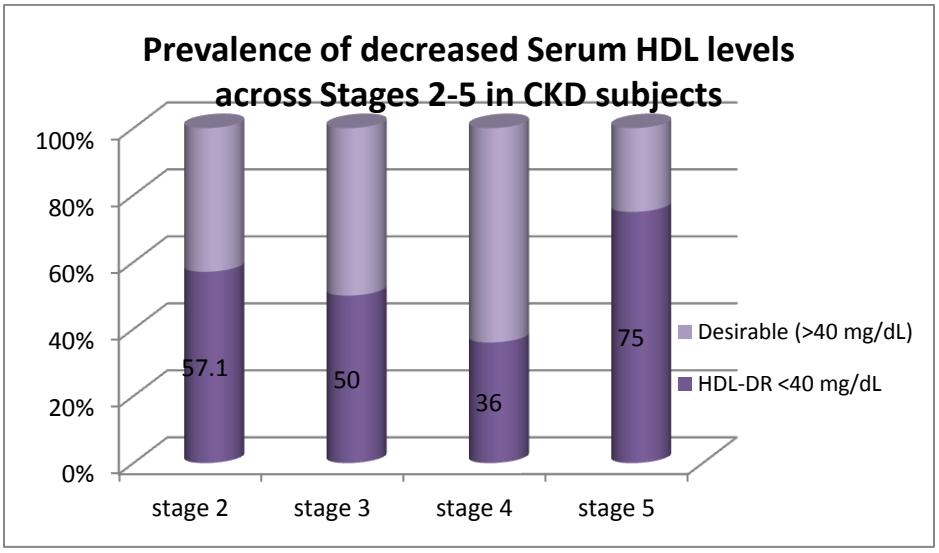
Graph 12 shows Increased Serum LDL levels is most prevalent in Stage 2(57.1%). Subjects in the Stages 3,4& 5 have a higher prevalence of Reduced Serum LDL levels.

Graph 13 : Prevalence of Hypertriglyceridemia across Stages 2-5 in CKD subjects



Graph 13 depicts that prevalence rates of Hypertriglyceridemia are higher in all stages of CKD, which are 85.7%, 70%, 84% and 50% in Stages 2,3,5 respectively.

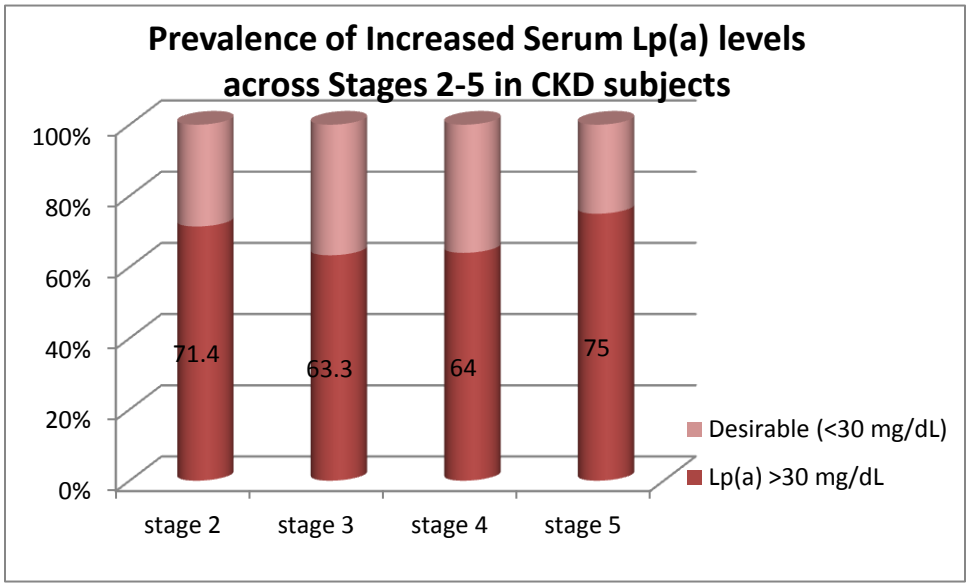
Graph 14 : Prevalence of decreased Serum HDL levels across Stages 2-5 in CKD subjects



Graph 14 indicates that reduced serum HDL levels are more prevalent in Stages 2& 5 of CKD (57.1% & 75%)

Table 8 & Graphs 10-14 demonstrate that the pattern of dyslipidemia in CKD subjects- Hypertriglyceridemia (the most common lipid abnormality), reduced serum HDL levels, reduced Serum Total cholesterol and Serum LDL levels.

Graph 15 : Prevalence of Increased Serum Lp(a) levels across Stages 2-5 in CKD subjects



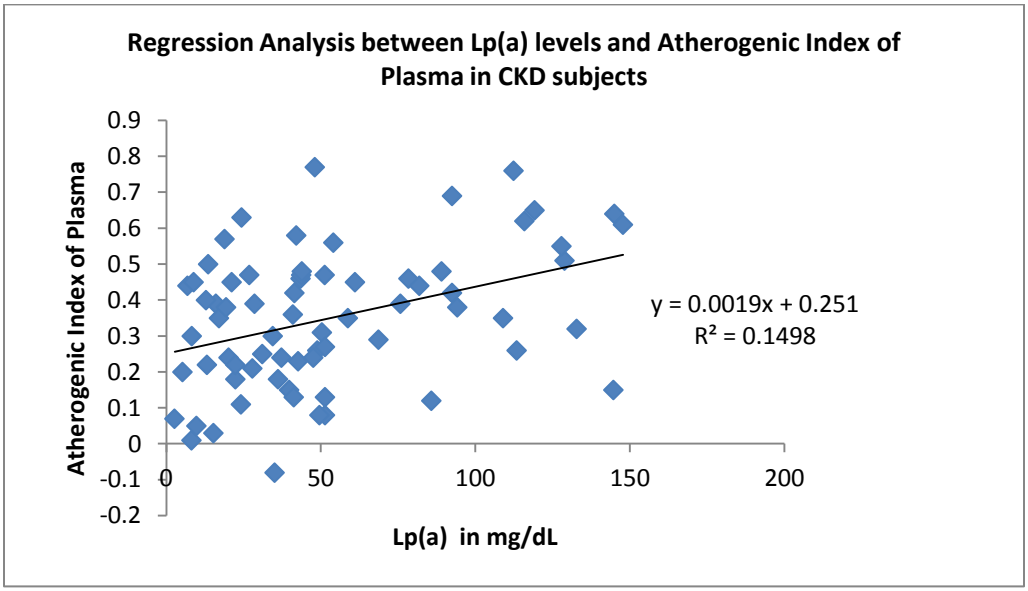
Graph 15 shows that the prevalence of increased Serum Lp(a) levels is higher in all stages of CKD subjects.

Table 9: Pearson's Correlation between Serum Lp(a) and Lipid Indices in CKD patients:

S. No.	Analytes	Pearson's correlation coefficient ('r value')	Significance
1.	Lp(a) Vs AIP	**0.388**	Positive correlation
2.	Lp(a) Vs LTI	**0.799**	Positive correlation
3.	Lp(a) Vs AC	0.327	Positive correlation
4.	Lp(a) Vs CRI- I	0.327	Positive correlation
5.	Lp(a) Vs CRI- II	0.171	Positive correlation
6.	Lp(a)VsNonHDL	0.410	Positive correlation
7.	Lp(a) Vs eGFR	0.071	Postive correlation

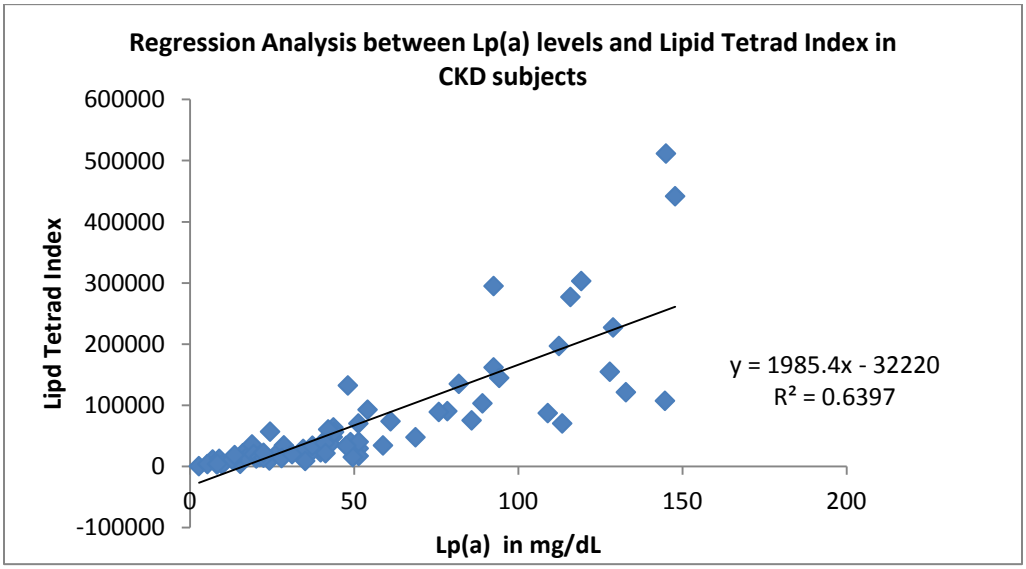
Table 9 demonstrates that in CKD subjects there exists a moderate positive correlation between Serum Lp(a) and Atherogenic Index of Plasma (r=0.388), Non HDL(r=0.41). A strong positive correlation exists between Serum Lp(a) and Lipid Tetrad Index(r=0.799). A weaker positive correlation is observed between Serum Lp(a) and Castelli's Risk Index I (r=0.17)

Graph 16:



