SERUM VISFATIN- A NOVEL MARKER IN CHRONIC KIDNEY DISEASE

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CERTIFICATE

This is to certify that dissertation titled "**SERUM VISFATIN –A NOVEL MARKER IN CHRONIC KIDNEY DISEASE"** is a bonafide work done by **Dr.S. SYED ALI FATHIMA** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2012 to 2015.

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I, **Dr. S. SYED ALI FATHIMA** hereby solemnly declare that the dissertation titled "**SERUM VISFATIN –A NOVEL MARKER IN CHRONIC KIDNEY DISEASE"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam,M.D(Bio).,DGO,**. This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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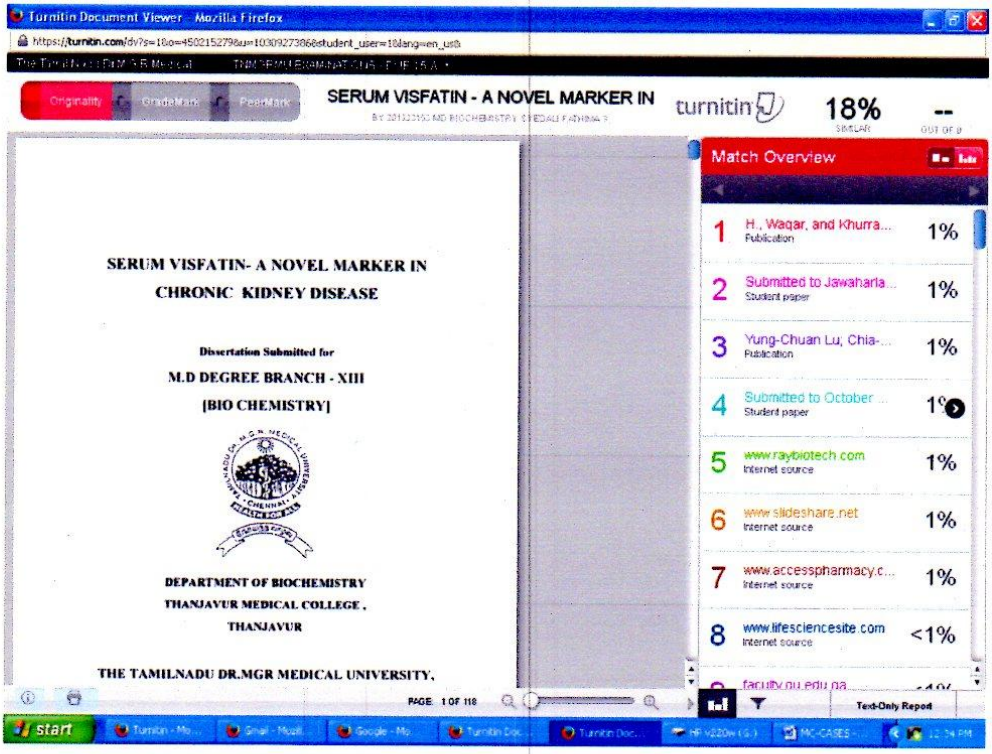

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CONTENTS

ABBREVIATIONS

CKD	Chronic Kidney Disease
GFR	Glomerular Filteration Rate
CVD	Cardio Vascular Disease
ESRD	End Stage Renal Disease
IL	Interleukin
TNF	Tumour Necrosis Factor
NKF-KDOQI	National Kidney Foundation Kidney Disease Outcomes Quality Initiative
eGFR	estimated Glomerular Filteration Rate
NSAIDS	Non Steroidal Anti Inflammatory Drugs
DM	Diabetes Mellitus
HT	Hypertension
PTH	Parathormone
PAI-1	Plasminogen Activator Inhibitor-1
RAAS	Renin Angiotensin Aldosterone System
TGF	Transforming Growth Factor
PDGF	Platelet Derived Growth Factor
PKD	Polycyctic Kidney Disease
ACE	Angiotensin Converting Enzyme
GN	Glomerulo Nephritis
TC	Total Cholesterol
TGL	Triglycerides
VLDL-C	Very Low Density Lipoprotein- Cholesterol

LDL-C	Low Density Lipoprotein- Cholesterol
HDL-C	High Density Lipoprotein- Cholesterol
LH	Luteinizing Hormone
CRP	'C' Reactive Protein
NAD	Nicotinamide Adenine Dinecleotide
SIRT	Silent Information Regulator
NMN	Nicotinamide Mono Nucleotide
VEGF	Vascular Endothelial Growth Factor
hsCRP	high sensitive 'C' Reactive Protein
HRP	Horse Radish Peroxidase

SERUM VISFATIN- A NOVEL MARKER OF CHRONIC KIDNEY DISEASE

BACKGROUND

Chronic Kidney Disease (CKD) is defined as Glomerular Filteration Rate < 60ml/min/1.73m^2 for a minimum of 3 months irrespective of the cause. Accelerated atherosclerosis is the main cause of morbidity and mortality in CKD patients. Visfatin is a 52KDa protein predominantly secreted by the visceral adipose tissue which has anti-inflammatory , Insulin-mimetic and antiapoptotic activities.

AIMS AND OBJECTIVE

To estimate the levels of serum Visfatin in patients with CKD and to compare it with serum hsCRP (high sensitive C reactive protein), Ccr(Creatinine clearance) and lipid profile.

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College Hospital . 50 patients of CKD as cases and 50 age and gender matched healthy individuals were selected as cases and controls respectively.Serum Visfatin and Serum hsCRP were estimated by Enzyme Immune Assay and Immunoturbidimetric method respectively. Serum Total Cholesterol (TC), Triglycerides (TGL) , Very Low Density Lipoprotein(VLDL-C), High Density Lipoprotein (HDL-C) were

estimated by enzymatic method. Creatinine Clearance (Ccr)and Low Density Lipoprotein (LDL-C) were calculated using formula.

RESULTS

The mean value of Visfatin in cases and controls were 27.42±8.92 and 10.62±1.57 ng/ml ($t=13.11$; $p<0.05$ significant) respectively. The level of serum visfatin is inversely correlated with Ccr ($r= -0.898$; $p< 0.01$) . Serum hsCRP, TGL and VLDL-C were significantly increased and HDL-C was significantly decreased in cases when compared to controls ($p < 0.05$).There is no significant difference of TC between cases and controls .

CONCLUSION

- The present study demonstrated that serum Visfatin levels are significantly increased in patients with CKD. This increase in serum Visfatin level is progressive from the early stages to the late stages of CKD .
- Visfatin may be considered as the novel marker of mortality predictor in CKD patients. Higher the visfatin level, higher the mortality of CKD patients.

Key words

Chronic kidney disease, glomerular filteration rate, Visfatin, high sensitive C reactive protein.

INTRODUCTION

Chronic Kidney Disease (CKD) encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function and a progressive decline in Glomerular Filteration Rate (GFR)[1]. CKD is defined as kidney damage or GFR < 60ml/min/1.73m^2 for a minimum of 3 months irrespective of the cause. The GFR is defined as the rate of plasma flow filtered across the glomerular basement membrane .CKD is a growing public health problem worldwide with increasing prevalence, high cost and poor outcomes like End Stage Renal Disease (ESRD), Cardio Vascular Disease (CVD) and Premature Death[2].

CKD affects 10-15% of general adult population in developed countries. In India, the prevalence of CKD is 0.79%[3]. It is an underestimate of the disease because most of the CKD patients die of CVD than to reach ESRD. CKD is the 12th cause of death and 17th cause of disability worldwide[4].

Accelerated atherosclerosis is the main cause of premature morbidity and mortality in patients with CKD. Over 80-90% of patients with CKD die primarily of CVD before reaching the need for dialysis. This necessitates the importance of early detection of CVD before the patient reach advanced stages of CKD[5].

Eventhough the traditional cardiovascular risk factors such as Diabetes Mellitus(DM) , Hypertension(HT) , Smoking and Dyslipidemia are highly

prevalent in CKD patients, they only partly explain the high cardiovascular risk of CKD patients. Nontraditional risk factors such as Inflammation, Endothelial dysfunction , Insulin resistance and Myocardial necrosis have also been associated with the increased cardiovascular event rates and mortality risk in CKD patients.

CKD is associated with chronic inflammation which promotes endothelial dysfunction , vascular remodelling and progression of atherosclerosis. In CKD , there is a progressive deterioration of renal function may also lead onto accumulation of uremic toxins and dyslipidemia which in turn stimulate inflammation and result in atherosclerosis[6]. The causes of inflammation among patients with CKD are complex and multifactorial[7].

Adipose tissue is no more considered as inert site of nutrient storage but rather a metabolically active site capable of producing soluble factors called Adipokines. Visfatin is one of the adipokine being the subject of intense research nowadays because of its pleiotropic actions[8,9]. Most important action is acting as a proinflammatory cytokine that stimulates the expression of inflammatory cytokines like IL (Interleukin) - 6, Tumour Necrosis Factor (TNF) α and β.

Because of the reduced renal function in CKD patients there will be accumulation of these inflammatory cytokines. In CKD there exists an active interplay between atherosclerosis and inflammation through the accumulation of these inflammatory cytokines. This in turn contributes to the development of

CVD in CKD. So measurement of serum level of Visfatin could therefore have a potential value to predict premature atherosclerosis and hence to assess the cardiovascular risk in CKD.

Hence in the present study the serum level of Visfatin is estimated in patients with different stages of CKD and the relationship between serum Visfatin, inflammation and dyslipidemia were analyzed.

REVIEW OF LITERATURE

The kidneys play a central role in the homeostatic mechanisms of the human body, and reduced renal function strongly correlates with increasing morbidity and mortality.

IMPORTANT COMPONENTS OF KIDNEY FUNCTION

- Filteration and preparation of an ultrafilterate.
- Reabsorption of Glucose, Aminoacids , Electrolytes and Proteins.
- Homeostasis of Extracellular volume, Acid base status, Blood pressure and Electrolytes.
- Synthesis of Erythropoietin, Glutathione ,Ammonia .
- Site of Gluconeogenesis.
- Catabolism of Hormones ,Cytokines.
- Activation of Vitamin D.
- Release of Renin.

REQUIREMENTS FOR NORMAL KIDNEY FUNCTION

The function of kidney depends upon the number and function of nephrons present in each kidney. For a nephron to function normally, the following conditions must be satisfied[10].

1. There must be free flow of blood through the glomerular capillaries.

2. An adequate volume of filterate must be produced which should not contain any blood cells or proteins.
3. The tubules must be able to selectively reabsorb some important substances from the filterate and to excrete other substrates into the filterate.
4. The urine formed by the nephron must be able to flow freely from the kidney into the bladder and out of the urethra.

Any derangement of the above function will result in kidney Disease.

RENAL GLOMERULAR FUNCTION

About 200L of plasma ultra filterate enter the renal tubular lamina daily by glomerular filteration[11]. GFR depends upon the following factors:

- Balance of pressures across the filteration barrier in the glomerulus (difference between the hydrostatic pressure in the glomerular capillaries which promote filteration, and the plasma oncotic pressure and hydrostatic pressure in Bowman's space which oppose filteration).
- Rate of renal plasma flow.
- Total surface area of the glomerular capillaries.

GLOMERULAR FILTERATION BARRIER

An ultra filterate of plasma passes from glomerular capillary blood into the space of Bowman's capsule, through the glomerular filteration barrier[12,13].

The glomerular filteration barrier is made up of three layers.

1.Capillary endothelium

2.Basement membrane

3.Podocytes

Glomerular endothelial cells and podocytes have glycocalyx, a negatively charged surface coat. Heparan sulphate, Sialic acid and Sialoproteins are present in the glomerular basement membrane. These negative charges impede the passage of negatively charged molecules through the glomerular filteration barrier by electrostatic repulsion.

RENAL TUBULAR FUNCTION[14,15,16]

When the plasma filtered into Bowman's space enters the proximal tubule, the process of reabsorption takes place. From the 200L of plasma filtered daily, only about 2L of urine is formed. Almost all the reusable nutrients and the bulk of electrolytes are reclaimed from the proximal tubules, with fine homeostatic adjustments taking place more distally.

The tubular cells do not actively deal with waste products like Urea and Creatinine to any significant degree.Most filtered Urea is passed in urine ,but some amount of it diffuses back passively from the collecting ducts with water; by contrast , some amount of Creatinine is secreted into the tubular lumen.

The tubular cells use Adenosine Tri Phosphate dependent active transport, sometimes selectively against physicochemical gradients. Transport of charged ions tends to produce an electrochemical gradient that inhibits further transport. This is minimized by two processes.

1. ISOSMOTIC TRANSPORT

This occurs mainly in the proximal convoluted tubules and reclaims the bulk of filtered essential constituents. Active transport of one ion leads to the passive movement of another ion of opposite charge in the same direction along the electrochemical gradient. For example, isosmotic reabsorption of Na^+ depends on the availability of negatively charged molecules like Cl^- .The process is isosmotic because the active transport of solute causes movement of equivalent amount of ion the same direction. Isosmotic transport also occurs to a lesser extent in the distal parts of the nephron.

2. ION EXCHANGE

This occurs mainly in the more distal parts of the nephrons and is important for fine adjustment after bulk reabsorption has taken place.Ions of the same charge , usually cations are exchanged and neither electrochemical nor osmotic gradients are created. For example, Na^+ may be reabsorbed in exchange for K^+ and H^+ ions.

CHRONIC KIDNEY DISEASE[17]

Chronic Kidney Disease refers to many clinical abnormalities that progressively worsen as kidney function declines. In 2002, The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF-KDOQI) has proposed a definition for CKD to create uniform terminology to improve communication among patients, physician and researchers. The definition of CKD is either

" Kidney damage for ≥ 3 months, as defined by structural (or) functional abnormalities of the kidney with or without decreased GFR manifested by either Pathological abnormalities (or) markers of kidney damage including the abnormalities in the composition of the blood or urine or abnormalities in Imaging tests".

(Or)

" Glomerular filtration rate < 60ml/min/1.73 m^2 for ≥3 months with (or) without kidney damage."

To define CKD , the GFR should be below 60ml/min/1.73m^2 because it represents over a 50% reduction in kidney function as compared to the level for young healthy adults.

End Stage Renal Disease is defined as either GFR<15ml/min/1.73m^2 (or) a need to start Renal Replacement Therapy either in the form of dialysis (or) renal transplantation. Most of the CKD patients will progress to ESRD and they require dialysis or kidney transplantation.

EPIDEMIOLOGY OF CKD IN WORLD[18,19]:

CKD is a clinical syndrome that occurs as a gradual decline in renal function overtime. As per United States Renal Data System (USRDS) annual data report on 2007, one in nine adult has been affected with CKD and 20 million people are at risk for CKD. Increasing incidence may be due to aging population, Metabolic Syndrome, DM and an increase in the prevalence of Obesity. Of about 45% of Type 1 Diabetes mellitus patients develop progressive

deterioration of kidney function within 15-20years after diagnosis. Hypertension has profound effects on renal system. Obesity plays an important role in the development of kidney disease apart from its role as a risk factor for DM and HT.

GLOBAL PREVALENCE OF CHRONIC KIDNEY DISEASE[20]

- Indicence of CKD is increased two fold in the last 15 years globally.
- In The United States of America , 30million people suffer from CKD and 6 lakh people will require Renal Replacement Therapy.
- Over 1 million people worldwide are alive on dialysis.
- The reported global annual growth of number of ESRD patients is 7%[21].

CURRENT SCENARIO IN INDIA[22,23]

Approximate prevalence of CKD in Delhi is 7852 per million population and the incidence of ESRD is 785 per million (10% of total CKD.) DM has emerged as the most frequent cause (30-40%) followed by Hypertension (14-22%).

STAGES OF CHRONIC KIDNEY DISEASE[24]

The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF-KDOQI) proposed a widely accepted classification for CKD in which CKD is divided into 5 stages. This classification system is based on the level of

estimated Glomerular Filtration Rate (eGFR). The higher the stage, the lower the GFR (or) vice versa.

This classification of staging provides the rough estimates of disease prevalence of different stages and the characteristics of individuals who are at increased risk for developing CKD (or) to allow the development of intervention plans for evaluation and management of each stage of CKD.