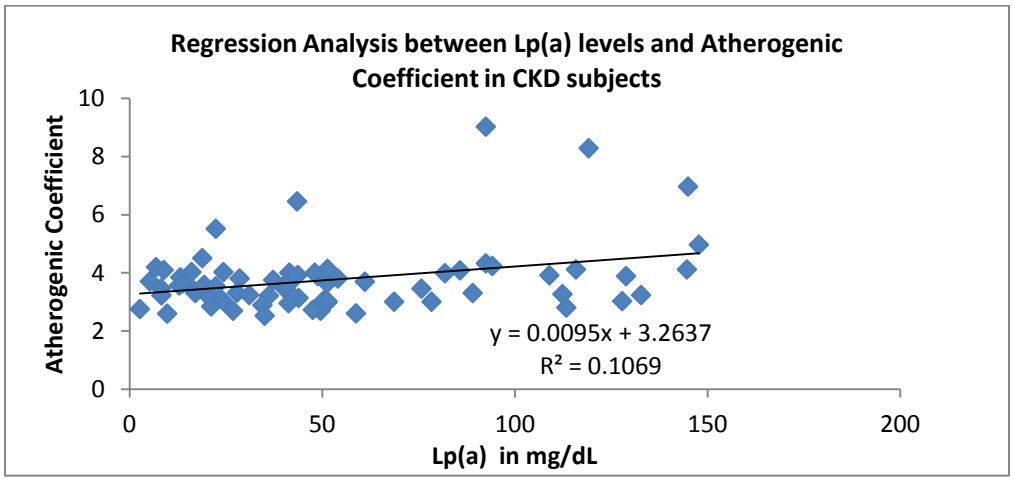
Graph 16 explains the correlation of Serum Lp(a) and Atherogenic Index of Plasma among CKD subjects. Linear regression analysis has an upward slope suggesting that AIP values have a positive correlation with Serum Lp(a) levels.

Graph 17:



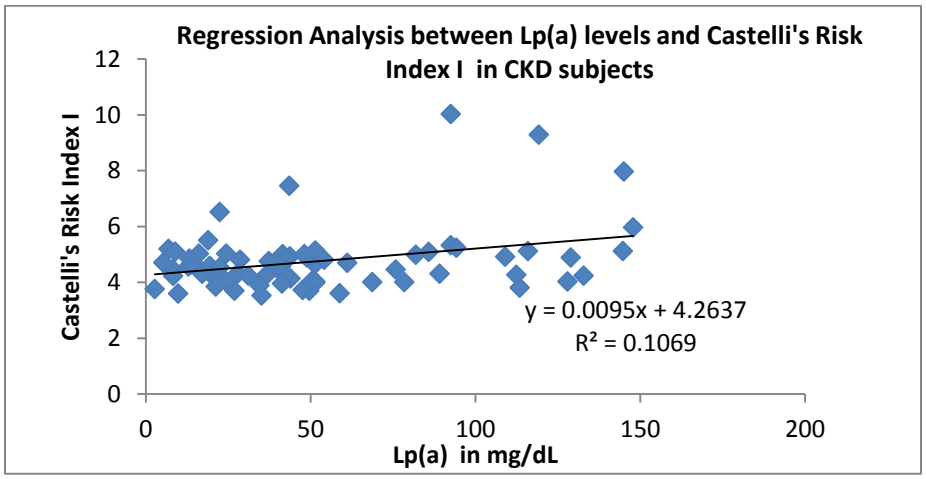
Graph 17 explains the correlation of Lipid Tetrad Index and Serum Lp(a) levels among CKD subjects. LTI values have positive correlation with Serum Lp(a) as the r value is equal to 0.788 & linear regression analysis has a steep upward slope.

Graph 18:



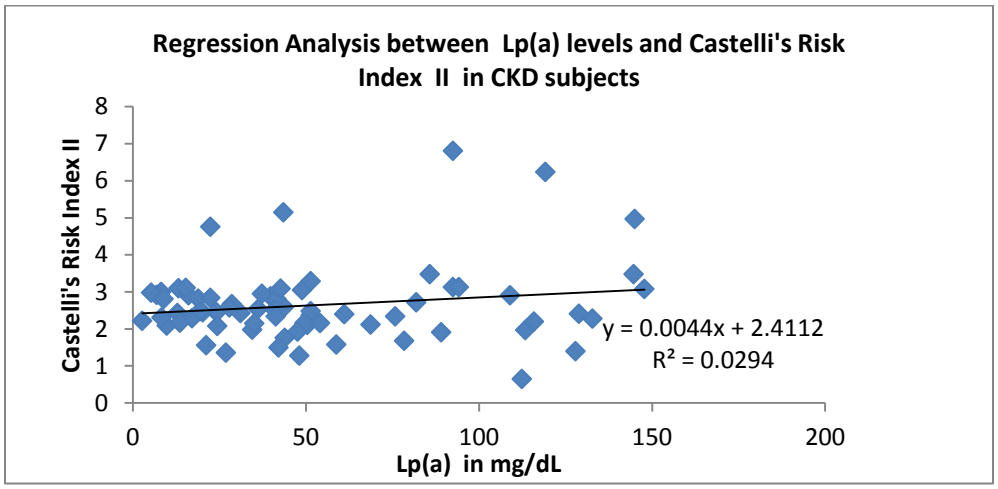
Graph 18 explains the linear regression analysis between Atherogenic coefficient and Serum Lp(a) levels, has an upward slope with a moderate correlation(r=0.33)

Graph 19:



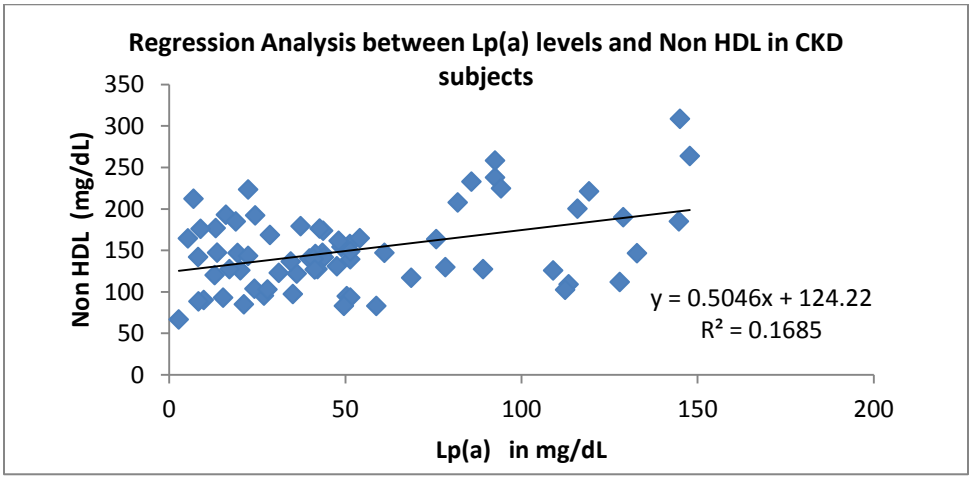
Graph 19 shows the linear regression analysis between Castelli's Risk Index I and Serum Lp(a) levels, has an upward slope with a moderate correlation (r=0.33)

Graph 20:



Graph 20 demonstrates that the Castelli's Risk Index II values have a weak positive correlation (r=0.17) with Serum Lp(a) levels and the linear regression analysis has an upward slope.

Graph 21:



Graph 21 explains the correlation of Non HDL values with Serum Lp(a) is positive(r=0.4) and the linear regression analysis between the two variables has a positive slope.

Table 10: Risk Stratification using AIP (Atherogenic Index of Plasma) across Stages 2-5 in CKD subjects

Risk Stratification using AIP	Stage 2	Stage 3	Stage 4	Stage 5
High Risk	**85.71%**	63.33%	72%	50%
Low Risk	14.29%	36.67%	28%	50%

Graph 22:

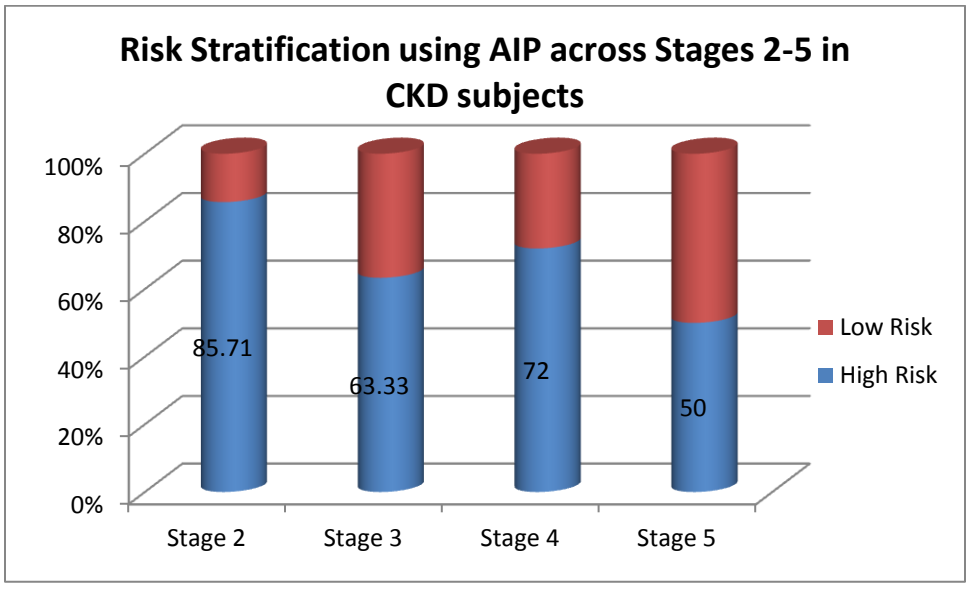


Table 10 & Graph 22 show that the prevalence of CKD subjects at high risk for CAD stratified by using Atherogenic Index of Plasma(AIP>0.1) are 85.7%, 63.3%, 72%,50% in Stages 2,3,4,5 of CKD respectively.