STAGES OF CKD	GFR (ml/min per $1.73m^2$)
0	$>90^a$
1	$\geq 90^b$
2	60-89
3	30-59
4	15-29
5	<15

'a' stands for associated risk factors of CKD. 'b' stands for demonstrated kidney damage (eg) Persistent proteinuria, Abnormal urinary sediment, Abnormal Blood and Urine chemistry or Abnormal Imaging studies.

GFR can be assessed by either 24 hours Urinary Creatinine Clearance or from serum Creatinine by using one of the following formulas[25,26]:

1. **<u>MODIFICATION IN DIET AND RENAL DISEASE(MDRD FORMULA)</u>**

$$eGFR = 186 \times (Pcr)^{-1.154} \times (\text{Age in years})^{-0.203}$$

- Multiply by 0.742 for women.
- Multiply by 1.21 for Blacks.
- Pcr – Plasma Creatinine in mg/dl.

2. **COCK CROFT – GAULT EQUATION**

$$\text{Estimated Creatinine Clearance} = \frac{(140\text{-Age}) \times \text{Wt in Kg}}{72 \times \text{Serum Creatinine}}$$

- Multiply by 0.85 for females.

3. CKD –EPI (EPIDEMIOLOGICAL COLLABORATION) FORMULA

$$eGFR = 141 \times \min(SCr/k,1)^{\alpha} \times \max(SCr/k,1)^{-1.209} \times 0.993^{Age}$$

- multiply by 1.018 for females
- multiply by 1.159 for black
- SCr - serum creatinine (mg/dL)
- k is 0.7 for females and 0.9 for males
- α is -0.329 for females and -0.411 for males
- min indicates the minimum of SCr/k or 1
- max indicates the maximum of SCr/k or 1

NATURAL HISTORY OF CKD[27]:

Many patients with CKD, stages 3-5 progress relentlessly to ESRD. The relationship between the reciprocal of serum Creatinine values (1/Scr) or the estimated GFR and time is linear. A significant percentage of patients have

breakpoints in their progression slopes leading to acceleration or slowing down of the rate of progression of CKD. They do not follow the predictable linear fashion. The breakpoints due to lack of adequate control in systemic Blood Pressure or exposure to Nephrotoxins , Non Steroidal Anti-Inflammatory Drugs (NSAIDS) or Radio Contrast. The rate of progression of CKD varies depending upon the underlying pathology and the individuals. In diabetic individuals the rate of progression of CKD is high. It is about 10ml/min/year reduction in GFR. In uncontrolled Hypertensive patients it is about 5ml/min/year. If both DM and HT are controlled, the reduction in GFR is only 2ml/min/year.

In non diabetic individuals, the rate of progression of CKD 2.5 times higher in chronic glomerulonephritis than in chronic interstitial nephritis and 1.5 times higher than in hypertensive nephrosclerosis.

ETIOLOGY OF CKD[28]

Location of Pathology (or) Anatomy	Systemic Diseases affecting the kidney	Primary kidney Diseases.
Glomerulus	Diabetes Mellitus, AutoImmune Disease,Systemic Infection,Drugs , Neoplasia.	Diffuse,focal, Crescentric and proliferative glomerulonephritis, focal and segmental glomerulo sclerosis, minimal change Disease.
Tubulo Interstitium	Systemic infections, Auto immune Diseases, Sarcoidosis, environmental toxins, Urea and drugs	Obstruction (or) stones and Urinary infection.
Vascular Diseases	Atherosclerosis, Decreased perfusion (Liver disease, Heart failure, Renal artery disease), Hypertension, Vasculitis, Thrombotic microangiopathy.	ANCA (AntiNeutrophil Cytoplasmic Antibodies) associated vasculitis, Fibromuscular dysplasia.
Congenital	Alport Syndrome, Polycystic Kidney Disease, Oxalosis, Fabry disease.	Medullary cystic disease ,Renal dysplasia

PERCENTAGE OF PRIMARY DISEASE CAUSING CKD

Diseases	Percentage
Diabetes mellitus	31.2%
Hypertension	14.1%
Glomerulo neptritis (GN)	14.4%
Tubulo Interstitial neptritis	7.0%
Hereditary (or) cystic diseases	2.1%
Miscellaneous	15.9%
Unknown	15.3%
Total	100%

The above datas are collected from the CKD registry of Indian Society of Nephrology Cumulative Report 2008.

RISK FACTORS OF CKD[29,30]

NON MODIFIABLE RISK FACTORS

- Old age
- Race and ethnicity
- Gender
- Low birth weight
- Low socio economic status

MODIFIABLE RISK FACTORS

- Obesity and Metabolic Syndrome
- Diabetes Mellitus
- Hypertension
- Uric Acid
- Proteinuria

MISCELLANEOUS FACTORS

- Smoking
- Alcoholism
- Caffeine
- Analgesic Abuse
- Dietary Phytoestrogens
- Lead and other heavy metal poisoning

CLINICAL PRESENTATION OF CKD[31]:

STAGE 1:

Represents kidney damage when GFR is normal or high. This includes patients with proteinuria (or) those with abnormal imaging studies.

STAGE 2:

- There is evidence of kidney damage with mild decrease in GFR.
- In both stages 1 and 2, patients are usually asymptomatic.

- Blood Urea Nitrogen and Serum Creatinine are normal.
- Acid Base, Fluid and Electrolyte balance are maintained by an adaptive increase of function in the remaining nephrons.

STAGE 3:

It includes patients with moderate decline in GFR. This is the stage where Serum Creatinine starts to rise. Majority of the patients still remain asymptomatic. Nocturia and polyuria are early symptoms that appear at this stage. Serum Creatinine and Blood urea nitrogen are increased and the level of Erythropoietin, Calcitriol and Parathormone (PTH) are usually abnormal.

STAGE 4:

They present with severe fall in GFR, and overt Uremic symptoms like loss of appetite, nausea, anemia and recurrent infections. They also have hypocalcemia, acidosis, hyperphosphatemia and hyperkalemia.

STAGE 5:

When GFR < 15ml/min, there is worsening of all the aforementioned symptoms in these patients. At this stage they require Renal Replacement Therapy.

FACTORS AFFECTING INITIATION AND PROGRESSION OF CHRONIC KIDNEY DISEASE

INITIATION FACTORS

- GENETIC PREDISPOSITION

CKD often runs within families. Polymorphism of the gene encoding RAAS(Renin Angiotensin Aldosterone System), Nitric Oxide Synthase, Kallikrein, IL-1, TNFα , Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β1), Plasminogen Activator Inhibitor-1(PAI-1), Complement factors and Immunoglobulins are the possible links of CKD.

- RACIAL FACTORS

Racial predisposition attributed to a number of factors such as DM, HT Susceptibility and also genetic susceptibility . Social deprivation or low social economic status are linked to the higher prevalence of CKD in the developing countries.

- MATERNAL AND FETAL FACTORS

Maternal malnutrition during pregnancy and resulting fetal malnutrition may contribute to the development of HT, Metabolic Syndrome, DM, CKD in adult life. Reduction in the number of nephron at birth (oligonephronia) and their ability to handle increased solute and salt load leads to glomerulosclerosis and CKD.

- OTHER FACTORS

Males and elderly people are more prone to develop CKD.

INITIATION MARKERS

- Hypertension – Elevated BP in both men and women is a risk factor for ESRD.
- Diabetes Mellitus

- Hyperlipidemia – Increased Triglycerides(TGL) is associated with CKD.
- Obesity
- Smoking is associated with increased risk for proteinuria in men.

PROGRESSION FACTORS

The progression of CKD is variable and associated with the variety of risk factors and markers:

AGE

Rate of progression of CKD is influenced by age. Elderly Patients affected by GN having a faster rate of GFR decline than young people except Type1 DM, in which young individuals having a faster rate of GFR decline.

GENDER:

Male gender was often associated with more rapid GFR decline and rapid progression.

RACE:

In United Kingdom , Indo-Asian patients with Diabetic Nephropathy may have a faster rate of decline of GFR than Caucasians.

GENETICS:

Patients with Polycystic Kidney Disease (PKD) carrying the genotype PKD1, have a worse prognosis than others. Angiotensin Converting Enzyme (ACE) gene polymorphism, either deletion (or) Insertion also involved in linking between susceptibility and progression of CKD.

LOSS OF RENAL MASS:

The threshold for nature progression in terms of GFR loss appears to be crossed when loss of nephron function exceeds 50%.

MODIFIABLE RISK FACTORS AND MARKERS.

HYPERTENSION

Transmission of systemic hypertension into glomerular capillaries and the subsequent development of glomerular hypertension contributes to the initiation and progression of glomerular sclerosis.

PROTEINURIA

Degree of proteinuria is associated with the rate of progression of CKD. Heavy proteinuria is associated with faster rate of progression. Non selective proteinuria is mainly responsible for the natural progression of CKD whereas the highly selective proteinuria (eg) Albuminuria can persist for more than 10 years in the nephrotic range without causing structural damage to the kidney.

METABOLIC MARKERS AND FACTORS

- GLYCEMIC STATUS

  Degree of glycemia is associated with the rate of progression of CKD. Higher the degree of glycemia , faster the rate of progression of CKD.

- LIPIDS

Dyslipidemia is the contributory factor for glomerulosclerosis and tubulointerstitial fibrosis.

- OBESITY

Excess body weight and high body mass index have been linked to a rapid progression of CKD.

- URIC ACID

Hyperuricemia may cause hypertension and renal injury through stimulation of Renin Angiotensin System.

MISCELLANEOUS FACTORS

- **SMOKING**

Cigarette smoking increases systemic blood pressure and alters the renal hemodynamics leading to rapid progression of CKD.

- **ALCOHOL AND RECREATIONAL DRUGS**

Alcohol consumption increases the rate of progression of CKD through the effect of hypertension. The use of recreational drugs such as Opiates is associated with the progression of CKD.

- **CAFFEINE**

Excessive exposure to Caffeine leads to progression of renal scarring.

- **ANALGESICS AND NSAIDS**

Ingestion of Phenacetin , Paracetamol, Aspirin and NSAIDS are associated with the increased risk of ESRD.

- **LEAD EXPOSURE:**

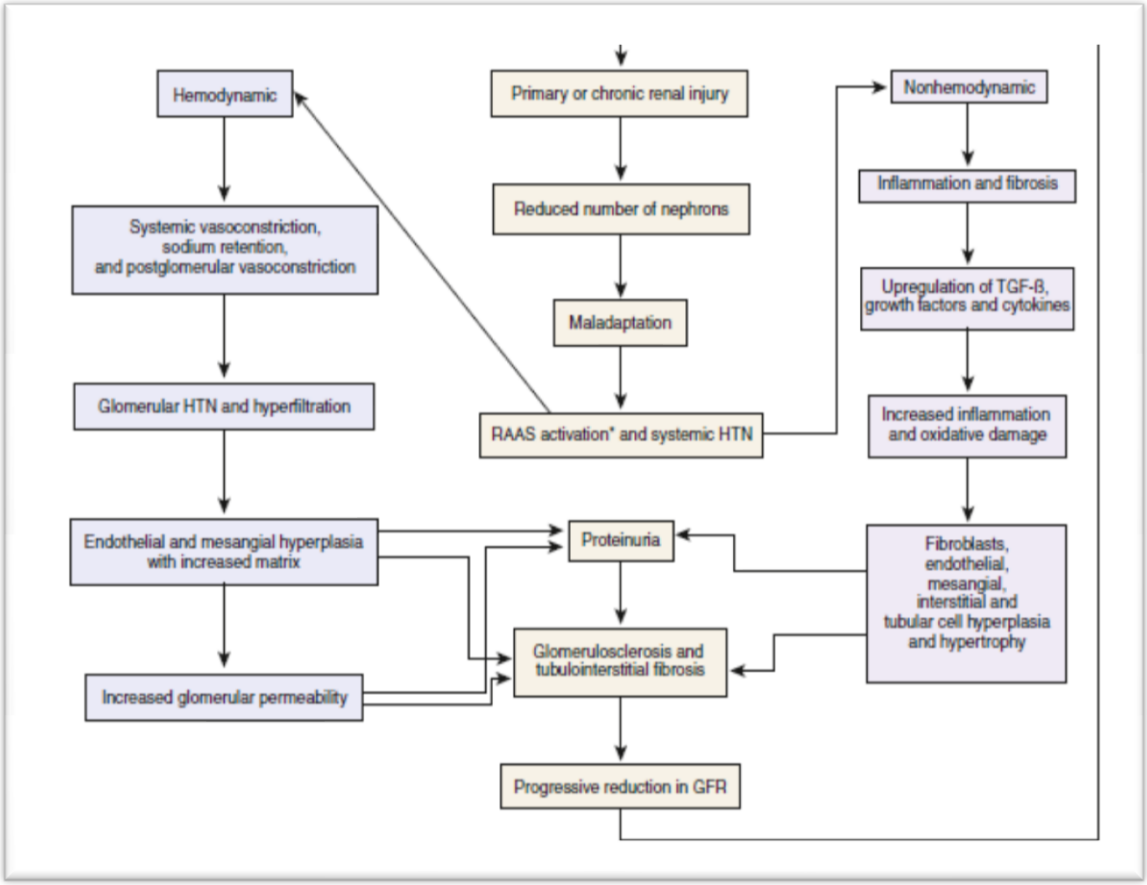
Chronic Lead exposure is implicated in the development of ESRD.

PATHOPHYSIOLOGY[28]

The pathophysiology of CKD is a complex process and is dependent on the primary cause. After an acute or chronic insult, many common pathways are activated to perpetuate glomerular and tubulointestinal injury. There are two types of injuries.

FIGURE.1

PATHOGENESIS OF CHRONIC KIDNEY DISEASE



1. Hemodynamic injury
2. Non-hemodynamic injury

HEMODYNAMIC INJURY

This process occurs at a linear rate in proportion to the greater reduction in kidney mass resulting in an increase in renal plasma flow and hyperfilteration of the remaining nephrons. Systemic hypertension and RAAS mediated glomerular hypertension cause progressive glomerular damage and proteinuria, resulting in decreased afferent arteriolar tone than the efferent tone. This net efferent vasoconstriction increases intraglomerular pressure and filteration pressure further more, perpetuating hyperfilteration injury.

With loss of functioning nephrons, Renin is released from the Juxta Glomerular Apparatus due to decreased perfusion pressure and low Sodium delivery to the Macula Densa. Renin converts Angiotensinogen to Angiotensin I. This Angiotensin I is converted into angiotensin II with the help of Angiotensin Converting Enzyme. Angiotensin II is the main perpetrator of glomerular hemodynamic maladaptation.

Angiotensin II is the potent vasoconstrictor in the post glomerular arterioles.It also increases proximal tubular Sodium reabsorption directly and distal tubular Sodium reabsorption indirectly through Aldosterone. Lastly, it also stimulates posterior pituitary to release AntiDiuretic Hormone.

All these mechanisms are an integral component of autoregulation helping to maintain GFR when perfusion is decreased. The increase in

glomerular hypertension increases the filteration fraction and radius of the pores in the glomerular basement membrane resulting in clinical proteinuria and glomerular destruction.

NON-HEMODYNAMIC INJURY

Non hemodynamic maladaptive pathways lead to inflammation and fibrosis of kidney. Angiotensin II level is increased in virtually every compartment of the kidney such as mesangial cell, endothelial cells, podocytes, the urinary space (Bowman's capsule) and the tubulointerstitium.

Increased Angiotensin II which upregulates several growth factors and their receptors like Connective Tissue Growth Factor, Epidermal Growth Factor, Insulin Like Growth Factor-1, PDGF ,Vascular Endothelial Growth Factor(VEGF), Transforming Growth Factor-β and Monocyte Chemotactic Protein -1. The activation of these factors leads to over production of extracellular matrix by upregulating other factors such as Type1 procollagen, PAI-1 and Fibronectin. In addition to that , excess adhesion molecules like Integrins or Vascular Cell Adhesion Molecule 1 allow the increased extracellular matrix and hypercellularity to accumulate resulting in cell proliferation, extra cellular matrix accumulation, adhesion of these cells and functional changes ultimately resulting in fibrosis.

Inflammation is the key factor in the progression of all types of kidney disease and it is mediated partly by RAAS. Angiotensin II recruits macrophages and T cells by stimulating Endothelin -1 and increased the production of Nuclear Factor k light chain enhancer of activated B cells. These molecules will release cytokines creating more inflammation. TGF-β is also responsible for cellular recruitment. Free radical oxygen species creates an additional injury which enables further inflammation and fibrosis.

Through primary stimulation of the RAAS, a cascade of events beginning with inflammation occurs which is perpetuated by accumulation of cells and matrix, and is exacerbated by adhesion of these cells and matrix resulting in glomerulosclerosis and tubulointerstitial necrosis. This creates a progressive course of CKD, proteinuria, decline in GFR and a vicious cycle of continuous RAAS activation.

Figure.1. shows the pathophysiology of progressive chronic kidney disease.It represents both hemodynamic and non hemodynamic mediated injury to the kidney to the development of CKD.

MECHANISMS OF PROGRESSION OF CHRONIC KIDNEY DISEASE

The progression of CKD is associated with the progressive sclerosis of glomeruli irrespective of the nature of underlying nephropathy. Both intra and extra glomerular cells contribute to the initiation and progression of glomerulo sclerosis.

INTRA GLOMERULAR CELLS

ROLE OF GLOMERULAR ENDOTHELIAL CELLS:

Glomerular endothelium performs an important role in preserving the integrity of vascular beds of glomeruli. They are the first exposed to the damage caused by hemodynamic injury, immunologic and metabolic injury. This endothelial injury is associated with the loss of their anticoagulant and anti inflammatory characteristics and gain of procoagulant and inflammatory properties leading to attraction and activation of platelets and microthrombus formation.

It is further associated with the initiation of glomerular micro inflammation with the attraction, adhesion and infilteration of glomerular tufts by monocytes. Then platelets and monocytes interact with mesangial cells resulting in production of extra cellular matrix (ECM).

ROLE OF MESANGIUM

After endothelial injury, the infiltrating monocytes interact with the mesangial cells and stimulate them either through direct cell to cell interaction or through release of mitogens like PDGF. The transcription factor kappa B (NF-κB) and a variety of kinases [Mitogen Activated Protein kinase (MAPK) and Jun N-terminal kinase or stress activated protein kinase] are involved in the proliferation of mesangial cells.

Activated mesangial cells have the capacity to revert to a myofibroblasts expressing markers such as α smooth muscle Actin, under the control of

Fibrogenic Growth Factor like TGF-β1 and synthesizes interstitial Type III collagen which is not a normal component of glomerular extra cellular matrix. Resolution of glomerular and mesangial sclerosis depends upon the balance between the increased extra cellular matrix and its breakdown by metalloproteinases and glomerular collagenases.

ROLE OF GLOMERULAR EPITHELIAL CELLS

The relative inability of podocytes to replicate with respect to injury may cause their stretching along the glomerular basement membrance. This will expose the areas of denuded glomerular basement membrane. Attraction and interaction of denuded glomerular basement membrance with the parietal epithelial cells forms capsular adhesions and subsequent segmental glomerulosclerosis. Tuft-to-capsule adhesions allow the influx of periglomerular fibroblasts into the glomerualr tuft causing glomerulosclerosis.

EXTRA GLOMERULAR CELLS:

PLATELETS

The stimulation of the coagulation cascade by the activation of platelets and their release products will activate the mesangial cell and promote its sclerosis.

- Thrombin stimulates TGF-β1, resulting in progression of mesangial extra cellular matrix production .

- Upregulation of plasminogen activator inhibitor -1 within the damaged glomeruli may lead to extra cellular matrix accumulation and glomerulo sclerosis because of its inhibition of proteolytic enzyme plasmin.

Degree of glomerulosclerosis depends upon the balance between thrombotic- antiproteolytic and anticoagulant- proteolytic activities.

LYMPHOCYTES, MONOCYTE AND MACROPHAGES

The release of Cytokines, Growth Factors and Procoagulant factors by lymphocytes as well as monocytes and macrophages is likely to contribute to the pathogenesis and progression of glomerulosclerosis.

BONE MARROW – DERIVED CELLS

Hematopoietic stem cells are involved in the normal glomerular cell turnover and response of glomeruli to injury.

TUBULO INTERSTITIAL SCARRING:

Tubulo interstitial fibrosis is developed in three stages.

1. Inflammation of tubulointerstitium.
2. Proliferation of interstitial fibroblasts.
3. Excessive deposition of interstitial extracellular matrix

Renal tubular cells play an important role in the pathogenesis of tubulointerstitial fibrosis. Injured tubular cells act as antigen presenting cells

expresses cell adhesion molecules and releases inflammatory mediators and growth factors resulting in increased synthesis of ECM.

Loss of complementary proteins in the glomerular proteinuria may damage the tubular cells. Tubular cells may also be stimulated by the spillover of hormones such as Angiotensin II, Growth Factors and Cytokines from injured glomeruli.

Activation of tubular cells and their release of chemotactic factors can attract inflammatory cells including monocytes to the tubules and renal interstitium with subsequent activation of renal fibroblasts.

Activated renal fibnoblasts acquire myofibroblast characteristics [eg. express α – smooth muscle Actin and synthesize interstitial Type I and III collagen] proliferate and invade the periglomorular and peritubular spaces. The resolution of deposited extracellular matrix depends on activation of Matrix Metalloproteinases and Plasmin. Inhibition of these two protolytic enzymes results in tubulo interstitial scarring.

Tubular cells contribute to fibrogenesis through their transformation into a myofibroblastic phenotype is called epithelial mesenchymal transformation. This is a form of reverse embryogenesis because proximal tubules are derived ontogenetically from the metanephric mesenchyme.

VASCULAR SCLEROSIS

This is an integral feature of the renal scarring process. Renal arteriolar hyalinosis is associated with progression of CKD. This may be present at the

early stage of CKD, even in the absence of severe hypertension. Hyalinosis of afferent arterioles may be implicated in the pathogenesis of glomerulosclerosis. Hyalinosis of the post glomerular arterioles may exacerbate interstitial ischemia and fibrosis. Ischemia and the ensuing hypoxia stimulate tubular cells and kidney fibroblasts to produce extra cellular matrix components and reduce their collagenolytic activity.

COMPLICATIONS OF CKD

- **ANAEMIA**[32]

Several factors implicated in the development of anaemia.

They are

- Erythropoietin deficiency.
- Retention of Bone marrow toxins .
- Bone marrow fibrosis secondary to hyperparathyroidism.
- Haematinic deficiency – Iron, Vitamin B_{12}, Folate.
- Increased red cell destruction.
- Abnormal red cell membranes causing increased osmotic fragility.
- Increased blood loss – occult gastro intestinal bleeding, blood loss during haemodialysis or because of platelet dysfunction.
- Drugs like ACE inhibitors may cause anaemia in CKD by interfering with the control of endogenous Erythropoietin release.

- **RENAL OSTEODYSTROPHY**

- Decreased renal production of the 1α hydroxylase enzyme results in reduced conversion of 25-OH cholecalciferol to 1,25 dihydroxy cholecalciferol.
- Reduced activation of vitamin D receptors in the parathyroid gland leads to increased release of PTH.
- Calcium sensing receptors expressed in the parathyroid glands react rapidly to acute changes in serum Calcium concentrations and a low Calcium also leads to increased release of PTH.
- 1,25 dihydroxy cholecalciferol deficiency also results in gut Calcium malabsorption.
- Phosphate retention owing to reduced excretion by the kidneys lowers ionised Calcium, results in an increase in PTH synthesis and release.
- PTH promotes reabsorption of Calcium from bone and increased proximal renal tubular reabsorption of Calcium and this opposes the tendency to develop hypocalcemia induced by 1,25 dihydroxy cholecalciferol deficiency and phosphate retention.

DEFINITION OF CKD-MBD[33,34]

A systemic disorder of mineral and bone metabolism due to CKD is manifested by either one or a combination of the following:

- Abnormalities of Calcium, Phosphorous, Parathormone and vitamin D metabolism.
- Abnormalities in bone turn over, mineralization volume, linear growth or strength.
- Vascular or other soft tissue calcification.

- **FLUID, ELECTROLYTE AND ACID-BASE DISORDERS**

  - **SODIUM AND WATER HOMEOSTASIS**

    - In most patients with stable CKD, the total-body content of Sodium and water is modestly increased.
    - Normal renal function guarantees that the tubular reabsorption of filtered Sodium and water is adjusted so that urinary excretion matches net intake of Sodium and water.
    - Many forms of renal disease (e.g., Glomerulonephritis) disrupt this glomerulotubular balance such that dietary intake of Sodium exceeds its urinary excretion, leading to sodium retention and attendant extracellular fluid volume expansion.
    - This expansion may contribute to hypertension, which itself can accelerate the nephron injury.

- As long as water intake does not exceed the capacity for water clearance, the extracellular fluid volume expansion will be isotonic and the patient will have a normal plasma Sodium concentration and effective osmolality.
- Hyponatremia is not commonly seen in CKD patients.

POTASSIUM HOMEOSTASIS

- In CKD, the decline in GFR is not necessarily accompanied by a parallel decline in urinary Potassium excretion, which is predominantly mediated by Aldosterone-dependent secretory events in the distal nephron segments.
- Another defense against Potassium retention in these patients is augmented Potassium excretion in the gastro intestinal tract.
- Against defense mechanisms s hyperkalemia may be precipitated by increased dietary Potassium intake, protein catabolism, hemolysis, hemorrhage, transfusion of stored red blood cells, and metabolic acidosis.
- Hypokalemia is not common in CKD and usually reflects markedly reduced dietary potassium intake, especially in association with excessive diuretic therapy or concurrent gastro intestinal losses.
-

- **METABOLIC ACIDOSIS**

❖ Metabolic acidosis is a common disturbance in advanced CKD.

❖ This is a non-anion-gap metabolic acidosis.

❖ With worsening renal function, the total urinary net daily acid excretion is usually limited to 30–40 mmol, and the anions of retained organic acids can then lead to an anion-gap metabolic acidosis.

❖ The non-anion-gap metabolic acidosis that can be seen in earlier stages of CKD may be complicated by the addition of an anion-gap metabolic acidosis as CKD progresses.

❖ In most patients, the metabolic acidosis is mild; the pH is rarely <7.35 .

- **SKIN DISEASE**

Pruritus is common in severe CKD and is due to retention of nitrogenous waste products of protein catabolism. It improves following dialysis.

OTHER CAUSES OF PRURITUS IN CKD

- Hypercalcemia
- Hyperphosphataemia
- Elevated calcium x phosphate product
- Hyperparathyroidism
- Iron deficiency

- **NEPHROGENIC SYSTEMIC FIBROSIS**

It is seen only in patients with moderate to severe CKD particularly in patients on dialysis . Skin is predominantly involved.

- **GASTROINTESTINAL COMPLICATIONS**

  - Decreased gastric emptying
  - Increased risk of reflux oesophagitis
  - Peptic ulceration
  - Acute pancreatitis
  - Constipation
  - Elevated serum amylase of upto three times normal, due to retention of high molecular weight forms of amylase in the body.

- **METABOLIC ABNORMALITIES**

  - Gout – Uric acid retention is a common feature of CKD.
  - Insulin- Insulin is catabolized by kidney and to some extent it is excreted by kidneys. End organ resistance to insulin is a feature of advanced CKD resulting in modestly impaired glucose tolerance.
  - **ABNORMALITIES OF LIPID METABOLISM[35,36]**

Progressive deterioration of renal function results in altered composition of blood lipids which in turn predisposes to the development of cardio vascular disease. Renal dyslipidemia is characterized by the following features:

- Hepatic apo AI synthesis is decreased and Lecithin cholesterol acyl transferase activity is reduced. This leads to decreased HDL-C levels.
- Increased synthesis of apo C III, a competitive inhibitor of Lipoprotein Lipase leads to elevated levels of VLDL-C and Chylomicrons, which results in hypertriglyceridemia. Further, uremic toxins and secondary hyperparathyroidism reduces the levels of lipoprotein lipase which results in impaired catabolism of Triglyceride rich lipoproteins. Insulin resistance associated with CKD also increases the VLDL-C levels.
- Total and LDL- Cholesterol levels are usually normal but may be low in patients with concomitant inflammation and malnutrition. There is characteristic accumulation of small dense atherogenic LDL-C.
- As GFR declines, the levels of high molecular weight isoforms of Lipoprotein (a) increase which is associated the increased cardiovascular risk.
- The changes in lipoprotein composition and structure in CKD stimulate and amplify the already existing inflammatory mechanisms which in turn results in endothelial dysfunction and atherosclerotic progression.

ENDOCRINE ABNORMALITIES

- Hyperprolactinaemia
- Increased Luteinizing hormone (LH) levels in both sexes and abnormal pulsality of LH release.

- ❖ Decreased serum Testosterone level, so erectile dysfunction and decreased spermatogenesis are common.
- ❖ Absence of normal cyclical changes in the female sex hormones resulting in oligo-menorrhoea or amenorrhoea.
- ❖ Abnormalities in Growth Hormone secretion and action resulting in impaired growth in uraemic children.
- ❖ Abnormal Thyroid hormone levels, partly because of altered protein binding.

- **MUSCLE DYSFUNCTION**

  Uremia interferes with muscle energy metabolism.

- **NERVOUS SYSTEM**

  Severe uremia causes an depressed cerebral function and decreased seizure threshold, asterixis , tremor and myoclonus.

- **CARDIO VASCULAR DISEASE**

The overall mortality rate from cardiovascular disease in CKD patients has been found to be about 30 times greater than that of general population[37]. Patients with all stages of CKD are considered as the highest risk group for CVD. CKD is therefore considered as a “cardiovascular risk equivalent.

Cardiovascular disease is characterized by left ventricular hypertrophy which results largely due to expansion of extracellular volume, anemia and

hypertension. Left ventricular remodelling and fibrosis may accompany left ventricular hypertrophy which ultimately results in severe complications[38].

Cardiovascular complications associated with CKD include Myocardial infarction, Angina pectoris, Arrhythmias, Cardiac failure, Peripheral Vascular Disease, Stroke and Sudden death. The risk increases from early stages to advanced stages of CKD.

CARDIOVASCULAR RISK FACTORS IN CKD[39,40]

TRADITIONAL RISK FACTORS

- Age
- Gender
- Diabetes Mellitus
- Hypertension
- Smoking

NON-TRADITIONAL RISK FACTORS

- Inflammation
- Oxidative stress
- Endothelial dysfunction
- Anaemia
- Hyperphosphatemia
- Secondary hyperparathyroidism

- Vascular calcification
- Advanced glycation end products
- Hyper homocystinemia

- **INFLAMMATION**[41,42,43]

Most CKD patients are in a state of chronic inflammation. Various factors may be associated with a sustained inflammatory response in CKD which include

- Genetic background
- Persistence of inflammatory conditions
    - Exogenous (bacteria, viruses)
    - Endogenous (Reactive oxygen species , glycated and oxidized adducts , Renin Angiotensin Activating System)
- Failure of clearance of inflammatory mediators (cytokines and other mediators and glycated, oxidized adducts)
- Dysmetabolic states
    - Dyslipidemia
    - Central obesity
    - Insulin resistance

The most commonly used biomarker of inflammation is C-reactive protein (CRP) which is one of the member of Pentraxin family and the prototypic acute phase reactant. Other acute phase reactants include Serum

Amyloid A, Ferritin and Fibrinogen.These proteins are the forward or positive acute phase reactants whose serum levels increase during inflammation. The negative phase reactants include Albumin and Prealbumin and the level of these proteins fall during inflammation.

CRP, Interleukin-6 and Fibrinogen are the independent predictors of mortality in CKD patients. These markers have been attributed to their pro-atherogenic properties such as endothelial dysfunction, promotion of vascular calcification and oxidative stress.

- **NUTRITIONAL ABNORMALITIES**

- Protein-energy malnutrition, a consequence of low protein and caloric intake, is common in advanced CKD and is often an indication for initiation of Renal Replacement Therapy.
- These patients are resistant to the anabolic actions of insulin and other hormones and growth factors. Metabolic acidosis and the activation of inflammatory cytokines can promote protein catabolism.

VISFATIN

DISCOVERY[44,45,46,47]

In 1960s, researchers found a protein with enzymatic activity in liver extracts which was named as Nicotinamide Phosphoribosyl Transferase (Nampt). In 1994, an another group of researchers identified the gene with same action in cDNA library from human peripheral blood lymphocytes and named it as Pre-B- cell colony Enhancing Factor-1 (PBEF-1).