Table 11 : Risk Stratification using Lipid Tetrad Index across Stages 2-5 in CKD subjects

Risk Stratification using LTI	Stage 2	Stage 3	Stage 4	Stage 5
High Risk	100%	63.33%	72%	62.5%
Low Risk	0	36.67%	28%	37.5%

Graph 23:

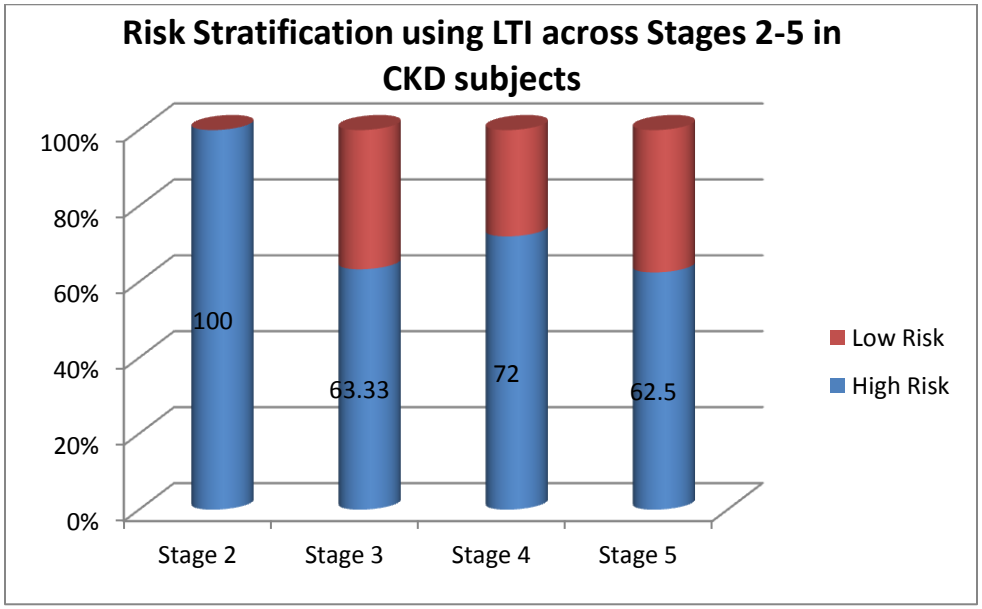
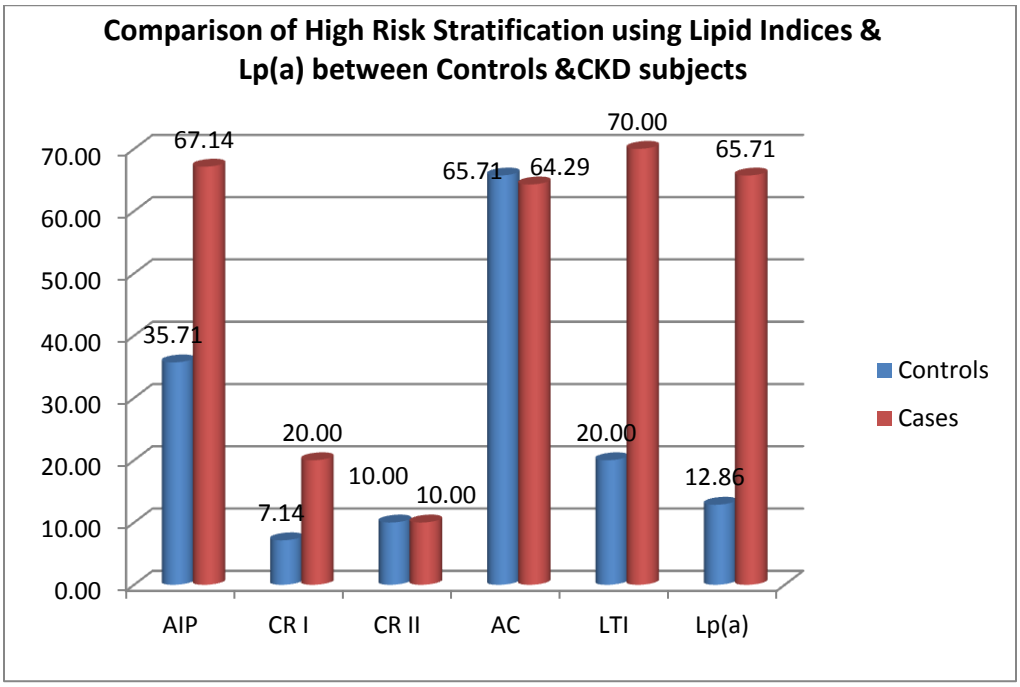


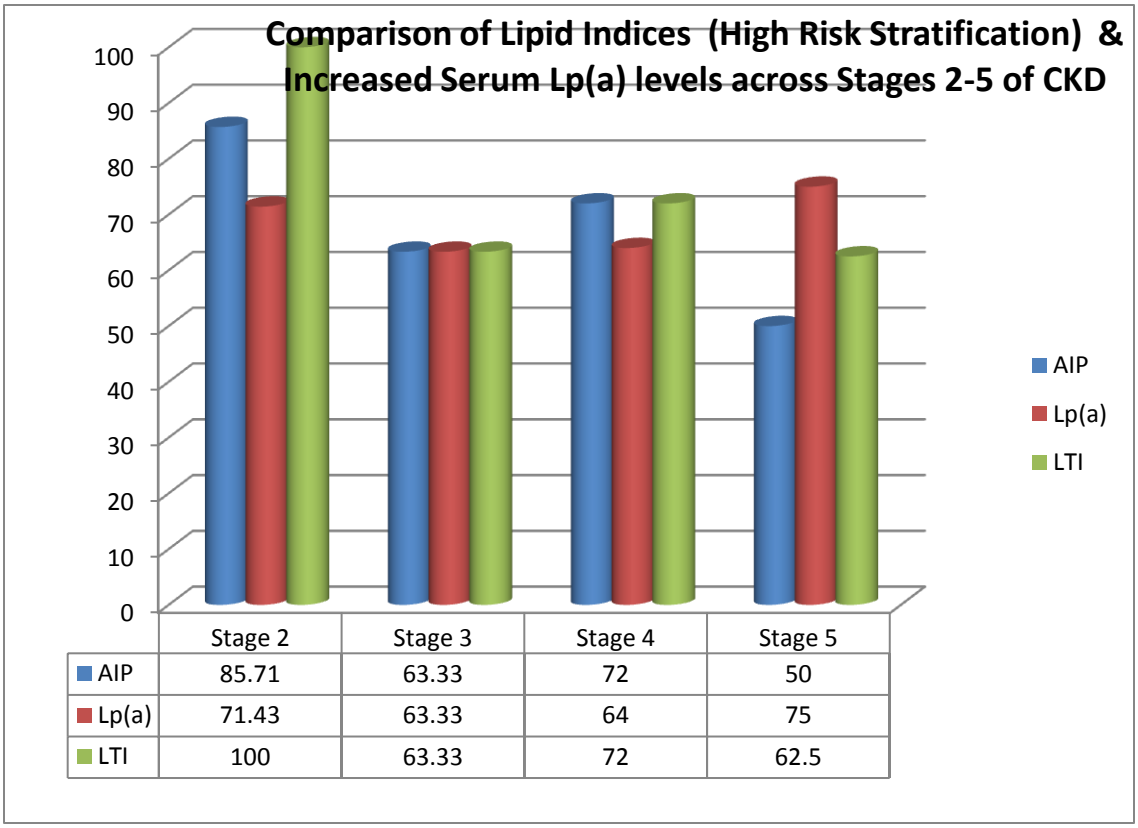
Table 11& Graph 23 show that the prevalence of CKD subjects at high risk for CAD stratified by using Lipid Tetrad Index(LTI > 20,000) are 100%, 6.3%, 72%, 62.5% in Stages 2,3,4,5 of CKD respectively.

Graph 24:



Graph 24 compares the prevalence of individuals classified as high risk for CAD, using Lipid Indices and Serum Lp(a) levels between Controls and CKD subjects. The Prevalence rate of CKD subjects at high risk for CAD predicted by AIP, LTI and Serum Lp(a) are 67.14%, 70% and 65.71% which are significantly higher than that in the control subjects. There is no significant difference in the prevalence rates of AC,CRI-I, CRI-II, at high risk for CAD, between cases and controls.