The protein which is secreted by peripheral blood lymphocytes is also found to be secreted by visceral adipose tissue. Since the primary source of protein is visceral adipose tissue ,it is renamed as **visfatin(visceral fat derived adipokine**) by Fukuhara and colleague in 2005[48].

MOLECULAR GENETICS

Visfatin is a 52 KDa protein. The sequence of visfatin is highly conserved among vertebrates, invertebrates, bacteria and bacteriophages[49,50]. The gene for Visfatin is locacted on chromosome 7 between 7q 22.1 and 7q31.33. This gene

has a pseudogene on chromosome 10. It is composed of 11 exons and 10 introns. It is encoding 491 Amino acid residues. It spans 34.7 kb length of human genomic DNA[51,52]. Three mRNA transcripts exist at sizes of 2.0, 2.4 and 4.0kb. Among these three, 2.4kb is the predominant one. The variations in size may be due to alterations in either exon splicing or sites of polyadenylation. The Visfatin gene has 2 distinct promoters and so the gene and gene products may be differentially expressed in different tissues.

SECRETION AND EXPRESSION[53,54]

Visfatin is ubiquitously expressed and associated with a variety of functions in different cell types. Apart from leucocytes and adipocytes, Visfatin is also expressed in hepatocytes, skeletal muscle, heart, brain, placenta, kidney, lung, pancreas and bone marrow. In addition to tissue localization, It is also identified in the plasma of humans. The exact mechanism of its secretion into the plasma is unknown, because it lacks a signal peptide. It occurs through a non classical secretory pathway.

In mammals, there are two forms of Visfatin have been identified.

1. Intracellular Visfatin / Nampt:

   Involved in the regulation of cellular metabolism in response to nutrient availability, cell maturation and survival.

2. Extracellular Visfatin/Nampt:

   Secreted by different cell types acting as a proinflammatory molecule.

Visfatin secretion is a highly regulated process. Its secretion is cell type dependent because brown adipose tissues secrete more Visfatin than white adipose tissues. The plasma level of Visfatin correlates with the volume of visceral fat but not with the quantity of subcutaneous fat[55]. Cellular distribution of Visfatin varies with the growth phase of the cell, being predominantly nuclear in non proliferating cells and cytoplasmic in proliferating cells[56].

STRUCTRUE[57,58,59]

The crystal structure of Visfatin with regard to its enzymatic function is a homodimeric protein. It has two active sites at the interface of the dimeric protein. So homodimerization is essential for the catalytic activity of the enzyme. The extensive dimeric interface with a total surface area of $8077A^{0\,2}$ is formed by 10 segments from each unit.

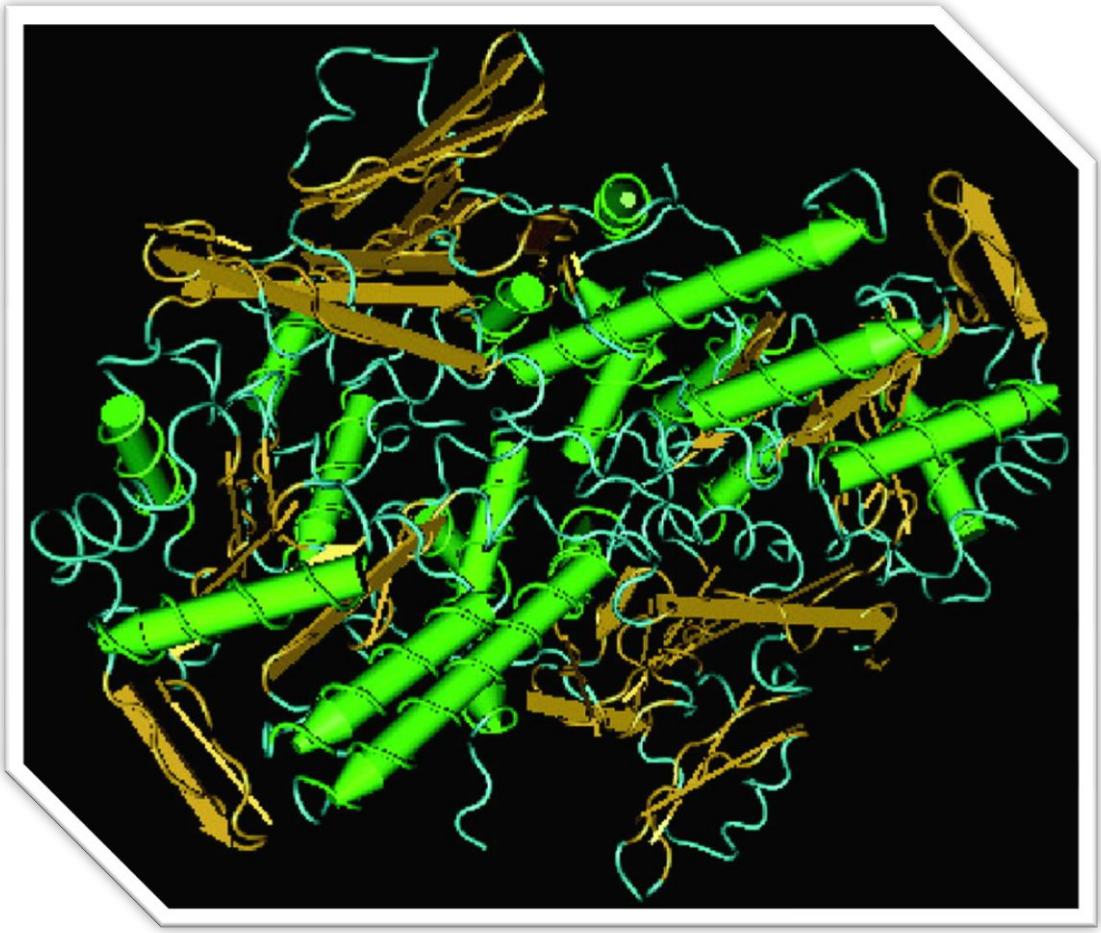
Of about 89 polar and hydrophobic residues are distributed along the interface and 42 hydrogen bonds are involved in the intra molecular interactions.

Each monomer is composed of 491 residues that form 13 helices and 19 strands consisting of 2 structural domains. The first structural domain is organized into 7 stranded anti parallel β sheets, 2 anti parallel β strands and one α helix. The second domain is arranged into alternative folding of the classical (β/α)s barrel. Alignment of 13 residues in the active site of Visfatin / Nampt is highly conserved.

Figure.2. represents the crystal structure of Visfatin.

FIGURE.2

CRYSTAL STRUCTURE OF VISFATIN



SCHEMATIC RIBBON DIAGRAM OF VISFATIN SHOWS THE THE PRESENCE OF TWO MONOMERS, COMPRISING OF 491 AMINO ACIDS. α HELICES ARE REPRESENTED BY GREEN CYLINDERS. β SHEETS ARE REPRESENTED BY ORANGE ARROWS.

BIOLOGICAL FUNCTIONS OF VISFATIN.

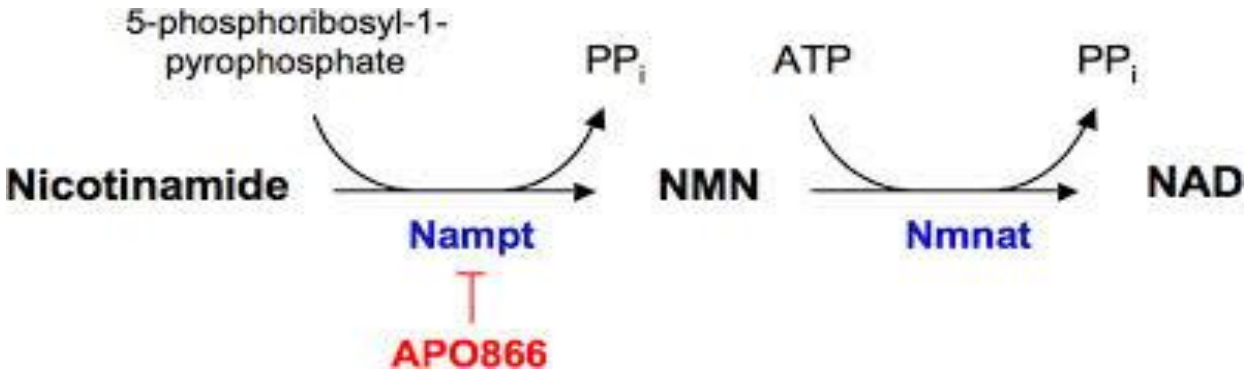
Numerous biological functions have been attributed to Visfatin. Most important functions are

1. Visfatin catalyzes the first step in synthesis of NAD –Enzymatic action of Visfatin.
2. Pro inflammatory cytokine – paracrine effect of Visfatin.
3. Insulin mimetic action – Endocrine effect of Visfatin.
4. Intracellular Visfatin enhances the insulin sensitivity in liver – Autocrine effect of Visfatin.

The immune, inflammatory and metabolic responses of Visfatin depends on both extracellular (cytokine like) and intra cellular (enzymatic) isoforms[60]. Visfatin is upregulated by hypoxia, inflammation and hyperglycemia. Visfatin is downregulated by Insulin, Somatostatin and Statins. Visfatin is an endocrine, autocrine as well as paracrine peptide with many functions[61].

ACTS AS AN ENZYME

Visfatin participates in Nicotinamide metabolism by acting as an enzyme named Nampt which belongs to the family of Pentosyltransferases and catalyzes the following chemical reaction:



Nampt is the rate limiting enzyme, involved in both extra and intracellular formation of Nicotinamide Mononucloeotide(NMN). An another enzyme Nicotinamide mononucloeotide adenylyl transferase (Nmnat) converts NMN to NAD thus replenishing the NAD pool within the cell.

NAD^+ is an essential cofactor of fundamental intracellular processes such as

- Transfer of electrons during redox reactions, DNA repair mechanisms, transcriptional regulation.
- To modulate the activity of key regulators of cellular longevity.
- To serve as a substrate for the generation of other biologically important molecules.
- Regulation of intracellular signaling.
- Involved in energetic metabolism by influencing the activity of NAD/NADH dependent enzymes.

NAD^+ acts as a cofactor a family of Class 3- NAD^+-dependent Histone deacetylases known as SIRTs (silent information regulator 2) or Sirtuins. It binds to NAD^+ and a target protein that contains an acetylated lysine. It catalyzes the formation of acetylated ADP-ribose by deacetylation of the lysine residue of the target protein. These sirtuins are essential for certain cell survival reactions[62].

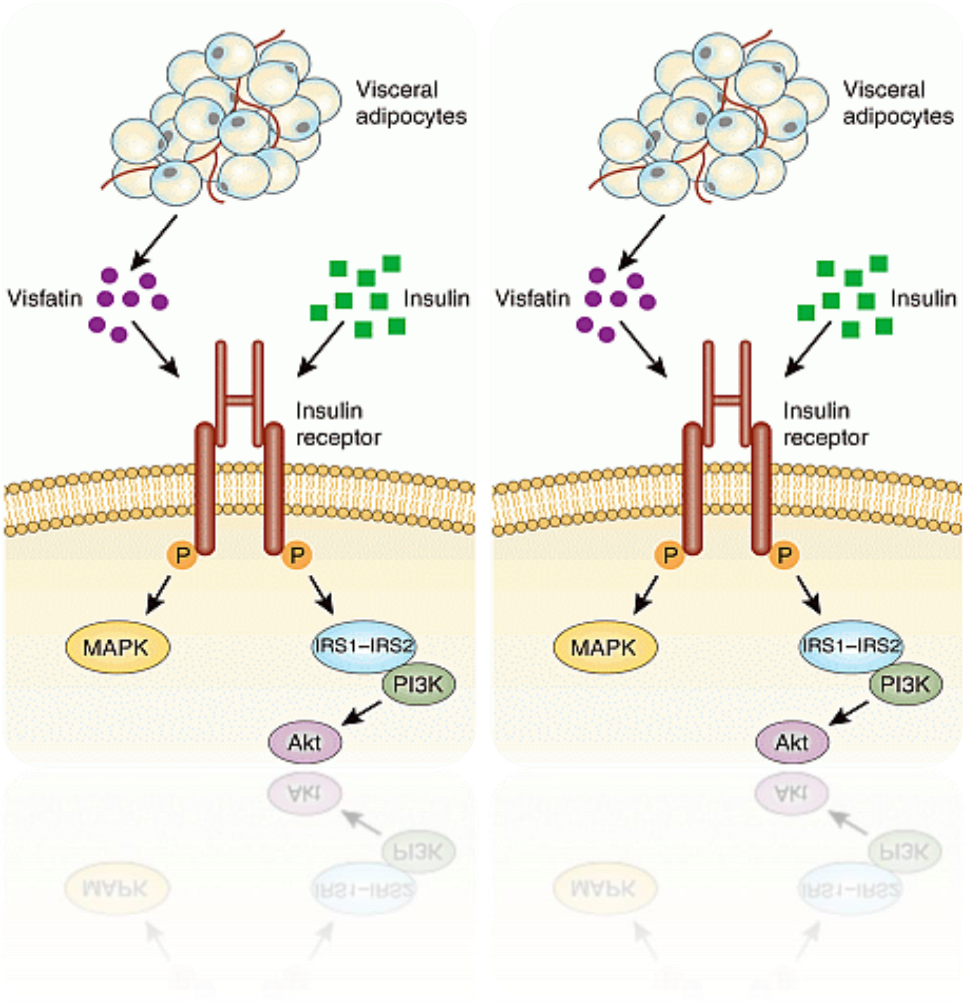
Sirtuins have been implicated in influencing aging and regulating transcription, apoptosis and stress resistance. Nampt indirectly helps in the longevity of the cells life span. Nampt extends the lifespan and promotes the maturation of human smooth muscle cells by activating SIRT1. All these reactions will cleave NAD and utilize it. So a salvage pathway is essential to replesnish the cellular NAD pool[63].

ROLE AS A PRO INFLAMMATORY CYTOKINE[64]

Visfatin increases the effect of IL-7 and stemcell factor on pre B cell colony formation so it is named as PBEF-1. It also increases the expression of inflammatory cytokines such as IL-6, IL-1β and TNF α.

FIGURE.3

INSULINO-MIMETIC ACTION OF VISFATIN



ACTS AS AN ANTI APOPTOPIC[65,66]

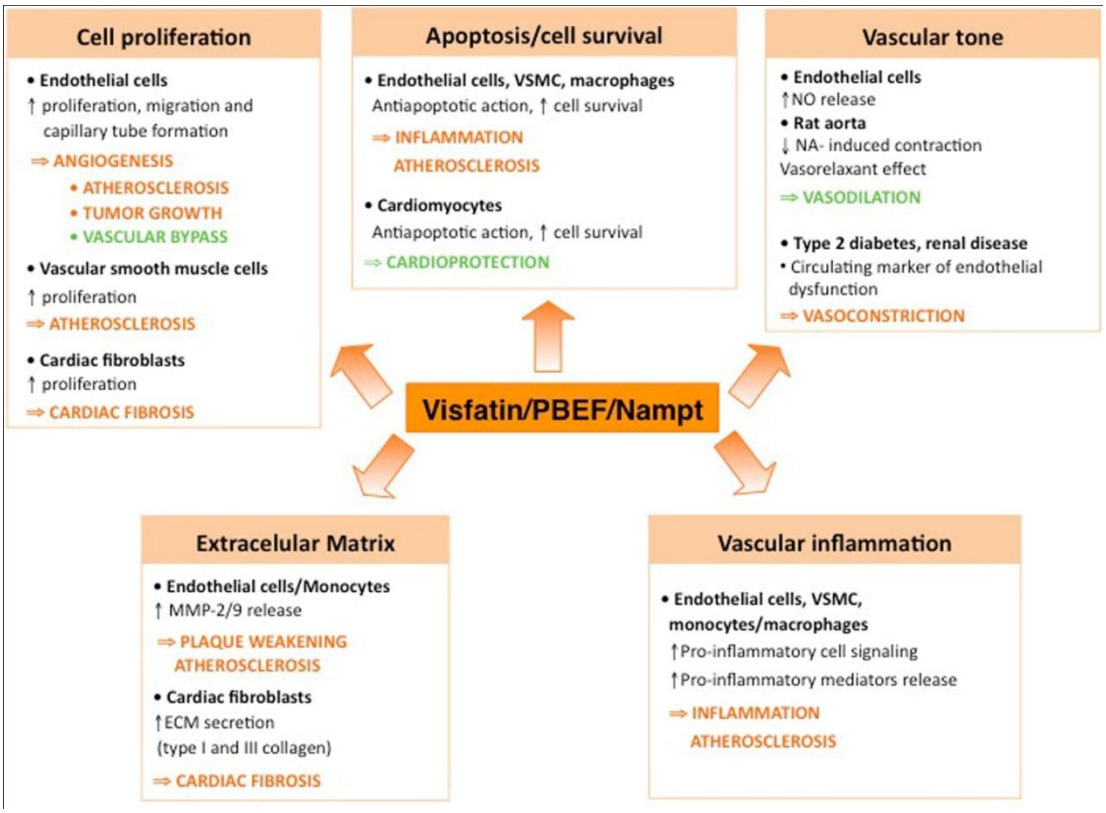
Apoptosis is programmed cell death. Removal of intact neutrophils is necessary to prevent chronicity of the disease, because it leads to recognition of intact senescent neutrophils that have not necessarily disgorged their granule contents. Visfatin is also secreted by neutrophils in response to inflammatory stimuli and acts as an inhibitor of apoptosis resulting from a variety of inflammatory stimuli.