Graph 25:



	Stage 2	Stage 3	Stage 4	Stage 5
AIP	85.71	63.33	72	50
Lp(a)	71.43	63.33	64	75
LTI	100	63.33	72	62.5

Graph 25 compares the prevalence rates of CKD subjects stratified as high risk for CAD by Atherogenic Index of Plasma and Lipid Tetrad Index across various stages of CKD with the prevalence rate of increased Serum Lp(a) levels, which is a standard risk factor for CAD. The prevalence rates of high risk subjects predicted by AIP, LTI are equal and comparable to Serum Lp(a) in Stage 3(63.33%), higher than Serum Lp(a) in Stage 2. The prevalence rates of increased AIP and LTI values are equal in Stage 4 (72%) and higher than Serum Lp(a) [64%]. The prevalence rate of raised Serum Lp(a) levels in Stage 5 (75%) is higher than that of AIP(50%) and LTI(62.5%)

Table 12: Positive Predictive Value and Negative Predictive value of Lipid Indices and Serum Lp(a) in the study population:

Parameters	**Positive Predictive value (PPV)**	**Negative Predictive value (NPV)**
Serum Lp(a)	**83.6%**	**71.8%**
AIP	**65.3%**	**66.2%**
LTI	**77.8%**	**72.7%**
AC	49.5%	48.9%
CRI-I	73.7%	53.7%
CRI-II	50%	50%

Table 12 indicates that Serum Lp(a) has the highest positive predictive value(83.6%). Among the Lipid Indices, Lipid Tetrad Index(LTI) has the highest positivie predictive value(77.8%) and the highest negative predictive value(72.7%), followed by Atherogenic Index of Plasma with PPV 65.3% and NPV 66.2%

Table 13: Unpaired 't' test between different analytes among CKD subjects

Parameters	**'t' value**	**p value**
AIP Vs Lp(a)	11.19	<0.001,statistically significant
LTI Vs Lp(a)	10.46	<0.001,statistically significant
AC Vs Lp(a)	6.26	<0.001,statistically significant
CRI- I Vs Lp(a)	10.25	<0.001,statistically significant
CRI- II Vs Lp(a)	10.70	<0.001,statistically significant
Non HDL Vs Lp(a)	13.05	<0.001,statistically significant
eGFR Vs Lp(a)	3.61	<0.001,statistically significant

Table 13 explains the unpaired 't' test between different analytes among CKD patients. The 't' test between AIP, LTI, AC, CRI- I & CRI- II, Non HDL, eGFR and Lp(a) have significant differences between them and thereby suggesting that these variables are not independent of each other.

DISCUSSION

DISCUSSION

The present study examined the prevalence of dyslipidemia and evaluated Serum Lipoprotein(a) levels and the role of Novel Lipid Indices, namely, Atherogenic Index of Plasma, Lipid Tetrad Index, Atherogenic Coefficient, Castelli's Risk Index-I and Castelli's Risk Index-II among subjects with Chronic Kidney Disease in the predialysis phase, and compared the findings with those of healthy Control subjects. Adhering strictly to the inclusion and exclusion criteria, 70 diagnosed cases of Chronic kidney disease and 70 apparently healthy control subjects were selected for the study.

The mean of the age distribution in CKD population (48.19±9.24) years was more or less similar to the mean of the age distribution in controls(44.23±9.57)years. Age distribution in CKD subjects of the present study is similar to previous studies in Karnataka[13] and Nigeria[33].

In our study, Diabetes mellitus and Hypertension constituted the major aetiology of renal disease, accounting for 51.4%, 67.1% respectively. This is significantly higher than in controls, the proportion of diabetics being 17.1% and hypertensives being 21.4%.

GFR was estimated using MDRD formula and the CKD patients were classified into 6 stages based on National Kidney Foundation-K/DOQI Clinical Practice Guidelines for chronic kidney disease. Cases were 10%, 42.86%, 35.71%

& 11.43% in stages II, III, IV, V respectively. Majority of cases were in Stages III & IV and no cases in Stage 0 and I, suggesting a delay in seeking medical attention and thereby very late presentation of the patients to the OPD.

Derangements involving all lipoprotein classes were observed in all the Stages of CKD with disease progression. Vasilis et al[9] explained in their review, that abnormalities in lipoprotein metabolism manifest even at earlier stages of CKD, following a downslope course with worsening of renal function.

In our study, the most prevalent lipid abnormality observed among CKD subjects, in predialysis stage, was Hypertriglyceridemia, the overall prevalence being 74.3%. This prevalence is significantly greater than that of previous reports[35,36]. This could be due to a higher proportion of diabetics among the CKD subjects compared with the controls. Previous studies by Vaziri et al[34], Fliser et al[35] explain that it represents an early feature of renal failure, the predominant mechanism being delayed clearance of ApoB containing lipoproteins.

The Serum Total Cholesterol and Serum LDL values were statistically insignificant [p:0.34,(>0.001)] in CKD subjects (mean±SD: 191.28±53.62) in comparison to controls (mean±SD: 184.26±30.7). This is in concordance with studies by Mannangi et al[13] and Cabarkapa et al[36]. The prevalence of Hypercholesterolemia is 57.1% in Stage I of CKD and decreases with renal disease progression. Most previous studies have also reported similar or lower values[8,9,37]. Unlike general population, lower plasma cholesterol in CRF has been

associated with a higher cardiovascular mortality [24]. This is explained by a phenomenon known as "reverse epidemiology". Studies by Shlipak et al[36]., Kalantar et al[37] have proposed that it is likely due to inflammatory lowering effect of cholesterol, and presence of malnutrition. These factors confound the relationship between traditional risk factors and risk of cardiovascular disease.

In our study, we observed no statistically significant difference in Non HDL cholesterol values [p value: 0.2, (>0.001)] between cases and controls. This is in concordance with previous studies by Mannangi et al[13] and Schreier et al[38]. Similar to their studies, the results of our study show that Non HDL may not be an appropriate marker for risk assessment among patients with CKD.

A statistically significant difference was observed in Serum Lipoprotein(a) levels (p value:<0.001) between Cases (mean±SD: 53.26±39.56) and Controls(mean±SD: 17.88±14.5). This concords with the studies done by Mannangi et al[13], Cabarkapa et al[36]. Increased Serum Lp(a) levels is a recognized independent risk factor for premature atherosclerotic disease. However, there is a poor correlation between Serum Lp(a) and eGFR values($r=0.072$, $p<0.001$). This is in concordance with study done by Cabarkapa et al[36], but contrary to Sechi LA et al study[39].

In The CRIC study by Mahboob Rahman et al, Serum Lp(a) levels were higher in CKD patients with decreased eGFR. After adjustment for other known risk factors was made, the association of Serum Lp(a) with decline in renal

function as indicated by fall in eGFR values, was markedly decreased and was not statistically significant[40].

In the present study, among lipid indices, statistically significant differences($p<0.001$) between CKD cases and controls were observed for Atherogenic Index of Plasma (AIP) and Lipid Tetrad Index (LTI). This is in concurrence with Mannangi et al[13], Cabarkapa et al[36] studies. Previous Studies of Gaziano et al[41], Dobiasova et al[42] demonstrate that AIP strongly predicts the occurrence of infarction. In individuals whose Serum Triglyceride levels and/or Serum HDL levels appear normal, AIP can serve as a diagnostive alternative[43].

As described in the study by Enas et al,[44] LTI is a novel way to assess the risk of cardiovascular disease. It incorporates the product of three atherogenic risk factors namely, Serum Total Cholesterol, Serum Triglycerides and Serum Lp(a) and relates this product to non-atherogenic protective HDL particle; thus expresses the overall lipid profile of patients[13].

No statistically significant differences were observed for other lipid indices-Atherogenic coefficient, Castelli's Risk Index I, Castelli's Risk Index II. This concords with Mannangi et al[13], except for CRI- II.

Pearson's correlation was studied between Serum Lp(a) and Lipid Indices in CKD subjects. A strong positive correlation ($r=0.799$, $p<0.001$) was observed between Serum Lp(a) and Lipid Tetrad Index, which is concordant with study by Mannangi et al[13], followed by Atherogenic Index of Plasma($r=0.388$, $p<0.001$). Between Serum Lp(a) and Lipid indices namely, Atherogenic coefficient&

Castelli's Risk Index I, moderate positive correlation was observed. A weak correlation exists between Serum Lp(a) and Casteli's Risk Index II.

In our study, the prevalence rates of individuals stratified as high risk for CAD by AIP and LTI, in stage III was 63.3% which equals the prevalence rate of increased level of Serum Lp(a). In the other stages of CKD, the prevalence rates of raised AIP and LTI values were more or less similar to that of Serum Lp(a) values, implying that these Indices are comparable in risk assessment with Serum Lp(a), a standard risk factor for cardiovascular disease. Whereas, other Indices such as AC, CRI- I & CRI- II are not comparable with Serum Lp(a) in risk assessment, as the prevalence rates of increased values of AC,CRI- I & CRI- II are much lower than that of Serum Lp(a).

In our Study, among the lipid indices, Lipid Tetrad Index has the highest positive predictive value (77.8%) and negative predictive value (72.7%); Atherogenic Index of Plasma has PPV of 65.3% and NPV of 66.2%. Mahantesh et al[12] demonstrated in their study that LTI had maximum accuracy among the lipid indices, about 80% to the CAD risk followed by AIP (70%).

There is an increased prevalence of dyslipidemia in the early stages of CKD and raised Serum Lp(a) levels. The novel lipid indices serve as a link between lipid profile parameters and CAD risk assessment. LTI and AIP correlate well with Serum Lp(a) levels, a widely accepted independent risk factor for CAD. Lipid Indices such as AC, CRI- I &CRI- II may not be appropriate for assessment of risk of cardiovascular disease in subjects with Chronic Kidney disease.

SUMMARY

Summary

The study of evaluation of Serum Lipoprotein(a) levels and the role of novel Lipid Indices among CKD subjects in the predialysis phase who attend the regular OPD was conducted in our institution.

Adhering strictly to the inclusion and exclusion criteria, 70 cases were selected for study. Among them, 31 were males and 39 were females. 70 healthy control subjects were selected for study with equivalent age distribution for comparison of study parameters.

After getting careful history and examination, fasting blood sample was collected in red topped vacutainers with clot activator for renal function tests, fasting lipid profile and serum Lp(a) estimation. Blood sample was processed and serum separated and stored at -20ºC.

No interference was done in their routine treatment during the study.

Serum urea and serum creatinine were estimated after standardization using IDMS reference calibrator and eGFR calculated & staging of CKD done based on NKF-K/DOQI guidelines using MDRD formula. Serum Lipoprotein(a) levels were estimated by Immunoturbidimetry method. Subjects with dyslipidemia were identified based on NCEP-ATP III guidelines. Novel Lipid Indices were calculated and the cardiovascular risk assessment was done.

Dyslipidemia was evident in all the stages of CKD, with hypertriglyceridemia being the most common lipid abnormality. Dyslipidemia in

CKD actively participates in the pathogenesis of cardiovascular disease. Non HDL cholesterol is not a suitable marker in CKD subjects. Among the lipid indices, only Atherogenic Index of Plasma and Lipid Tetrad Index showed significant contribution in high risk prediction, when compared with Serum Lp(a) levels. Out of which AIP is preferred because it is the best cost effective marker and it's increased pathological values act as an indirect indicator of small dense LDL particles which are relatively more atherogenic. These novel lipid indices can be better screening tools for monitoring cardiovascular disease risk in CKD subjects in the predialysis stage.

CONCLUSION

CONCLUSION

This study concludes that

- Chronic kidney disease results in profound dysregulation of lipoprotein metabolism and subjects with chronic impaired renal function constitute a high risk group for developing cardiovascular events.
- In developing countries like India, owing to factors like high cost of standard tests such as Serum Lipoprotein(a),measurement of Apoprotein levels, evaluation of cardiovascular risk assessment can be undermined, in CKD.
- Novel Lipid Indices especially Atherogenic Index of Plasma and Lipid Tetrad Index can be used to measure burden of dyslipidemia especially in a setting when the absolute values of individual parameters seem normal.
- Use of Lipid Indices in CKD subjects can complement the routinely done lipid profile to identify the individuals at high risk for cardiovascular disease, in the early stages of CKD.

FUTURE PERSPECTIVE:

Further studies are required to improve these results in a larger sample size in the predialysis stage of CKD, taking into consideration the other putative risk factors for cardiovascular disease risk in CKD. Robust multivariate analyses to ascertain independent association between lipoprotein measures, isoforms of Lipoprotein(a) in CKD, novel lipid indices and cardiovascular outcomes may be performed.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, SaranR, Wang AY, Yang CW. Chronic kidney disease: global dimension and perspectives. *Lancet* 2013; 382: 260-272 [PMID: 23727169 DOI: 10.1016/S0140-6736(13)60687-X]
2. National Kidney Foundation-K/DOQI Clinical Practice Guidelines for chronic kidney disease: Evaluation, Classification and Stratification. *Am J Kidney disease 2002;39;S1*
3. Agarwal SK, Srivastava RK: Chronic kidney disease in India: challenges and solutions. *Nephron Clin Pract 2009.*
4. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, et al. What do we know about chronic kidney disease in India: First report of the Indian CKD registry. *BMC Nephrol. 2012;13:10.*
5. Ghanshyam Palamaner Subash Shantha, Anita Ashok Kumar et al; Prevalence of subclinical hypothyroidism in patients with end-stage renal disease and the role of serum albumin: a cross-sectional study from South India :*Cardiorenal Med 2011;1:255–260*
6. Varma PP, Raman DK, Ramakrishnan TS, Singh P, Varma A. Prevalence of early stages of chronic kidney disease in apparently healthy central government employees in India.*Nephrol Dial Transplant. 2010;25:3011–7.*

7. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med 2004; 351:1296–1305*.
8. Vasilis Tsimihodimos Evangelia Dounousi Kostas C. Siamopoulos: Dyslipidemia in Chronic Kidney Disease: An Approach to Pathogenesis and Treatment. *Am J Nephrol 2008;28:958–973*
9. Vasilis Tsimihodimos, Zoi Mitrogianni and Moses Elisaf: Dyslipidemia Associated with Chronic Kidney Disease. *The Open Cardiovascular Medicine Journal, 2011, 5, 41-48.*
10. Sarnak MJ, Levey AS, Schoolwerth AC, *et al*. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation 2003;108: 2154-69*.
11. William F Keane, Joanne E Tomassini, David R Neff: Lipid Abnormalities in patients with Chronic Kidney Disease: Implications for the pathophysiology of Atherosclerosis –Review. *Journal of Atherosclerosis and Thrombosis, 2013; 20:123-133*
12. Mahantesh Patil, Shubha Jayaram, Meera S, Kantharaj N: Role of novel lipid indices and lipoprotein (a) in Type 2 Diabetes Mellitus with Coronary Artery Disease. *Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online) 2015 Vol. 5 (2) April-June, pp.41-47*

13. Neela B Mannangi, Shubha Jayaram, Viruprakash HS, Sangamesh Kashinakunti, Manjula R: Novel lipid indices in chronic kidney disease. *National Journal of Medical Research-print ISSN: 2249 4995*
14. Dan L. Longo,Dennis L.Kasper, J. Larry Jameson,Anthony S. Fauci,Stephen L. Hauser, Joseph Loscalzo. Harrison's Textbook of internal medicine *18thedition (2012) ; vol 2: page numbers 1761-1762.*
15. Brenner and Rectors: The kidney text book, *9th edtn, vol 2, page 861-862*.
16. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for chronic kidney disease: Evaluation, Classification and Stratification. *Am J Kidney disease 2002;39;S1*
17. Fless GM, ZumMallen ME, Scanu AM (1985) Isolation of apolipoprotein(a) from lipoprotein(a). *J. Lipid Res. 26: 1224-1229.*
18. Kronenberg F, Trenkwalder E, Lingenhel A, Friedrich G, Lhotta K, Schober M, Moes N, König P, Utermann G, Dieplinger H (1997) Renovascular arteriovenous differences in Lp[a] plasma concentrations suggest removal of Lp[a] from the renal circulation. *J.Lipid Res. 38: 1755-1763*
19. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature 330: 132-137*.

20. Carl A. Burtis, Ph.D., Edward R. Ashwood, M.D., David E. Bruns, M.D**:** Tietz' Textbook of Clinical chemistry and Molecular Diagnostics *5th Edition* page : 731-805, 1544-1554, 2132-2188.
21. Bihari-Varga M, Gruber E, Rotheneder M, Zechner R, Kostner GM (1988) Interaction of lipoprotein(a) and low-density lipoprotein with glycosaminoglycans from human aorta. *Arteriosclerosis 8: 851-857*.
22. Eaton DL, Fless GL, Kohr WJ, McLean JW, Xu QT, Miller CG, Lawn RM, Scanu AM(1987) Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. *Proc. Natl. Acad. Sci. USA 84: 3224-3228 (1987)*
23. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF (1989) A potential basis for the thrombotic risks associated with lipoprotein(a). *Nature 339: 301-303.*
24. Hajjar KA, Gavish D, Breslow JL, Nachman RL (1989) Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. *Nature 339:303-305*.
25. Harpel PC, Gordon BR, Parker TS (1989) Plasmin catalyzes binding of lipoprotein(a) to immobilized fibrinogen and fibrin. *Proc. Natl. Acad. Sci. USA 56: 3847-3851.*
26. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, Ginsberg H, Amarenco P, Catapano A, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lesnik P, Masana L, Reiner Z, Taskinen MR,

Tokgözoglu L, Tybjærg-Hansen A; European Atherosclerosis Society Consensus Panel. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J. 2010; 31(23):2844-53*.

27. Erqou S, Kaptoge S, Perry PL, Di AE, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA 2009;302:412-423*.
28. Anna Gries, Institute of Physiology, Medical University of Graz, Austria. Lipoproteins – Role in Health and Diseases;Chapter 32:Lipoprotein (a) – An Overview© 2012 Gries, licensee. *InTech. http://dx.doi.org/10.5772/45986*
29. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285(19):2486
30. Vishwam Pandya, Akhilesh Rao, Kunal Chaudhary: Lipid abnormalities in kidney disease and management strategies. *World J Nephrol2015 February 6; 4(1): 83-91*
31. Vaziri ND, Norris K: Lipid Disorders and Their Relevance to Outcomes in Chronic Kidney Disease. *Blood Purif, 2011; 31:189-196*

32. Vaziri ND: Dyslipidemia of chronic renal failure: the nature, mechanisms and potential consequences. *Am J Physiol Renal Physio, 2006; 290:F262-272*

33. Oluseyi A. Adejumo, Enajite I. Okaka and Louis I. Ojogwu: Lipid profile in pre-dialysis chronic kidney disease patients in southern Nigeria. *www.ghanamedj.org Volume 50 48 Number 1 March 2016*

34. Vaziri ND, Moradi H: Mechanisms of dyslipidemia of chronic renal failure. *Hemodial Int 2006; 10: 1–7*

35. Fliser D, Pacini G, Engelleiter R, Kautzky- Willer A, Prager R, Franek E, Ritz E: Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. *Kidney Int 1998; 53: 1343–1347.*

36. Velibor Cabarkapa, Mirjana Djeri, Zoran Stosi, Vladimir Saka, Zagorka Lozanov-Crvenkovi, Biljana Vu kovi: Evaluation of lipid parameters and bioindices in patients with different stages of chronic renal failure. *Vojnosanit Pregl 2012; 69(11): 961–966.*

37. Guerrero A, Montes R, Muñoz-Terol J, Gil-Peralta A, Toro J, Naranjo M, et al. Peripheral arterial disease in patients with stages IV and V chronic renal failure. *Nephrol Dial Transplant 2006; 21(12): 3525-31.*

38. Schreier L, González AI, Elbert A, Berg G, Wikinski R. Utility of non-high-density lipoprotein cholesterol in hemodialyzed patients. *Metabolism 2004; 53(8): 1013-5.*

39. Sechi LA, Zingaro L, De Carli S, Sechi G, Catena C, Falleti E, et al. Increased serum lipoprotein(a) levels in patients with early renal failure. *Ann Intern Med 1998; 129(6): 457-61.*