ACTS AS AN INSULIN – MIMETIC HORMONE

Visfatin binds to Insulin receptor at a site different from that of Insulin. After binding with insulin receptor, it causes tyrosine phosphorylation as well as phosphorylation of Insulin Receptor Substance (IRS) -1 and 2 and down stream signaling kinases (like Protein Kinase B and Mitogen Activated Protein Kinase) leading to enhanced glucose uptake. The affinity of Visfatin and Insulin for insulin receptors are same but the circulating concentration of Visfatin is 10 times lower than that of Insulin. Acting as Insulin mimetic hormone, Visfatin increases glucose transport and lipogenesis by adipose tissues and myocytes and decreases glucose production by hepatocytes.Figure.3. shows the Insulino-mimetic action of Visfatin.

FIGURE.4

ACTIONS OF VISFATIN IN CARDIO VASCULAR SYSTEM



ROLE OF VISFATIN IN CKD[67,68,69,70]

There is impaired renal filteration function in CKD leading to accumulation of uremic toxins within the body.There are three groups of uremic toxins such as water soluble, protein bound and middle molecule uraemic retention solutes.

Visfatin is a middle molecule uraemic retention solute of wt 52 KDa , synthesizes NAD ,which is important for vascular smooth muscle maturation suggesting its potential role in vascular pathology. Proliferation of vascular smooth muscle cell is a hallmark of development of Atherosclerosis . Visfatin stimulates the Vascular Endothelial Growth Factor (VEGF) synthesis and secretion and also enhances the expression of VEGF receptor 2 that promotes endothelial proliferation.

Visfatin also upregulates other proangiogenic soluble factors such as Fibroblast Growth Factor 2, MCP -1 (Monocyte Chemotactic Protein-1) and IL-6 in endothelial cells.Figure.4. shows the various actions of Visfatin in Cardiovascular system to cause atherosclerosis.It acts through cell proliferation of endothelial cell and vascular smooth muscle cell resulting in angiogenesis and atherosclerosis.

Visfatin activates human leukocyte expression of Interleukin- 1β, Tumor Necrosis Factor-α, Interleukin-6 as well as Matrix Metalloproteinase-9 activity suggesting that there is a potential link between Visfatin and Inflammation.

Inflammation is an ubiquitous feature of CKD associated with an adverse outcome of CVD. It is therefore reasonable to propose that Visfatin is contributing to the pathogenesis of Atherosclerosis and Cardiovascular disease in CKD patients as it acts as a pro- inflammatory cytokine and plays a role in chronic inflammation.

AIMS AND OBJECTIVES

AIM:

To estimate the levels of serum Visfatin in patients with CKD and to compare them with healthy controls.

OBJECTIVES:

1. To study the relationship of serum Visfatin with the inflammatory biomarker hsCRP.
2. To evaluate the association of serum Visfatin with Creatinine clearance.
3. To find out the correlation of serum Visfatin with lipid profile.

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College Hospital, Thanjavur after getting approval from the ethical committee.

50 patients of known CKD (25 males and 25 females) were selected as cases from the outpatients and wards of the Department of Nephrology. 50 age and gender matched healthy individuals were selected as controls.

INCLUSION CRITERIA

- Patients with established diagnosis of CKD.
- Age > 18 years.

EXCLUSION CRITERIA

- Acute/chronic inflammatory diseases(sepsis, infection, malignancy and liver disease).
- Previous history of Coronary Artery Bypass Graft surgery.
- Patients on lipid lowering drugs.
- Acute kidney injury.
- Patients on immuno therapy.
- Previous history of cerebrovascular diseases.
- Patients who underwent renal transplantation.
- Nephrotic syndrome.

Informed consent was obtained from all subjects prior to the study. Under aseptic precautions, 5ml of venous blood sample was collected after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000rpm for 15 minutes for separation of serum.

An aliquot of the serum was taken for the estimation of Visfatin and stored at -20°C in the deep freezer. The remaining serum was used for the estimation of Glucose, Urea, Creatinine, hsCRP, Total cholesterol (TC), Triglycerides (TGL) and High Density Lipoprotein Cholesterol (HDL-C).

ANALYSIS OF BLOOD SAMPLES

The serum collected above was used for the estimation of the following parameters.

A. ESTIMATED PARAMETERS

1. Serum Visfatin	Enzyme Immuno Assay.
2. Serum hsCRP	Turbidimetric Immunoassay.
3. Blood Urea	Urease –Glutamate Dehydrogenase (GLDH) Method.
4. S.Creatinine	Modified Jaffe's method.
5. Total Cholesterol	Cholesterol Oxidase- PAP method
6. Triglycerides	Glycerol Phosphate Oxidase –PAP method

7. HDL-C Phosphotungstate /Magnesium precipitation method.

8. Glucose Glucose-oxidase/ peroxidase method.

B. CALCULATED PARAMETERS

1. LDL-C and VLDL-C were calculated using Freidwald's formula:

VLDL-C = TGL/5

LDL-C = TC-{HDL-C+VLDL-C}

2. Creatinine clearance was calculated using Cockcroft- Gault formula

$$\text{Estimated Creatinine Clearance} = \frac{(140\text{-age}) \times \text{wt in kg}}{72 \times \text{serum Creatinine}}$$

Multiply by 0.85 for females.

ESTIMATION OF SERUM VISFATIN

Method:

Enzyme Immuno Assay Kit Purchased from Ray-Biotech. Inc

Principle:

Anti-rabbit secondary antibody is precoated onto a micro-titer plate. After a blocking step and incubation of the plate with anti-Visfatin antibody, Biotinylated Visfatin and standard Visfatin (or) sample are added to all wells. There is a competitive binding between biotinylated Visfatin and standard (or) serum Visfatin with anti-Visfatin antibody. Streptavidin – Horseradish Peroxidase (HRP)was added to the well which reacts with the uncompleted (or)

free biotinylated Visfatin to produce a colour. The Intensity of the colour is directly proportional to the amount of biotinylated Visfatin and inversely proportional to the amount of Visfatin peptide in the standard or sample. The concentration of Visfatin in the serum is calculated from a standard curve of different Visfatin concentrations accordingly.

REAGENTS:

1. Visfatin Microtiter plate : 96 wells coated with secondary antibody.
2. Lyophilized standard Visfatin peptide - 2 vials
3. Lyophilized anti-Visfatin polyclonal antibody – 2 vials
4. Lyophilized biotinylated Visfatin peptide – 2 vials
5. Lyophilized positive control – 1 vials
6. IX Assay Diluent for both standard and samples.
7. HRP – streptavidin concentrate : 600ml
8. Wash buffer concentrate 20X : 25ml
9. Tetra methyl Benzidine (TMB) one step substrate reagent 12ml of 3,3', 5, 5' Tetra Methyl Benzidine in buffered solution.
10. Stop solution : 8ml of 0.2M sulphuric acid.

Reagent Preparation:

1. Kit reagents were kept on ice during reagent preparation.
2. Preparation of anti-Visfatin antibody

AntiVisfatin antibody vial was briefly centrifuged and reconstituted with 5µl of deionised water. Then 50µl of IX Assay diluents was added to prepare a detection antibody concentrate. Then it was diluted 100 fold with IX Assay diluents to get antiVisfatin antibody working solution.

1. Preparation of Biotinylated Visfatin

   Biotinylated Visfatin vial was briefly centrifuged and reconstituted with 20µl of deionised water before use. Then 5µl of this was added to 5ml of IX Assay diluents to get biotinylated Visfatin of 10 ng/ml final concentration.

2. Preparation of 10 fold dilution of Biotinylated Visfatin:

   2µl of reconstituted Biotinylated Visfatin was mixed with 18µl of IX Assay diluents to get 10 fold dilution of Biotinylated Visfatin. This was used for positive control preparation and sample preparation.

3. Positive control Preparation:

   Positive control vial was briefly centrifuged and reconstituted with 100µl of deionised water. To this 101µl of IX Assay diluent and 2 µl of 10 fold diluted Biotinylated Visfatin were added and mixed up. This is the two fold dilution of positive control.

4. Sample Preparation

2.5µl of 10 fold diluted Biotinylated Visfatin and 247.5µl of sample was mixed so that the final concentration of biotinylated Visfatin concentration should be 10ng/dl in each sample.

5. Preparation of Standards:

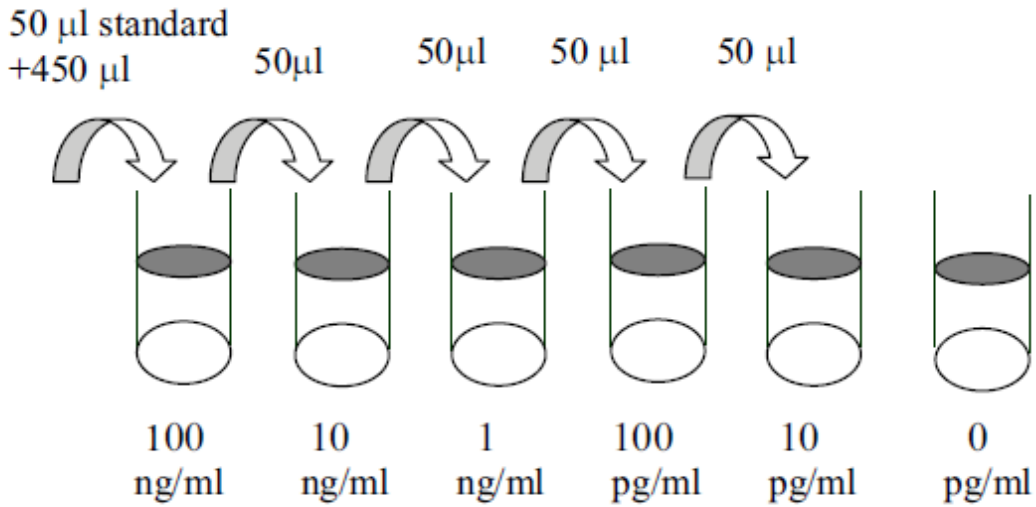
6 micro-tubes were labeled with the following concentrations:

Tube no 1	100ng/ml
Tube no 2	10ng/ml
Tube no3	1ng/ml
Tube no 4	100pg/ml
Tube no 5	10pg/ml
Tube no 6	0pg/ml

450µl of biotinylated Visfatin was added to all tubes. In a separate tube, 10 µl of standard Visfatin Peptide was added into 990 µl of biotinylated Visfatin solution to prepare a 1000 ng/ml standard. This solution served as the Visfatin stock solution. From this solution 50µl was added to the tube 1 labeled as 100ng/ml to get 100ng/ml Visfatin standard which served as the first standard solution.This step was repeated after thorough mixing before each transfer with each successive concentration, except the last tube preparing a dilution series as shown in the illustration below:

Tube	Concentration of Standard	Biotinylated Visfatin	Standard Visfatin
1	100ng/ml	450µl	50µl of 1000ng/ml standard visfatin
2	10ng/ml	450µl	50µl of tube 1
3	1ng/ml	450µl	50µl of tube 2
4	100pg/ml	450µl	50µl of tube 3
5	10pg/ml	450µl	50µl of tube 4
6	0pg/ml	450µl	-

The final tube served as the Zero standard (or) total binding the concentration of which is 0pg/ml of visfatin. The Concentration of Biotinylated Visfatin is all tubes should be 10ng/ml.



6. Wash buffer Preparations

20X wash concentration contains visible crystals, which was brought to room temperature and mixed gently until dissolved. Then 375ml of Deionised water was added to yield 400ml of IX wash buffer.

6. Preparation of HRP – Streptavidin:

HRP – streptavidin vial was briefly centrifuged. Then it was diluted 100 fold with IX Assay diluent.

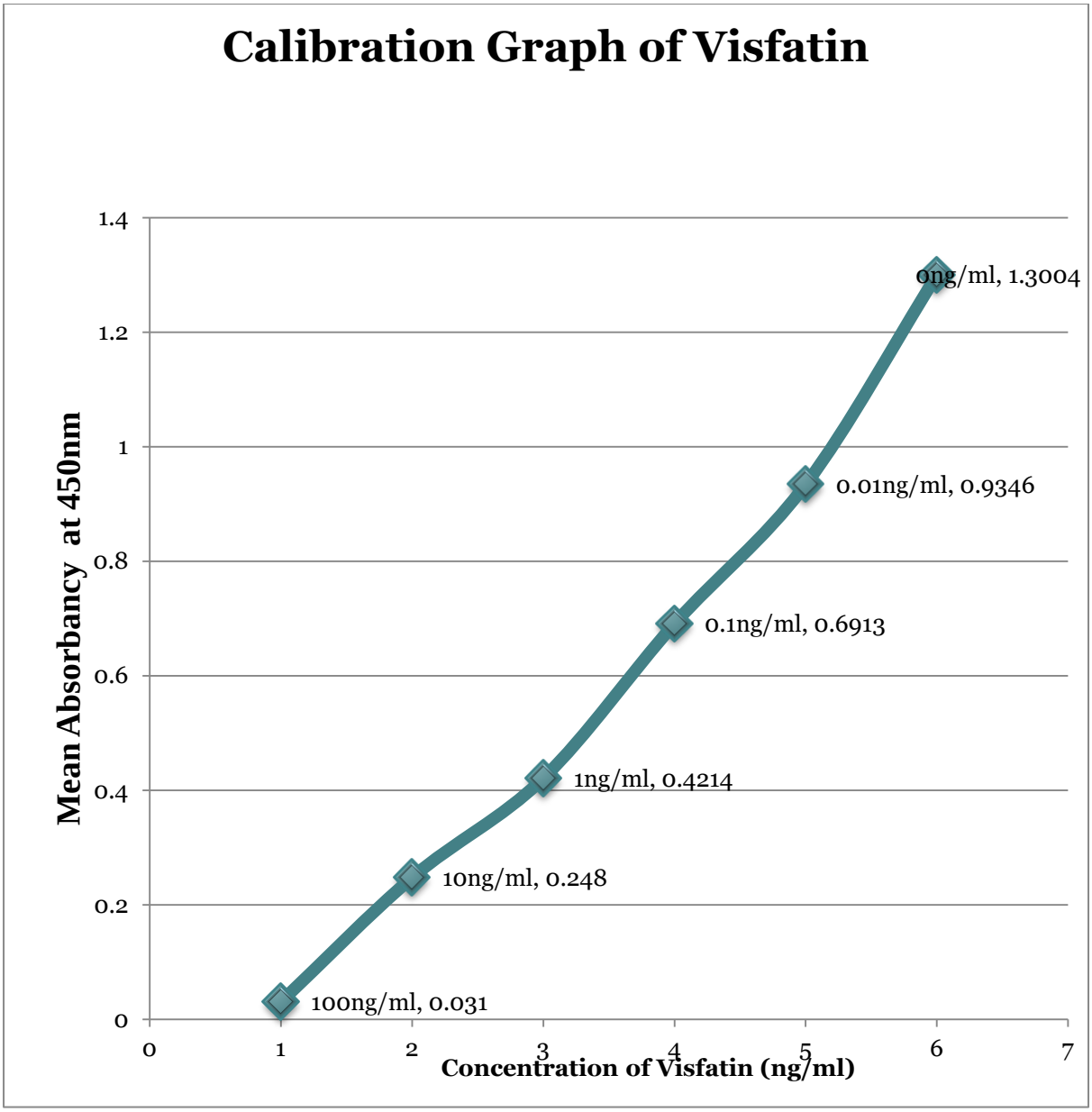
Assay Procedure:

1. Micro-titre plate was equilibrated to room temperature before opening the sealed pouch.
2. 100µl of anti Visfatin antibody was added into each well. Then the plate was incubated for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec.)
3. After incubation the solution was discarded and wells were washed 4 times with IX wash buffer of 300µl each well using an automated plate washer. Then the plate was inverted and blotted against clean paper towels.
4. 100µl of each standard positive control and sample was added into appropriate wells. Then the wells were covered and incubated overnight at 4^0 c.

5. After Incubation the solution was discarded and washed 4 times with wash buffer.
6. 100µl of prepared HRP-streptavidin solution was added to each well and incubated for 45min with gentle shaking at room temperature.
7. After Incubation the solution is discarded and washed 4 times with wash buffer.
8. 100µl of TMB substrate was added to each well and incubated for 30 min at room temperature in the dark with gentle shaking (1-2 cycles/sec.)
9. 50µl of stop solution was added and absorbancy was read at 450nm immediately.

Calibration Graph:

By plotting the mean absorbance of each standard on the y-axis against the concentration of Visfatin (ng/ml) in each standard on the x-axis, a calibration curve was constructed.



Sensitivity:

The minimum detectable concentration of Visfatin is 379pg/ml .

Linearity:

The linearity of serum Visfatin lies between 1 -100ng /ml.

ESTIMATION OF SERUM hs-CRP:

METHOD:

Turbidimetric Immuno Assay

PRINCIPLE:

Serum C-reactive protein causes agglutination of the latex particles washed with Antihuman C-reactive protein. The latex agglutination is proportional to the concentration of CRP and can be measured by turbidimetry.

Reagents required:

1. hsCRP standard 13.8mg/L
2. Reagent A: Glycine buffer 0.1mol/L, Sodium Azide 0.95g/L, pH 8.6
3. Reagent B: Suspension of latex particles coated with Anti-human CRP antibodies, Sodium Azide 0.95g/L

Reagent Preparation

hs CRP standard was prepared by reconstituting it with 5ml of distilled water to get a stock solution of 13.8mg/L. A dilution series was prepared as follows:

Standard tubes	Normal saline(µl)	Volume of hs-CRP standard (µl)	Concentration of hs-CRP standard (mg/L)
1	__	100µl of stock	13.8
2	100µl	100µl of stock	6.9
3	100µl	100µl of tube 2	3.45
4	100µl	100µl of tube 3	1.725
5	100µl	100µl of tube 4	0.86
6	100µl	100µl of tube 5	0.43
7	100µl	-	0

PROCEDURE:

Reagents and samples were brought to room temperature prior to use.

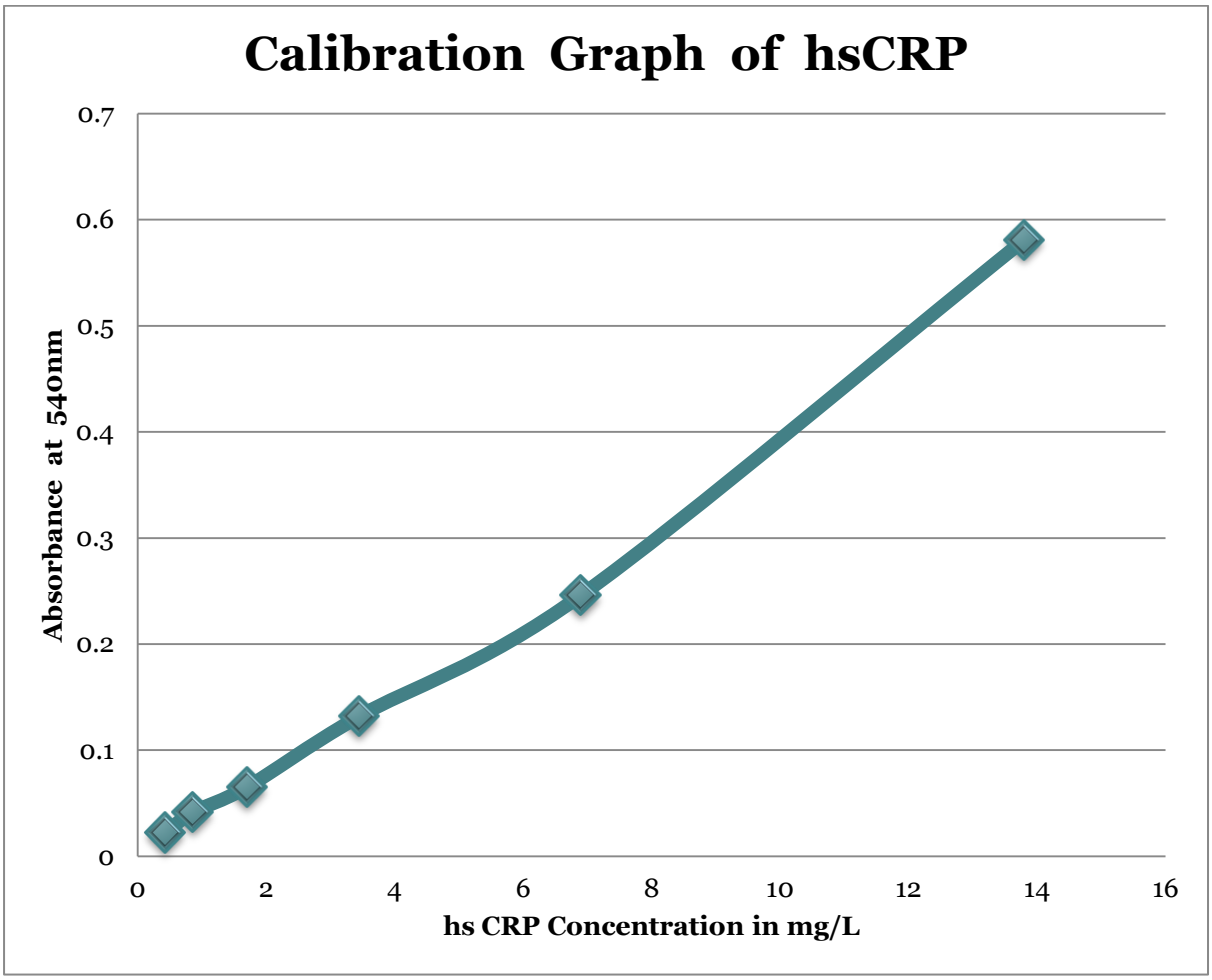
1. Zero correction of the instrument was done with distilled water.
2. Working reagent 1.5ml was pipetted into the cuvette, to which either standard (or) sample 20µl was added and mixed up.
3. Then the cuvette was inserted into the instrument immediately.
4. Absorbance was recorded at 540nm after 10seconds (A_1) and after 5 minutes(A_2)

Calibration:

The difference in the Absorbance ($A2-A_1$) for each standard was calculated and plotted in y-axis against the corresponding standard hs-CRP concentration in the x-axis. Thus the calibration graph was constructed. The hs-

CRP concentration in the sample was calculated by interpolation of its absorbance ($A2-A_1$) in

calibration graph.



Detection limit:

Minimum detection level is 0.06mg/L

Measurement Interval:

Measurement Interval lies between 0.06-15mg/L

Reference Values:

Men

Age	hs CRP(mg/L)
5-13 yrs	<1.4
14-18	<2.13
19-39	<2.68
40-49	<4.8
50-64	<7.9
65-99	<6.8

Women

Age	Hs CRP(mg/L)
5-18 yrs	<1.9
19-49	<3.33
50-64	<8.5
65-99	<6.6

ESTIMATION OF UREA

Method:

Urease – GLDH method

Principle:

Urea is hydrolyzed to ammonia and carbon dioxide by Urease. Then Glutamate dehydrogenase (GLDH) converts Ammonia and α Keto glutarate to Glutamate and water with the concurrent oxidation of NADH to NAD^+ . Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{Urease} 2NH_4^+ + CO_2 .$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{GLDH} \text{Glutamate} + NAD^+.$$

The initial rate of decrease in absorbance at 340nm is proportional to the Urea concentration in the sample.

Reagent composition:

Reagent 1:

α-Keto Glutaric Acid 99.8mmol/L

Urease 23.5kU/L, GLDH 3.5KU/L, Adenosine diphosphate

7.6mmol/L, Sodium Azide 0.2%.

Reagent 2:

NADH 2.95mmol/L, sodium Azide 0.1%

Concentration of Urea standard 50mg/dl.

Reagent Preparation:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

Procedure:

3 test tube were labeled as Blank, standard and test and the procedure is done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	10µl
Standard	1000µl	10µl	-	-
Test	1000µl	-	10µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 60 sec (A_2) at 340nm.

Calculation:

$\Delta A = A_2 - A_1$.

$$\text{Urea in mg/dl} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard}$$

Linearity:

The method is linear upto 200mg/dl.

Reference Interval:

Adults 15-30mg/dl.

ESTIMATION OF SERUM CREATININE

Method:

Modified Jaffe's Method.

Principle:

Creatinine reacts with alkaline picrate to produce an orange-yellow colour. The intensity of the colour is directly proportional to the concentration of Creatinine and is measured photometrically at 510nm.

Reagent Composition

Reagent No	Composition	Concentration
1	Picric acid	25.8mmol/L
2	Sodium hydroxide	95mmol/L

Concentration of standard creatinine 2 mg/dl

Reagent Preparation

Equal Volumes of reagent 1 and reagent 2 were mixed and waited for 15 minutes before use.

Procedure

3 test tubes were taken and labeled as Blank, Standard and test and the procedure was done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	100µl
Standard	1000µl	100µl	-	-
Test	1000µl	-	100µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 80 sec (A_2) at 510nm, against reagent blank with distilled water.

Calculation

$\Delta A = A_2 - A_1$.

$$\text{Serum Creatinine (mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard}$$

Linearity

This assay is linear upto 20mg/dl.

Reference Range

Males: 0.7-1.4 mg/dl

Females: 0.6-1.2mg/dl.

ESTIMATION OF TOTAL CHOLESTEROL:

Method:

Cholestrol Oxidase – PAP method, End Point Analysis.

Principle:

1.Cholestrol Ester + Water $\xrightarrow{Esterase}$ Cholestrol + fatty acid

2.Cholesterol + oxygen $\xrightarrow{Cholesterol\ Oxidase}$ Cholest – 4- en-3 one + H_2O_2

3. $2H_2O_2$ + 4AAP + Phenol $\xrightarrow{Peroxidase}$ Quinoneimine dye + 4 H_2O

4AAP - 4 Amino antipyrine

Absorbance of quinoneimine is directly proportional to cholesterol concentration.

Reagent composition:

Goods buffer (PH 6.4) : 100mmol/L

Cholesterol Oxidase : >100U/L

Cholesterol Esterase : >200U/L

Peroxidase : >3000 U/L

4- Amino anti pyrine : 0.3mmol/L

Phenol : 5mmol/L

Procedure:

Reagents	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

The tube were mixed well and incubated for 10min at room temperature. The absorbance of the test and standard were read against reagent blank at 505nm.

Calculation:

$$\text{Cholesterol in mg/dl} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard (mg/dl).}$$

Concentration of cholesterol standard 200mg/dl.

Reference Range

AGE	SERUM CHOLESTEROL(mg/dl)
2-12 months	60-190
≥1year	110-230
Adults	<200

Linearity:

This method is linear upto 700mg/dl

ESTIMATION OF TRIGLYCERIDES

Method:

Enzymatic method with glycerol Phosphate oxidase (GPO – PAP method)

Principle:

$$\text{Triglycerides} + H_2O \xrightarrow{lipase} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg2+]{Glycerol\ kinase} \text{Glycerol 3 Phosphate} + \text{ADP}$$

$$\text{Glycerol 3 Phosphate} + O_2 \xrightarrow{GPO} \text{DAP} + H_2O_2$$

ATP – Adenosine Triphosphate, 4 AAP 4 Amino Antipyrine, DHBS—3,5 Dichloro 2-Hydroxy Benzene Sulfonate

The Intensity of the Quinoneimine dye formed is proportional to the Triglyceride concentration in the sample when measured at 540nm.

Reagent Composition:

Reagent 1 (Enzymes/Chromogen):

Lipoprotein lipase	4000U/L
4-Amino Antipyrine	0.4 mmol/L
ATP	2 mmol/L
Glycerol Kinase	1500U/L
Peroxidase	2200U/L
Glycerol Phosphate Oxidase	4000U/L

Reagent – 2:

Pipes buffer pH7.0: 40mmol/L

DHBS : 0.2 mmol/L

Magnesium salt: 2.5 mmol/L

Working Reagent Preparation:

The working reagent was prepared by mixing 4 parts of R_1 with 1 part of R_2, and is stable for 90 days at 2-8^{0}C.

Procedure:

Three test tubes were taken and labeled as Blank (B), Test (T) and Standard (S). The procedure was as follows:

Reagents	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-		10µl

The tubes were mixed and incubated for 10 minutes. Absorbance was read at 540nm for standard and sample against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (mg/dl)}$$

Reference Values: 25-160mg/dl

Linearity: Upto 1000mg/dl

HDL CHOLESTEROL ESTIMATION

Method:

Phosphotungstic acid / Magnesium Precipitation method.

Principle:

Chycomicrons, VLDL-C and LDL-C are precipitated from the serum by phosphotungstate in the presence of divalent cations such as Magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent

$$\text{Serum} \xrightarrow{PRECIPITATION} \underset{\text{(Supernatant)}}{\text{HDL-C}} + \underset{\text{(Precipitate)}}{\{\text{LDL-C+VLDL–C+ Chylomiorons}\}}$$

Reagent Composition:

Precipitating reagent:

Phosphotungstic acid	2.4 mmol/L
Magnesium Chloride	40 mmol/L

HDL Cholesterol standard 25mg/dl.

Precipitation:

Precipitation of LDL-C, VLDL-C and chylomicrons were done by adding 500µl of precipitating reagent into 250µl of serum. The tube was mixed well and allowed to stand for 10 min. Then it was centrifuged at 3000 rpm for 10 min and a clear supernatant was obtained. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

Procedure:

Reagents	Blank	Standard	Sample
Cholesterol working reagent	1000µl	1000µl	1000µl
Distilled water	50µl	-	-
Standard	-	50µl	-
Supernatant	-	-	50µl

Tubes were mixed well and incubated for 10min at room temperature. The absorbance of the standard and the test samples were read at 505nm against reagent blank.

Calculation:

$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} \times \text{dilution factor.}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 25 \times 3$$

Linearity: Upto 125mg/dl

Normal Values:

Males: 30-65mg/dl

Females: 35-80 mg/dl.

Estimation of Glucose:

Method:

Glucose oxidase – peroxidase (GOD-POD) method.

Analysis:

End Point Analysis

Principle:

Glucose is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide Oxidatively couples with 4-amino Antipyrine and phenol to produce red quinoneimine dye in the presence of peroxidase. This red dye has maximum absorbance at 505nm. The intensity

of the colour complex is directly proportional to the concentration of glucose in specimen.

D-Glucose + O_2 + H_2O $\xrightarrow{GOD}$Gluconic acid + H_2O_2

H_2O_2 + 4- Amino Antipyrine + Phenol $\xrightarrow{POD}$ red dye + H_2O

Specimen:

Fresh unhemolysed serum

Assay Procedure:

Enzyme reagent and standard were brought to the room temperature before performing the assay.

Reagents	Blank	Standard	Sample
Glucose enzyme reagent	1000µl	1000µl	1000µl
Standard	-	10µl	-
Sample	-	-	10µl
Distilled water	10µl	-	-

The tubes were mixed thoroughly and incubated at 37^o for 10min. The absorbance was read against reagent blank at 505nm.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dl)}$$

Glucose Standard: 100mg/dl

Linearity: Upto 500mg/dl

Normal Values:

Glucose fasting: 65-100mg/dl

Glucose postprandial: 90-140mg/dl

RESULTS

A total of 100 subjects were selected as the study group for the present study. This includes 50 cases with CKD and 50 healthy controls.