40. Mahboob Rahman, Wei Yang,SanjeevAkkina, Arnold Alper: Relation of Serum Lipids and Lipoproteins with Progression of CKD:The CRIC Study.*Clin J Am SocNephrol 9: 1190–1198, 2014. doi: 10.2215/CJN.09320913*

41. Gaziano JM, Henne kens CH, O'Donnell CJ, Breslow JL and Buring JE (1997). Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. *Circulation 96(8) 2520-2525.*

42. Dobiasova M (2006). AIP-atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice. *Vnitrní lékarství 52(1) 64-71.*

43. Nwagha UI, Ikekpeazu EJ, Ejezie FE, Neboh EE and Maduka IC (2010). Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. *African Health Sciences 10(3) 248–252.*

44. Enas EA (1993).The Coronary Artery Disease in Asian Indians (CADI) Study. *Asian American and Pacific Islander Journal of Health 1(2) 161-2.*

45. Michael L. Bishop, MS, CLS, MT(ASCP), Edward P. Fody, MD, Larry E. Schoeff, MS, MT(ASCP):Clinical Chemistry-techniques, principles, correlations*:6th edition(2010) pages 262-269.*

46. Kaysen GA: Lipid and lipoprotein metabolism in Chronic kidney disease. *J Ren Nutr, 2009; 19:73-77*

47. Stegmayr B, Olivecrona T, Olivecrona G: Lipoprotein lipase disturbances induced by uremia and hemodialysis. *Simn Dial, 2009;22:442-444*

48. Chan DT, Dogra GK, Irish AB, Ooi EM: Chronic kidney disease delays VLDL-Apo B 100 particle catabolism: potential role of ApoC III. *J Lipid Res, 2009; 50:2524-31*.

49. Klin M, Smogotzewski M, Ni ZM< Massry SG: Abnormalities in hepatic lipase in chronic renal failure- Role of excess parathyroid hormone. *J Clin Investig, 1996; 97:2167-73.*

50. Vaziri ND, Liang KH: Downregulation VLDL receptor expression in chronic experimental renal failure. *Kidny Int, 1997; 51:913-919*

51. Danesh J, Collins R, Peto R: Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation,2000;102:1082-1085*.

52. Kronenberg F, Kuen E, Ritz E, *et al*. Lipoprotein(a) serum concentrations and apolipoprotein(a) phenotypes in mild and moderate renal failure. *J Am Soc Nephrol 2000; 11: 105-15.*

53. Longenecker JC, Klag MJ, Marcovina SM, *et al*. Small apolipoprotein(a) size predicts mortality in end-stage renal disease: The CHOICE study. *Circulation 2002; 106: 2812-8.*

54. Vaziri ND, Deng G, Liang K Hepatic HDL receptor, SR-B1 and ApoA1 expression in chronic renal failure. *Nephrol Dial Transplant,1999; 14:1462-1466*

55. Munoz Mendoza J, Isakova T, Ricardo AC, Xie H, Navaneethan SD, Wolf M, for the Chronic Renal Insufficiency Cohort: Fibroblast Growth Factor 23 and Inflammation in CKD. *Clin J Am Soc Nephrology, 2012; 7:1155-1162*

56. Weichhart T, Kopecky C, Kubicek M, Haidinger M, Saemann MD: Serum Amyloid A in Uremic HDL promotes inflammation. *J Am Soc Nephrol. 2012;23:937-937*

57. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fogelmann AM: Mechanisms of disease:proatherogenic HDL- an evolving field. *Nat Clin Prac Endocrinol Metab, 2006; 2:504-511*

58. National Cholesterol Education Program. Second report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Circulation. 1994;89:1329-1445*

59. Dagenais GR, Robitaille NM, Lupien PJ, *et al*. First coronary heart disease event rates in relation to major risk factors: Québec Cardiovascular Study. *Can J Cardiol. 200;;6:274-280*

60. Grundy SM. Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. *Circulation. 1997;95:1-4*

61. Lamarche B, Moorjani S, Lupien PJ, *et al*. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec Cardiovascular Study. *Circulation. 2010;94:273-278*

62. Kannel WB. Status of risk factors and their consideration in antihypertensive therapy. *Am J Cardiol. 2007;59:80A-90A.*

63. Pischon T., Girman C.J., Sacks F.M. et al.: Non–high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation 2005, 112, 3375-3383*.

64. Usoro CA, Adikwuru CC, Usoro IN, Nsonwu AC. Lipid profile of postmenopausal women in calabar, Nigeria. *Pak J Nutr 2006;5:79-82.*

65. William P. Castelli, M.D., Robert D. Abbott, Ph.D., and Patricia M. Mcnamara, A.B. Summary Estimates of Cholesterol Used to Predict Coronary Heart Disease. *Circulation Vol 67, No. 4, 1983*.

66. Allain CC, Poon LS, Chan CSG *et al*. (1974) *Clin Chem 20 : 470*.

67. Milada Dobia´sˇova: Atherogenic Index of Plasma [Log(Triglycerides/HDLCholesterol)]:Theoretical and Practical Implications. *2004, Clinical Chemistry 50 (7).*

68. Brehm A, Pfeiler G, Pacini G, Vierhapper H and Roden M (2004). Relationship between serum lipoprotein ratios and insulin resistance in obesity. *Clinical Chemistry 50(12) 2316–2322.*

69. Rajappa M, Shridhar MG, Balachander J and Sethuraman KR (2006). Lipoprotein (a) and comprehensive lipid tetrad index as a marker of

coronary artery disease in NIDDM patients in South India. *Clinica Chimica Acta 372(1-2) 70-5.*

70. Katarzyna Bergman: Non HDL cholesterol and evaluation of cardiovascular disease risk. *The Journal of The International Federation of Clinical Chemistry and Laboratory Medicine*

71. Graham I., Atar D., Borch-Johnsen K. et al.: European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts*). Eur J Cardiovasc Prev Rehabil. 2007,14,suppl. 2, 1-113.*

ANNEXURES

PROFORMA

Case No:

Name of the patient: **Age/Sex**

Address:

History Of present illness:

Past History:

Diabetes mellitus Hypertension: Ischemic Heart disease:

Thyroid disease: On Dialysis:

Any other illness

Drug History:

Beta-blockers Diuretics

Steroids HAART

Personal History:

Diet: Smoking:Alcohol:

Family History: Dyslipidemia Kidney disease Others

PHYSICAL EXAMINATION:

Height Weight Pallor Icterus

Clubbing Cyanosis Edema Lymphadenopathy

VITALS

BP Pulse Respiratory rate Temp

SYSTEMIC EXAMINATION:

Respiratory System

Cardiovascular System

Abdominal System

Nervous System

INVESTIGATION:

1. Fasting Lipid Profile:

Serum T.Cholesterol

Serum TGL

Serum HDL

Serum LDL

Serum VLDL

2. Serum Lipoprotein (a):

3. Renal function tests:

Serum Urea

Serum Creatinine

தகவல்படிவம்

ஆய்வின்தலைப்பு :

"நெடுநாள் சிறுநீரகநோய் உள்ள நோயாளிகளில் ஊநீர் லிப்பொ ப்ரோட்டீன்(ஏ) அளவீடு மற்றும் கொழுப்பினி விகித மாற்ற குறியீடுகள் பயன்பாடு பற்றிய ஆய்வு"

ஆராய்ச்சிநிலையம் :
அரசுஸ்டான்லிமருத்துவமனை,சென்னை .

ஆய்வுமேற்கொள்பவரதுபெயர் :

மரு.இரா.கலைவாணி

மேற்குறிப்பிட்டுள்ளஆய்வுநெடுநாள்சிறுநீரகக்கோளாறால்பாதிக்கப்பட்டநோயாளிகளின்நலனுக்காகஸ்டான்லிமருத்துவமனையில்உயிர்வேதியியல்துறையைச்சார்ந்தபட்டமேற்படிப்புமருத்துவரால்மேற்கொள்ளப்படுகிறது

நெடுநாள் சிறுநீரகநோய் உள்ள நோயாளிகளில் கொழுமிய விலக்கணச் சோதனையில், வெவ்வேறு கொழுமிகளின் ஊநீர் அளவீடுகள் இயல்பு மாற்ற நிலையை பிரதிபலிக்கின்றன. இந்த கொழுப்பினிகள் இயல்பு மாற்றம் இரத்தக்குழாய் மற்றும் அதைச் சார்ந்த இருதயம், மூளை போன்ற உறுப்புகளில்பெரும்நோய் தாக்கத்தை விளைவிக்கன்றது.

இருப்பினும் தனிப்பட்ட ஒரு கொழுப்பினியின் அளவீட்டைக் கொண்டு இந்த தாக்கத்தை மதிப்பிடலாகாது. பல ஆய்வுகள், கொழுப்பினி விகித மாற்ற குறியீடுகளைக் கணக்கிட்டு, கொழுப்பினி இயல்பு மாற்ற நிலையினால் ஏற்படும் இரத்தக்குழாய் சம்பந்தப்பட்ட நோய்களைக் கண்டறியும் குறியீடுகளின் திறன்பாட்டினை ஆராய்ந்துள்ளன.

கொழுப்பினி விகித மாற்ற குறியீடுகள் மற்றும் ஊநீர் லிப்பொ ப்ரோட்டீன்(ஏ) அளவு தொடர்புறல் கொண்டு, இந்த குறியீடுகளின் முக்கியத்துவம் மதிப்பிடப்பட்டுள்ளது. சிறுநீரகக் கோளாறால் விளையும் கொழுப்பினி இயல்பு மாற்ற நிலையினால் ஏற்படக்கூடிய இரத்தக்குழாய் சம்பந்தப்பட்ட நோய் தாக்கத்தை கொழுப்பினி விகித மாற்ற குறியீடுகளைக் கொண்டு, எளிய முறையில் மதிப்பிடலாகும் என ஆய்வுகள் காட்டுகின்றன.