Levels of serum Visfatin, Urea, Creatinine , hs CRP, TC, TGL, HDL-C and FBG were estimated for all the samples of the study group. VLDL-C, LDL-C and Creatinine clearance were calculated from the formula.

The values obtained in controls and cases are presented in the master chart I and II respectively.

MASTER CHART – I CONTROL GROUP																		
NO	SEX	AGE (Yrs)	HT (m)	WT (Kg)	BMI (Kg/m^2)	SBP (mm/Hg)	DBP (mm/Hg)	FBG (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	CCR (ml/min)	hsCRP (mg/L)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	VISFATIN (ng/ml)
1	M	38	1.68	62	36.90	110	70	86	20	1	87.83	0.83	168	110	42	22	104	9.8
2	M	31	1.64	68	41.46	104	80	90	22	1.1	93.58	0.29	186	150	46	30	110	10.2
3	M	37	1.6	72	45	120	76	80	18	0.9	114.44	0.21	164	122	46	24.4	93.6	8.9
4	M	35	1.56	66	42.30	90	70	98	28	0.9	106.94	0.58	150	112	45	22.4	82.6	11.2
5	M	37	1.7	70	41.17	110	80	100	20	1.1	91.03	0.81	180	135	42	27	111	11
6	M	26	1.68	68	40.47	114	70	102	24	0.8	134.58	0.46	174	140	40	28	106	10.2
7	M	32	1.7	65	38.23	100	80	78	24	0.8	121.87	0.89	150	124	38	24.8	87.2	8.8
8	M	33	1.56	67	42.94	96	80	82	18	0.9	110.63	0.33	160	150	46	30	84	10.2
9	M	35	1.65	55	33.33	112	74	96	26	0.8	100.26	0.42	176	142	42	28.4	106	10
10	M	34	1.62	68	41.97	98	80	72	20	1	100.11	0.22	178	135	50	27	101	9.1
11	M	36	1.65	60	36.36	120	76	78	20	0.9	96.29	0.56	184	140	40	28	116	8.8
12	M	38	1.68	67	39.88	116	70	100	22	1	94.91	0.92	160	138	45	27.6	87.4	8.9
13	M	36	1.56	65	41.66	90	80	104	28	1	93.88	0.18	208	122	41	24.4	143	8.8
14	M	28	1.65	56	33.93	118	78	84	20	0.9	96.79	0.67	188	120	40	24	124	10
15	M	58	1.69	68	40.23	110	70	90	26	0.8	96.8	0.72	175	154	48	30.8	96.2	11
16	M	68	1.68	73	43.45	100	76	86	26	0.8	91.25	0.32	182	160	44	32	106	8.9
17	M	64	1.58	69	43.67	96	74	92	24	0.8	91.04	0.88	168	110	42	22	104	8.8
18	M	61	1.63	60	36.80	126	70	76	24	0.7	94.04	0.41	200	124	45	24.8	130	10
19	M	62	1.67	68	40.71	90	72	84	28	0.8	92.08	0.29	165	115	42	23	100	11.2
20	M	53	1.7	68	40	108	80	98	22	0.9	91.29	0.47	152	148	40	29.6	82.4	10.2
21	M	63	1.52	68	44.73	110	74	86	26	0.8	90.9	0.73	188	134	46	26.8	115	10.5
22	M	61	1.57	67	42.67	100	70	100	24	0.8	91.89	0.91	164	140	38	28	98	10.7
23	M	62	1.65	70	42.42	96	72	78	24	0.8	94.79	0.53	166	128	45	25.6	95.4	10
24	M	61	1.63	72	44.17	94	80	104	20	0.9	87.77	0.25	194	136	40	27.2	127	11
25	M	62	1.69	68	40.23	106	76	92	18	0.8	92.08	0.36	202	120	36	24	142	10.8

MASTER CHART – I CONTROL GROUP

26	F	31	1.64	68	41.46	104	80	90	22	0.8	109.37	0.29	186	150	46	30	110	10.3
27	F	37	1.6	72	45	120	76	80	18	0.9	97.27	0.21	164	122	46	24.4	93.6	10.1
28	F	35	1.56	66	42.30	90	70	98	28	0.8	102.26	0.58	150	112	45	22.4	82.6	10.5
29	F	26	1.68	68	40.47	114	76	102	24	0.8	114.39	0.48	174	140	40	28	106	11
30	F	32	1.7	65	38.23	100	70	78	24	0.8	103.59	0.89	150	124	38	24.8	87.2	8.8
31	F	33	1.56	67	42.94	96	80	82	18	0.9	94.03	0.33	160	150	46	30	84	7.2
32	F	35	1.56	70	44.87	112	80	96	26	0.8	108.46	0.42	176	142	42	28.4	106	14.1
33	F	34	1.62	68	41.97	98	74	72	20	0.8	106.36	0.22	178	135	50	27	101	13.2
34	F	38	1.68	67	39.88	116	76	100	22	0.8	100.85	0.92	160	138	45	27.6	87.4	9.8
35	F	36	1.56	65	41.66	90	70	104	28	0.8	99.75	0.81	208	122	41	24.4	143	11
36	F	40	1.56	66	42.30	88	70	74	26	0.8	97.39	0.34	180	108	38	21.6	120.4	13.2
37	F	42	1.53	63	41.17	108	78	100	24	0.8	91.1	0.63	178	112	46	22.4	110	13.5
38	F	61	1.57	66	42.03	90	70	82	22	0.6	102.59	0.72	164	110	40	22	102	12
39	F	46	1.62	65	40.12	106	74	92	28	0.6	120.21	0.28	196	126	45	25.2	126	8.8
40	F	41	1.62	63	38.88	100	72	94	20	0.8	92.03	0.59	202	148	37	29.6	135	11.2
41	F	44	1.56	64	41.02	110	82	98	28	0.8	90.66	0.63	180	140	45	28	107	12.3
42	F	50	1.6	60	37.5	100	78	80	20	0.7	91.07	0.79	172	155	38	31	103	11
43	F	45	1.57	66	42.03	96	70	72	20	0.8	92.52	0.52	150	142	38	28.4	83.6	10.2
44	F	52	1.58	62	39.24	98	72	78	28	0.7	92.01	0.83	184	138	46	27.6	110	10.6
45	F	45	1.55	65	41.93	102	78	90	24	0.8	91.12	0.29	180	168	40	33.6	106	10.2
46	F	45	1.58	66	41.77	90	70	100	22	0.8	92.52	0.64	166	140	42	28	96	11
47	F	40	1.53	70	45.75	120	80	78	22	0.9	91.82	0.31	170	134	38	26.8	105	14.3
48	F	46	1.6	66	41.25	100	74	82	18	0.8	91.55	0.42	194	126	42	25.2	127	11.5
49	F	41	1.62	63	38.88	100	72	94	20	0.8	92.03	0.59	202	148	37	29.6	135	15
50	F	28	1.58	62	39.24	98	72	78	28	0.7	117.11	0.83	184	138	46	27.6	110	11.2

MASTER CHART – II STUDY GROUP																		
S.NO	SEX	AGE (yrs)	HT (m)	WT (Kg)	BMI (Kg/m^2)	SBP (mm/Hg)	DBP (mm/Hg)	FBG (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	CCR (ml/min)	hsCRP (mg/L)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	Visfatin (ng/ml)
1	M	65	1.53	35	14.95	126	82	120	92	3	12.15	10	180	180	28	36	116	32.2
2	M	65	1.64	43	15.99	152	84	112	108	3.8	11.79	7.44	150	200	38	40	72	39.6
3	M	47	1.73	57	19.05	118	80	86	88	2.5	29.45	3.69	174	184	38	36.8	99.2	31
4	M	55	1.67	65	23.31	114	76	146	92	2.7	28.42	4.53	162	152	34	30.4	97.6	29.6
5	M	32	1.62	55	20.96	150	100	110	104	3.14	26.27	8.7	182	192	42	38.4	102	28.5
6	M	75	1.78	60	18.94	150	90	132	96	2.3	23.55	7.61	172	180	46	36	90	26.9
7	M	73	1.73	52	17.37	120	80	106	90	2.81	17.22	10.4	184	208	32	41.6	110	30.5
8	M	65	1.67	72	25.82	110	70	72	114	3	25	2.9	176	166	36	33.2	107	28
9	M	38	1.62	53	20.2	140	70	114	130	4.9	15.32	5.58	172	152	40	30.4	102	31.8
10	M	35	1.7	86	29.76	124	82	120	126	5.7	22	8.89	160	176	42	35.2	82.8	25.7
11	M	45	1.63	57	21.45	110	80	112	80	1.6	47.01	3.2	160	184	40	36.8	83.2	20.8
12	M	45	1.55	62	25.81	110	74	74	88	2.2	37.18	0.69	190	160	33	32	125	22.8
13	M	32	1.57	50	20.28	160	110	120	90	2	37.5	3.72	174	240	37	48	89	24.2
14	M	38	1.63	60	22.58	140	100	70	70	1.4	60.71	0.93	174	210	42	42	90	19.5
15	M	55	1.76	68	21.95	140	100	140	60	1.3	61.75	1.54	180	220	36	44	100	19.2
16	M	48	1.7	74	25.61	112	80	132	68	1.3	72.74	2.06	166	150	38	31.2	96.8	18.9
17	M	23	1.66	55	19.96	120	80	74	72	1.4	63.84	1.2	174	198	38	39.6	96.4	19.4
18	M	48	1.59	76	30.06	140	82	104	64	1.3	74.7	3.13	162	202	34	40.4	87.6	17.5
19	M	37	1.45	36	17.12	120	80	122	142	8.9	5.78	10.56	216	180	32	36	148	42.5
20	M	53	1.62	70	26.67	118	74	90	136	8.3	10.19	9.7	160	170	36	34	90	40.5
21	M	57	1.55	58	24.14	124	80	106	128	4.9	13.65	6.91	220	208	28	41.6	150	39.6
22	M	45	1.6	48	18.75	128	86	142	110	4.3	14.73	6.73	170	216	40	43.2	86.8	39
23	M	53	1.57	62	25.15	170	100	92	134	7.8	9.27	5.82	166	192	32	38.4	95.6	39

24	M	45	1.62	45	17.15	130	70	116	90	1.94	30.61	2.99	160	230	35	46	79	31
25	M	55	1.7	55	19.03	110	80	130	110	2.03	31.99	3.96	196	160	38	32	126	31.2
26	F	58	1.54	44	18.55	110	80	128	78	1.3	27.84	2.97	178	154	4	30.8	102	31.6
27	F	49	1.56	68	27.94	126	82	80	84	1.9	32.68	3.98	160	170	37	34	89	22.1
28	F	54	1.52	65	28.13	170	100	112	70	1.56	35.95	2.63	150	168	42	33.6	74.4	18.9
29	F	70	1.59	75	29.67	102	90	86	68	1.8	29.26	3.7	172	208	40	41.6	90.4	19.9
30	F	33	1.55	45	18.73	120	80	74	74	1.6	30.2	4.67	184	176	34	35.2	115	20
31	F	58	1.62	55	20.96	124	72	72	80	1.4	32.32	5.3	160	188	34	37.6	88.4	21.5
32	F	55	1.56	45	18.49	130	80	110	92	2.69	14.27	8.6	162	200	35	40	87	33.2
33	F	55	1.5	40	17.78	120	80	80	86	1.7	20.66	5	170	194	28	38.8	103	25.7
34	F	57	1.56	60	24.65	114	72	128	98	2.9	17.22	4.93	192	168	46	33.6	112	29.1
35	F	63	1.61	46	17.75	156	90	136	130	4.6	7.72	4.89	208	192	28	38.4	142	42
36	F	53	1.54	47	19.82	160	92	70	148	58	7.07	5.6	200	184	40	36.8	123	41.8
37	F	50	1.57	52	21.1	150	100	102	126	4.7	9.99	8.26	186	178	34	35.6	116	40.6
38	F	48	1.54	45	18.97	120	80	76	150	7.3	5.69	2.15	152	190	37	38	77	42.8
39	F	61	1.5	43	19.11	110	80	110	134	5.3	6.42	9.8	176	184	48	36.8	91.2	41.7
40	F	62	1.66	56	20.32	114	82	90	104	2.2	19.92	10.9	170	212	46	42.4	81.6	25.7
41	F	57	1.7	68	23.53	130	80	138	110	2.5	22.65	9.26	162	168	38	33.6	90.4	33.5
42	F	42	1.61	68	26.23	110	80	72	68	1.3	51.44	1.73	174	134	40	26.8	107	19.5
43	F	33	1.67	67	24.02	120	84	86	80	1.4	51.38	4.92	198	140	45	28	125	19.7
44	F	37	1.7	70	23.53	110	80	100	56	1.1	77.38	1.81	180	135	42	27	111	15.5
45	F	61	1.63	72	27.1	120	72	104	66	0.9	74.61	1.07	216	180	32	36	148	16.4
46	F	45	1.7	62	21.45	140	100	70	70	0.9	77.26	0.93	174	210	42	42	90	16.5
47	F	62	1.69	68	23.81	150	100	140	60	0.8	78.26	1.54	180	220	36	44	100	15.2
48	F	48	1.7	74	25.61	112	80	132	68	1.3	61.82	2.06	166	156	38	31.2	96.8	19.4

49	**F**	**35**	**1.68**	**73**	**25.86**	**120**	**80**	**74**	**72**	**1.3**	**69.6**	**1.2**	**174**	**198**	**38**	**39.6**	**96.4**	**14.7**
50	**F**	**63**	**1.58**	**68**	**27.24**	**140**	**82**	**104**	**64**	**0.8**	**77.26**	**3.13**	**162**	**202**	**34**	**40.4**	**87.6**	**15.5**

STATISTICAL ANALYSIS

- Student's t-test, Chi-square test and oneway ANOVA were used for the statistical analysis of data.
- The datas were expressed in terms of mean and standard deviation.
- 'p' value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

Table 1 represents the minimum level, maximum level, mean and SD of all parameters of the study group.

DESCRIPTIVE STATISTICS OF THE STUDY GROUP.

S.NO	PARAMETERS	CONTROLS(n=50)				CASES (n=50)			
		MIN	MAX	MEAN	SD	MIN	MAX	MEAN	SD
1	Age (years)	26	68	43.08	11.83	23	75	50.76	11.74
2	Height (m)	1.52	1.7	1.61	0.05	1.45	1.78	1.61	0.73
3	Weight(kg)	55	73	66.06	3.76	35	86	58.6	11.83
4	BMI (kg/m^2)	20.2	29.9	25.39	2.18	14.9	30.06	22.26	3.93
5	SBP(mm/Hg)	88	126	103.6	9.95	102	170	125.68	24.1
6	DBP (mm/Hg)	70	82	74.84	4.04	70	110	83.76	9.59
7	FBG (mg/dl)	72	104	88.6	9.95	70	146	104.32	23.64
8	Blood Urea (mg/dl)	18	28	23.04	3.38	56	150	94.36	26.33
9	s. Creatinine (mg/dl)	0.6	1.1	0.83	0.1	0.8	8.9	2.87	2.05
10	Ccr(ml/min)	87.77	134.58	98.78	10.1	5.78	78.27	35.91	23.52
11	s.hsCRP (mg/L)	0.18	0.92	0.53	0.23	0.69	10.56	4.54	2.96
12	S.TC(mg/dl)	150	208	173	28.2	150	220	175.72	16.49
13	S.TGL (mg/dl)	108	168	133.54	14.57	134	216	171.86	23
14	S.HDL-C(mg/dl)	36	50	42.52	3.55	24	45	33.84	7.06
15	S.VLDL-C(mg/dl)	21.6	33.6	25.86	5.59	26.8	43.2	34.37	4.6
16	Serum LDL-C (mg/dl)	82.4	143	106.56	16.71	78.4	150.4	107.5	16.54
17	S.Visfatin (ng/ml)	7.2	15	10.62	1.57	14.7	42.8	27.42	8.92

Table 2

GENDERWISE DISTRIBUTION OF THE STUDY GROUP AND STATISTICAL ANALYSIS

GROUPS	GENDER		STATISTICAL INFERENCE
	MALES (%)	FEMALES(%)	
CONTROLS (n=50)	n=25 (50%)	n=25 (50%)	X^2 = 16.430; p value >0.05 not significant.
CASES (n=50)	n=25 (50%)	n=25 (50%)	
TOTAL	n=50 (50%)	n=50 (50%)	

The distribution of males and females in the study group were 50% and 50% respectively which shows equal distribution .There is no significant difference in gender between cases and controls.