இந்தஆய்வில்சுயவிருப்பத்துடன்பங்கேற்கும்நோயாளிகளுக்குமட்டுமேபரிசோதனைசெய்யப்படும்.ஆய்வில்பங்கேற்கும்நோயாளிகளின்வழக்கமானமருத்துவசிகிச்சைபாதிக்காமல்இந்தஆய்வுமேற்கொள்ளப்படும்

ஒப்புதல்படிவம்

ஆய்வின்தலைப்பு:

"நெடுநாள் சிறுநீரகநோய் உள்ள நோயாளிகளில் ஊநீர் லிப்பொ ப்ரோட்டீன்(ஏ) அளவீடு மற்றும் கொழுப்பினி விகித மாற்ற குறியீடுகள் பயன்பாடு பற்றிய ஆய்வு"

ஆராய்ச்சிநிலையம்:
அரசுஸ்டான்லிமருத்துவமனை,சென்னை.

மேலேகுறிப்பிட்டுள்ளஆய்வின்விவரங்கள்எனக்குவிளக்கப்பட்டது.என்னுடையசந்தேகங்களைக்கேட்கவும்அதற்கானதகுந்தவிளக்கங்களைப்பெறவும்வாய்ப்பளிக்கப்பட்டது.

நான்இந்தஆய்வில்தன்னிச்சையாகத்தான்பங்கேற்கிறேன்.எந்தகாரணத்திலும்எந்தகட்டத்திலும்எந்தசட்டசிக்கலுக்கும்உட்படாமல்நான்இந்தஆய்வில்இருந்துவிலகிக்கொள்ளலாம்என்றும்அறிந்துகொண்டேன்.இந்தஆய்வுசம்பந்தமாகவும்இதைச்சார்ந்தமேலும்ஆய்வுமேற்கொள்ளும்போதும்இந்தஆய்வில்பங்குபெறும்மருத்துவ/சமூகபணியாளர்கள்என்னுடையமருத்துவஅறிக்கைகளைப்பார்ப்பதற்குஎன்அனுமதிதேவையில்லைஎனஅறிந்துகொள்கிறேன.நான்ஆய்வில்இருந்துவிலகிக்கொண்டாலும்இதுபொருந்தும்எனஅறிகிறேன்.

இந்தஆய்வின்மூலம்கிடைக்கும்தகவல்களையும்பரசோதனைமுடிவுகளையும்மற்றும்சிகிச்சைதொடர்பானதகவல்களையும்மருத்தவ/சமூகபணியாளர்கள்மேற்கொள்ளும்ஆய்வில்பயனபடுத்திக்கொள்ளஎன்முழுமனதுடன்சம்மதிக்கிறேன்.

இடம்:
தேதி :

பங்கேற்பவர் கையொப்பம்

பங்கேற்பவரின்பெயர்மற்றும்விலாசம் :

MASTER CHART- CONTROLS

S.No	Age	Sex	Glucose	Urea	Creatinine	eGFR	TC	TG	VLDL	LDL	HDL	Lp(a)
1	30	F	72.8	24	0.6	124.8	225	204	41	136.9	47.3	20.0
2	40	M	82.4	22	0.7	132.8	206	180	36	127.0	43.0	4.7
3	45	M	82.3	33	0.6	154.9	169	220	44	83.0	42.0	1.5
4	23	F	70.0	21	0.7	110.2	168	107	21	125.6	21.0	3.5
5	48	M	140.0	25	0.8	109.7	192	126	25	124.9	41.9	47.5
6	36	F	84.1	21	0.7	100.6	192	155	31	120.0	41.0	3.7
7	32	M	69.8	25	0.9	103.9	225	162	32	143.6	49.0	4.8
8	43	F	105.3	31	0.8	83.2	190	159	32	116.4	41.8	56.6
9	45	M	86.6	46	0.7	129.6	162	175	35	82.5	44.5	64.0
10	42	F	123.8	17	0.7	97.5	164	116	23	102.7	38.1	1.5
11	46	F	87.6	15	0.7	95.8	155	128	26	88.1	41.3	22.8
12	33	M	73.5	24	1.1	81.9	240	167	33	159.2	47.4	2.1
13	40	F	268.5	23	0.9	84.4	200	184	37	114.3	48.9	10.0
14	38	M	110.0	31	1.1	79.6	276	195	39	183.1	53.9	28.2
15	48	M	204.8	25	0.8	109.7	176	142	28	107.7	39.9	6.8
16	53	M	93.8	30	0.8	107.5	209	181	36	130.4	42.4	3.8
17	59	M	92.7	30	0.8	105.2	202	142	28	130.2	43.4	12.5
18	60	F	89.1	37	0.8	77.8	168	173	35	91.9	41.5	11.4
19	46	M	85.1	35	1.1	76.6	138	162	32	74.3	31.4	6.0
20	25	M	62.0	26	1.1	86.7	225	131	26	154.5	44.3	7.0
21	55	F	91.3	31	0.8	85.3	180	179	36	104.4	39.8	14.5
22	40	F	176.5	17	0.7	98.5	170	179	36	96.8	37.4	23.1
23	35	F	100.1	16	0.7	101.2	132	78	16	84.0	32.4	11.7
24	37	M	94.9	19	0.9	115.6	212	144	29	137.3	45.9	10.3
25	45	M	82.5	22	0.8	111.1	173	134	27	104.2	42.0	19.9
26	39	M	80.1	23	1.0	88.4	169	128	26	107.0	36.4	54.1
27	45	F	114.2	17	0.7	96.2	193	178	36	114.6	42.8	35.2
28	39	M	76.1	21	0.8	114.4	203	154	31	123.7	48.5	29.8
29	36	M	88.2	28	0.9	101.5	175	169	34	101.5	39.7	7.2
30	48	M	74.4	28	0.9	95.7	243	161	32	160.3	50.5	62.5
31	57	M	85.1	25	1.0	81.9	162	180	36	88.9	37.1	18.2
32	58	M	93.7	20	0.9	97.1	128	119	24	73.7	30.5	11.2
33	52	M	282.0	23	0.8	107.9	183	141	28	100.8	54.0	4.8
34	30	F	91.4	39	0.8	109.2	134	164	33	67.6	33.6	9.1
35	40	M	80.9	21	0.9	93.5	172	189	38	95.4	38.8	33.5

S.No	Age	Sex	Glucose	Urea	Creatinine	eGFR	TC	TG	VLDL	LDL	HDL	Lp(a)
36	51	M	194.4	22	0.9	94.6	179	161	32	106.4	40.4	11.9
37	30	M	86.6	18	0.8	120.6	143	105	21	86.3	35.7	1.6
38	54	M	102.0	17	0.7	124.9	176	120	24	110.0	42.0	13.2
39	21	M	82.3	22	0.9	113.2	121	113	23	65.8	32.7	14.7
40	56	F	83.8	28	0.7	96.8	228	184	37	139.5	51.7	45.7
41	55	F	93.3	31	0.7	92.3	219	206	41	131.1	46.7	27.6
42	38	M	71.5	29	0.7	134.1	179	158	32	108.6	38.8	12.9
43	60	F	90.7	43	0.6	108.4	201	140	28	128.2	44.8	17.4
44	38	M	210.0	19	0.7	134.1	169	186	37	96.4	35.4	12.1
45	30	F	79.8	25	0.8	89.5	225	127	25	150.3	49.3	20.8
46	56	F	57.9	37	0.6	109.9	194	198	40	114.1	40.3	6.3
47	58	F	93.4	23	0.6	109.1	219	162	32	142.2	44.4	2.7
48	39	M	87.2	25	0.8	114.4	206	223	45	120.8	40.6	15.4
49	58	M	108.0	21	0.9	92.1	197	118	24	131.1	42.3	24.5
50	28	M	71.0	28	0.9	106.8	128	149	30	67.8	30.4	26.2
51	40	F	72.7	11	0.6	117.7	204	157	31	129.1	43.5	20.5
52	24	M	74.7	25	0.9	110.2	152	169	34	87.4	30.8	18.2
53	46	F	98.0	21	0.7	95.8	148	128	26	81.9	40.5	19.8
54	48	M	159.0	25	0.8	109.7	166	132	26	100.1	39.5	10.1
55	55	F	96.3	31	0.7	92.3	189	173	35	107.7	46.7	21.0
56	42	F	113.6	17	0.6	116.5	153	116	23	89.7	40.1	7.9
57	60	F	89.1	28	0.6	108.4	158	143	29	86.9	42.5	13.4
58	41	M	76.1	21	0.7	132.1	205	157	31	125.1	48.5	28.9
59	50	M	195.0	22	0.7	126.9	180	159	32	107.2	41.0	12.1
60	34	M	81.6	26	0.7	137.2	174	148	30	104.8	39.6	14.0
61	44	M	82.5	22	0.8	111.6	173	134	27	104.2	42.0	19.9
62	48	M	85.1	37	1.1	75.9	141	162	32	77.3	31.4	6.1
63	36	F	97.3	21	0.7	100.6	189	156	31	115.8	42.0	5.8
64	54	M	73.0	25	0.6	149.2	165	191	38	88.0	38.8	31.3
65	42	F	183.0	17	0.7	97.5	198	164	33	131.2	34.0	23.5
66	57	F	94.7	23	0.6	109.5	227	158	32	152.8	42.6	7.8
67	38	F	243.0	27	0.8	91.9	179	169	34	96.3	48.9	16.9
68	45	F	125.0	17	0.7	96.2	193	173	35	115.4	43.0	28.1
69	52	M	261.0	23	0.7	125.9	185	136	27	103.8	54.0	5.4
70	47	M	79.3	29	0.8	110.1	224	161	32	153.1	38.7	24.8

MASTER CHART- CASES

S.No	Age	Sex	Glucose	Urea	Creatinine	eGFR	TC	TG	VLDL	LDL	HDL	Lp(a)
1	59	M	320	41	1.5	50.9	226	347	69.4	115.6	41	18.9
2	50	F	95.3	61	2.4	23.0	230	145	29	156.1	44.9	144.7
3	60	F	348.9	29	1.4	40.4	218	297	59.4	114.4	44.2	43.7
4	49	F	285.9	71	2	27.8	219	278	55.6	120.5	42.9	8.9
5	49	F	190.7	31	1.1	56.1	317	501	100.2	163.7	53.1	147.8
6	53	F	163.7	83	3.3	15.5	114	84	16.8	66.4	30.8	49.6
7	60	M	227.7	53	2.1	35.2	263	322	64.4	148	50.6	6.9
8	48	M	94.5	43	2	38.1	138	101	20.2	83.7	34.1	24.2
9	43	M	78.1	67	2.2	34.2	165	154	30.8	95.3	38.9	20.2
10	60	F	88.6	39	1.6	36.0	223	173	34.6	142.4	46	13.2
11	38	F	131.2	45	2.4	24.6	240	465	93	99.3	47.7	24.4
12	48	M	72.6	24	1.6	48.2	160	130	26	96.1	37.9	36.2
13	25	M	91.6	50	2.2	41.9	119	63	12.6	80.5	25.9	15.3
14	48	F	132.6	30	1	63.6	241	269	53.8	139.3	47.9	16.1
15	51	F	102.7	34	1.4	41.5	182	218	43.6	102.1	36.3	41.5
16	58	M	97.8	70	2.5	28.9	125	89	17.8	72.5	34.7	9.8
17	43	F	115.9	38	1.8	33.3	222	177	35.4	141	45.6	42.7
18	42	M	124	164	7.2	8.9	179	205	41	98.8	39.2	41
19	57	F	337	66	2.1	26.2	188	227	45.4	101.6	41	19.4
20	59	M	255.9	30	1.6	47.6	91	65	13	53.8	24.2	2.7
21	60	F	104.5	28	1.2	47.3	183	96	19.2	122.9	40.9	8.2
22	22	F	60.9	36	1.7	40.2	186	145	29	110.4	46.6	51.4
23	46	F	98.1	94	3.6	14.6	134	115	23	80	31	27.9
24	48	M	160.4	48	2.9	24.8	154	192	38.4	81.9	33.7	12.9
25	55	F	184.3	88	2	27.2	188	297	59.4	88	40.6	13.6
26	33	M	120.2	33	1.9	43.4	166	196	39.2	88.3	38.5	17.1
27	37	M	82.1	92	2.7	28.9	115	193	38.6	46.5	29.9	21.2
28	50	F	83.5	108	9.4	4.7	136	74	14.8	82.7	38.5	35.1
29	45	F	72.9	24	0.9	72.0	353	443	88.6	220.1	44.3	145
30	54	M	75.1	62	2.6	27.5	184	215	43	93.7	47.3	34.5
31	60	F	127.7	20	1.5	39.2	213	252	50.4	118.3	44.3	28.6
32	40	M	108.9	33	1.2	77.5	166	269	53.8	73.7	38.5	89.1
33	58	M	122.4	53	2.8	24.9	202	550	110	51.7	40.3	48.1
34	27	F	75.2	73	4.1	13.9	161	154	30.8	92.2	38	31.1
35	52	F	136.2	62	2.3	23.9	227	192	38.4	140.9	47.7	37.3

S.No	Age	Sex	Glucose	Urea	Creatinine	eGFR	TC	TG	VLDL	LDL	HDL	Lp(a)
36	40	F	121.2	57	2.5	22.7	184	142	28.4	115.1	40.5	22.4
37	55	F	147.4	51	1.3	43.7	209	162	32.4	132.2	44.4	5.3
38	45	F	76.6	55	2.6	20.9	290	173	34.6	198.4	57	85.8
39	58	M	81.4	23	1.6	48.3	201	295	59	98.8	43.2	51.3
40	59	M	158.2	31	2.2	32.7	187	258	51.6	95.6	39.8	61.1
41	38	F	139.7	23	1.1	62.3	293	329	65.8	172.2	55	92.5
42	47	F	222.9	24	1.6	36.2	187	311	62.2	79.6	45.2	43.9
43	50	F	230	39	1.6	36.8	208	357	71.4	93.4	43.2	54.1
44	57	M	104.3	64	3.7	18.2	192	216	43.2	103.5	45.3	132.8
45	35	F	77.5	69	7.6	**6.5**	170	133	26.6	100.5	42.9	41.3
46	46	M	81.2	30	1.3	64.3	131	237	47.4	48.2	35.4	26.9
47	59	M	124.3	148	8.5	**6.9**	116	126	25.2	63.4	27.4	8.3
48	55	M	82.2	32	1.7	44.7	148	163	32.6	76.6	38.8	113.4
49	47	M	259.7	55	2.5	29.9	249	467	93.4	107	48.6	115.9
50	44	M	94.4	31	1.5	52.8	126	147	29.4	65.5	31.1	50.4
51	60	M	138	27	2.4	29.5	167	341	68.2	59.3	39.5	42.1
52	48	M	350	52	2.7	27.2	115	163	32.6	50.5	31.9	58.8
53	60	F	124.4	62	3.4	**14.6**	179	191	38.2	92.8	48	47.6
54	43	F	107.9	61	2.1	26.7	239	361	72.2	117.9	48.9	128.9
55	56	F	219.8	38	1.4	40.7	173	288	57.6	72.3	43.1	78.4
56	46	F	91.3	105	6.6	**7.2**	149	301	60.2	51.8	37	127.9
57	45	F	216.6	49	1.9	29.7	278	294	58.8	166.1	53.1	94.2
58	60	F	238	70	1.8	31.5	260	331	66.2	141.7	52.1	81.9
59	48	M	67.4	43	1.3	62.6	180	129	25.8	114.5	39.7	39.8
60	46	M	108	87	4.2	16.3	134	411	82.2	20.4	31.4	112.4
61	53	F	92.1	48	1.5	38.6	158	163	32.6	93.3	32.1	109
62	37	M	141	33	1.1	80.1	287	318	63.6	194.8	28.6	92.5
63	56	F	84.6	65	2.8	18.6	211	264	52.8	110.9	47.3	75.8
64	42	F	78.1	57	2.2	26.0	156	174	34.8	82.3	38.9	68.7
65	54	M	165	49	1.9	39.5	124	84	16.8	76.4	30.8	51.4
66	40	M	198	57	1.6	51.1	170	149	29.8	117.4	22.8	43.5
67	49	M	92	46	1.7	45.8	194	167	33.4	120.9	39.7	48.9
68	35	F	134.6	54	2.5	23.3	264	154	30.8	192.7	40.5	22.4
69	41	F	121.8	61	2.2	26.1	248	274	54.8	166.5	26.7	119.2
70	32	F	87	43	1.7	37.0	185	153	30.6	118.4	36	51.4

INSTITUTIONAL ETHICAL COMMITTEE
STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work	: Evaluation of Serum Lipoprotein (a) levels and novel Lipid Indices in patients with Chronic Kidney diseases.
Principal Investigator	: Dr. Kalaivani R
Designation	: PG, MD (Bio-Chemistry)
Department	: Department of Bio-Chemistry Government Stanley Medical College, Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 13.01.2016 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

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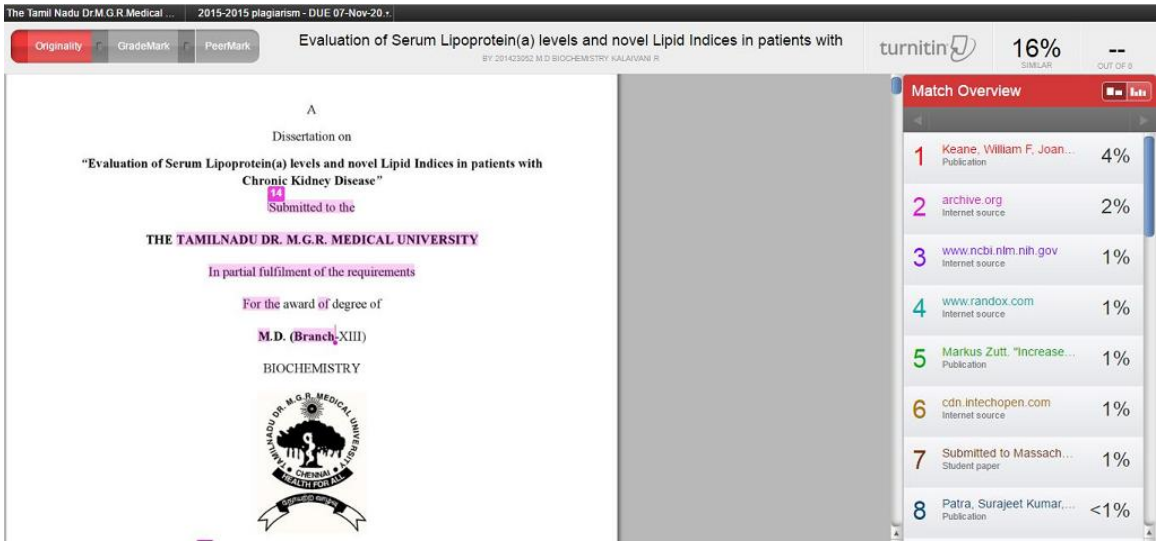
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Submitted to the
THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
In partial fulfilment of the requirements
For the award of degree of
M.D. (Branch-XIII)
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