Table 3

AGE WISE DISTRIBUTION OF THE STUDY GROUP

GROUPS	AGE (YEARS)		MEDIAN AGE (YEARS)	RANGE (YEARS)
	MEAN	SD		
CASES	50.76	11.89	50	23-75
CONTROLS	47.85	10.55	46.5	26-68

The age group of cases ranged from 23-75 years with a mean age of 50.76±11.89 years and median age of 50 years. The age group of controls ranged from 26-68 years, with the mean age of 47.85±10.55 years and median age of 46.5 years.

Table 4

COMPARISON OF SERUM VISFATIN LEVEL IN THE STUDY GROUP

S.NO	GROUPS	SERUM VISFATIN (NG/ML)		STATISTICAL INFERENCE
		MEAN	SD	
1	Controls (n=50)	10.62	1.57	t=13.11; P<0.05 significant
2	Cases (n=50)	27.42	8.92	

The mean value of Visfatin in cases was 27.42±8.92 ng/ml and this was significantly higher than that of the control group whose mean value was 10.62±1.57 ng/ml (t=13.11; p<0.05 significant).

Table 5

AGE MATCHED ANALYSIS OF SERUM VISFATIN LEVEL IN THE STUDY GROUP

AGE (Yrs)	SERUM VISFATIN(ng/ml)			STATISTICAL INFERENCE
	GROUPS	MEAN	SD	
< 30	Controls (n=4)	10.6	0.58	t=12.358 p <0.05 significant
	Cases (n=2)	19.7	0.42	
31-40	Controls(n=23)	10.35	1.82	t=7.577 P <0.05 Significant
	Cases (n=9)	24.67	8.80	
41-50	Controls(n=10)	11.47	1.76	t=4.888 P <0.05 Significant
	Cases (n=13)	26.3	9.41	
51-60	Controls (n=3)	10.6	0.40	t=4.493 p<0.05 significant
	Cases (n=14)	31.02	7.67	
>60	Controls(n=10)	10.39	0.99	t=5.54 P <0.05 Significant
	Cases (n=12)	27.8	9.85	

This table shows the comparision of serum Visfatin level in various age groups in the study subjects. Serum Visfatin is significantly higher in the cases than controls in all the included age groups.

Table 6

GENDER MATCHED COMPARISON OF SERUM VISFATIN LEVELS IN THE STUDY GROUP

GENDER	GROUPS	SERUM VISFATIN (ng/ml)		STATISTICAL INFERENCE
		MEAN	SD	
Males	Control(n=25)	9.96	0.85	t=12.358 P<0.05 significant
	Cases (n=25)	29.15	7.71	
Females	Control(n=25)	11.28	1.84	t=7.197 P<0.05 significant
	Cases (n=25)	25.70	9.84	

This table represent the gender-wise comparison of serum Visfatin in controls [males: mean 9.96±0.85, females: mean 11.28± 1.84] and cases [males: mean 29.15±7.71, females 25.7±9.84]. Serum Visfatin is significantly higher in both sexes of cases than in controls (P<0.05).

Table 7

GENDERWISE COMPARISON OF SERUM VISFATIN IN CASES

S.NO	GENDER	SERUM VISFATIN(ng/ml)		STATISTICAL INFERENCE
		MEAN	SD	
1	Males(n=25)	29.15	7.71	t=1.389 p>0.05 not significant
2	Females(n=25)	25.70	9.84	

This table shows the gender wise comparison of serum Visfatin level within cases [males:mean 29.15±7.71; females 25.7±9.84]. There is no significant difference of serum Visfatin level between genders in the cases (T = 1.389 ; p>0.05).

Table 8

COMPARISON OF FBG, BLOOD UREA, SERUM CREATININE AND CREATININE CLEARANCE IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	SD	STATISTICAL INFERENCE
FBG(mg/dl)	**Cases(n=50)**	**104.32**	**23.64**	**t=4.332 P<0.05 significant**
	Control(n=50)	**88.6**	**9.95**	
B.Urea(mg/dl)	**Cases(n=50)**	**94.36**	**26.33**	**t=19.00 P<0.05 significant**
	Control(n=50)	**23.04**	**3.33**	
Creatinine(mg/dl)	**Cases(n=50)**	**2.87**	**2.05**	**t=6.999 P<0.05 significant**
	Control(n=50)	**0.83**	**0.10**	
Ccr(ml/min)	**Cases(n=50)**	**35.91**	**23.51**	**t=17.363 P<0.05 significant**
	Control(n=50)	**98.78**	**10.10**	

There is a significant difference in FBG between the two groups (T= 4.332, $p<0.05$).FBG is significantly higher in cases than in controls indicates CKD is prevalent in DM. Blood urea and Serum Creatinine were found to be significantly higher in cases and Ccr was significantly lower in the cases than controls ($p <0.05$, significant).

Table 9

COMPARISON OF SERUM hsCRP IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	SD	STATISTICAL INFERENCE
s.hsCRP(mg/L)	Controls(n=50)	0.53	0.23	t=9.514; P<0.05 Not significant
	Cases(n=50)	5.54	2.96	

From this table it is obvious that hsCRP level was significantly higher in the cases than controls ($p<0.05$, significant).

TABLE 10

COMPARISON OF LIPID PARAMETERS IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	SD	STATISTICAL INFERENCE
TC (mg/dl)	**Cases(n=50)**	**175.72**	**16.492**	**t=0.589; P>0.05 not significant**
	Controls(n=50)	**173.00**	**28.202**	
TGL(mg/dl)	**Cases(n=50)**	**171.86**	**23.005**	**t=9.951; P<0.05 significant**
	Controls(n=50)	**133.54**	**14.570**	
HDL-C (mg/dl)	**Cases(n=50)**	**33.84**	**7.061**	**t=7.765; P<0.05 significant**
	Controls(n=50)	**42.52**	**3.553**	
VLDL-C (mg/dl)	**Cases(n=50)**	**34.372**	**4.600**	**t=8.301; P<0.05 significant**
	Controls(n=50)	**25.868**	**5.5957**	
LDL-C(mg/dl)	**Cases(n=50)**	**107.5080**	**16.5463**	**t=0.284; P>0.05 Not significant**
	Controls(n=50)	**106.5640**	**16.7122**	

We observed a significantly higher serum TGL and VLDL-C levels and a significantly lower HDL-C levels in the cases than controls ($p<0.05$). There is no significant difference in the serum TC and LDL-C levels between the two groups.

Table 11**COMPARISON OF VISFATIN IN RELATION TO CCR IN CASES.**

Ccr (ml/min)	**VISFATIN (ng/ml)**		**STATISTICAL INFERENCE**
	MEAN	**SD**	
60-90 (n=14)	**17.63**	**1.92**	**F=102.921; P<0.05 Significant**
30-59 (n=12)	**24.58**	**5.08**	
15-29 (n=12)	**29.06**	**2.80**	
<15 (n=12)	**40.10**	**2.82**	

As the Creatinine clearance declines, we observed a significant progressive increase in the serum Visfatin levels in cases ($p<0.05$).

Table 12**COMPARISON OF hsCRP IN RELATION TO Ccr IN CASES**

Ccr (ml/min)	**hsCRP(mg/L)**		**STATISTICAL INFERENCE**
	MEAN	**SD**	
60-90 (n=14)	**1.56**	**0.58**	**F=43.09; P<0.05 Significant**
30-59 (n=12)	**2.88**	**0.90**	
15-29 (n=12)	**6.73**	**2.20**	
<15 (n=12)	**7.48**	**2.05**	

This table represents the comparision of serum hsCRP levels in the study group in relation to Ccr. We observed a significant progressive increase in the hs CRP value in the cases as the renal function declined ($p<0.05$).

Table 13

COMPARISON OF UREA IN RELATION TO Ccr IN CASES

Ccr (ml/min)	UREA(mg/dl)		STATISTICAL INFERENCE
	Mean	SD	
60-90 (n=14)	67	6	F=55.858; P<0.05 Significant
30-59 (n=12)	83.33	11.26	
15-29 (n=12)	103.5	14.145	
<15 (n=12)	128.17	17.17	

This table represents the comparison of Blood Urea levels in the study group in relation to Ccr. There is a significant progressive increase in the Blood Urea values with declining renal function in cases ($p<0.05$).

Table 14 **COMPARISON OF CREATININE IN RELATION TO Ccr IN CASES**

Ccr (ml/min)	Creatinine(mg/dl)		Statistical inference
	Mean	SD	
60-90 (n=14)	1.17	0.22	F=41.449; P<0.05 Significant
30-59 (n=12)	1.81	0.34	
15-29 (n=12)	3.04	1.13	
<15 (n=12)	5.72	1.9	

This table shows the comparison of serum Creatinine levels in the study group in relation to Ccr. We observed a significant progressive increase of Creatinine values as the renal function decreases in cases serum Creatinine levels ($p<0.05$) were within normal reference range for controls.

Table 15

COMPARISON OF LIPID PARAMETERS IN RELATION TO Ccr IN THE CASES

PARAMETERS	CREATININE CLEARANCE (ml/min)				STATISTICAL INFERENCE
	60-90	30-50	15-29	<15	
TC (mg/dl)					
Mean	177.14	171.50	172	182.00	F=1.086 p>0.05
SD	14.48	13.96	10.05	24.24	Not significant
TGL(mg/dl)					
Mean	144.07	165.67	180.50	201.83	F=117.907 P<0.05
SD	6.00	8.60	6.66	10.49	Significant
HDL(mg/dl)					
Mean	42.64	35.92	29.83	25.50	F=119.012 P<0.05
SD	1.73	2.15	3.76	1.73	Significant
VLDL(mg/dl)					
Mean	28.81	33.13	36.10	40.36	F=117.907 P<0.05
SD	1.20	1.72	1.33	2.09	Significant
LDL(mg/dl)					
Mean	105.68	102.45	106.06	116.13	F=1.605 P>0.05
SD	14.21	14.68	9.00	23.84	Not Significant

Serum TC and LDL-C levels were found to be within the normal reference range in the cases irrespective of Ccr. We observed a serial significant increase in the serum TGL and VLDL-C levels and a serial significant decrease in the HDL-C levels with declining renal function ($p<0.05$).

TABLE 16

KARL PEARSON COEFFICIENT CORRELATION BETWEEN SERUM VISFATIN AND OTHER BIOCHEMICAL PARAMETERS IN CASES (n=50)

S.NO	PARAMETERS	CORRELATION VALUE (r)	STATISTICAL INFERENCE
1	Urea	0.902(**)	p<0.01 ;significant
2	Creatinine	0.832(**)	p<0.01 ;significant
3	Ccr	-0.898(**)	p<0.01 ;significant
4	hsCRP	0.746(**)	p<0.01; significant
5	TC	0.164	p>0.05; NS
6	TGL	0.877(**)	p<0.01; significant
7	HDL	-0.889(**)	p<0.01; significant
8	VLDL	0.877(**)	p<0.01; significant
9	VDL	0.298(**)	p<0.05 ;significant

This table shows the Pearson's correlation coefficient between serum visfatin and other studied biochemical parameters in the cases. There is highly positive correlation of serum Visfatin with hsCRP, Blood Urea, Serum Creatinine, TGL and VLDL-C ($p<0.01$). We also observed a highly negative correlation of serum Visfatin with HDL-C and Ccr ($p<0.01$).

Figure 5

Bar diagram showing the serum Visfatin level in the study group.

Figure 6

Bar diagram showing the comparison of serum hsCRP in the study group.

Figure 7

Bar diagram showing the comparison of serum Visfatin levels in the study group in relation to Creatinine Clearance.

Figure 8

Bar diagram showing the comparison of serum hsCRP in the study group in relation to Creatinine Clearance.

Figure 9

Scatter diagram of serum Visfatin vs hsCRP in controls.

Figure 10

Scatter diagram of serum Visfatin vs hsCRP in cases.

Figure 11

Scatter diagram of serum Visfatin vs Creatinine Clearance in controls.

Figure 12

Scatter diagram of serum Visfatin vs Creatinine Clearance in cases.

Figure 13

Scatter diagram of serum Visfatin vs TGL in controls.

Figure 14

Scatter diagram of serum Visfatin vs TGL in cases.

Figure 15

Scatter diagram of serum Visfatin vs HDL-C in controls.

Figure 16

Scatter diagram of serum Visfatin vs HDL-C in cases.

FIGURE 5

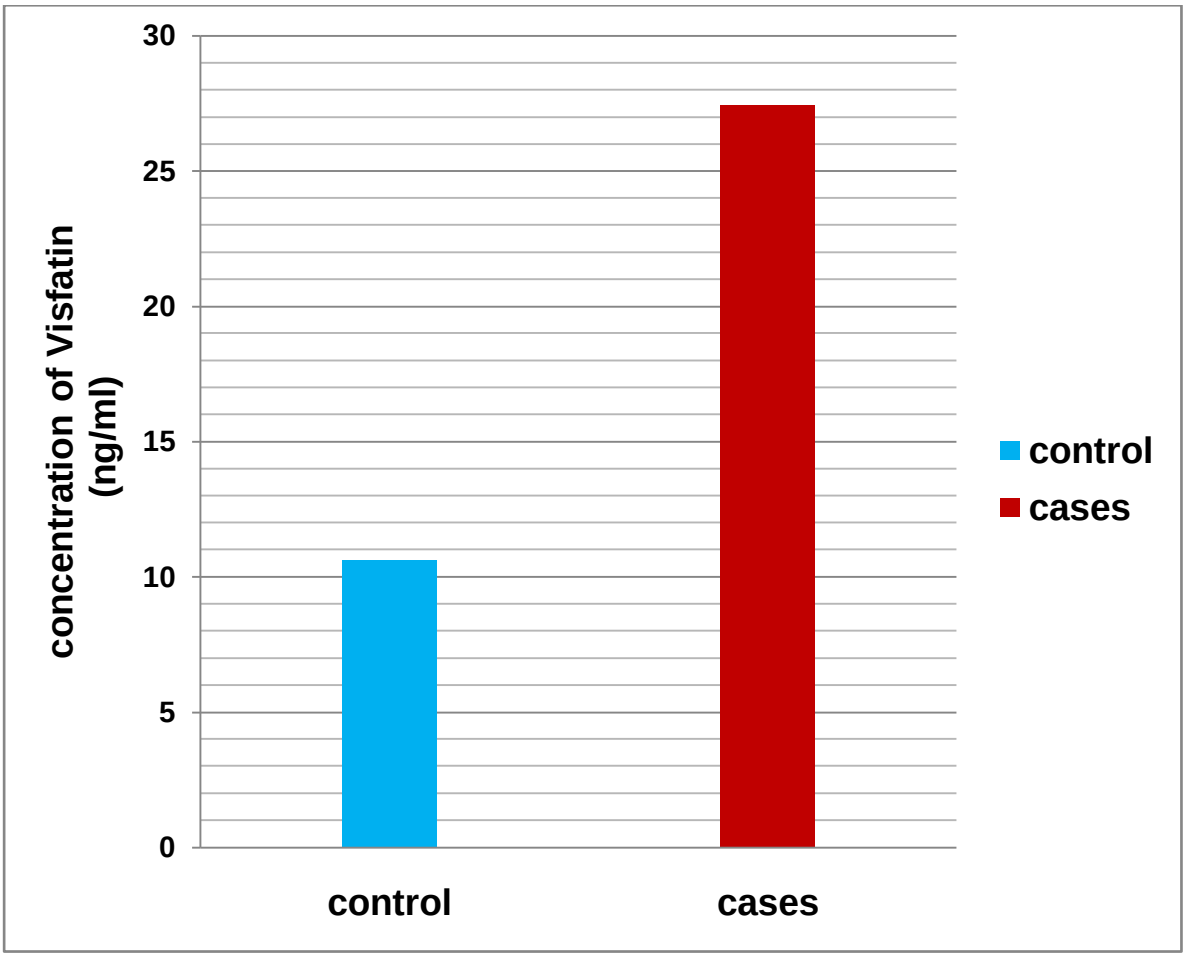

concentration of Visfatin (ng/ml)
30
25
20
15
10
5
0
control
cases
control
cases

FIGURE 6

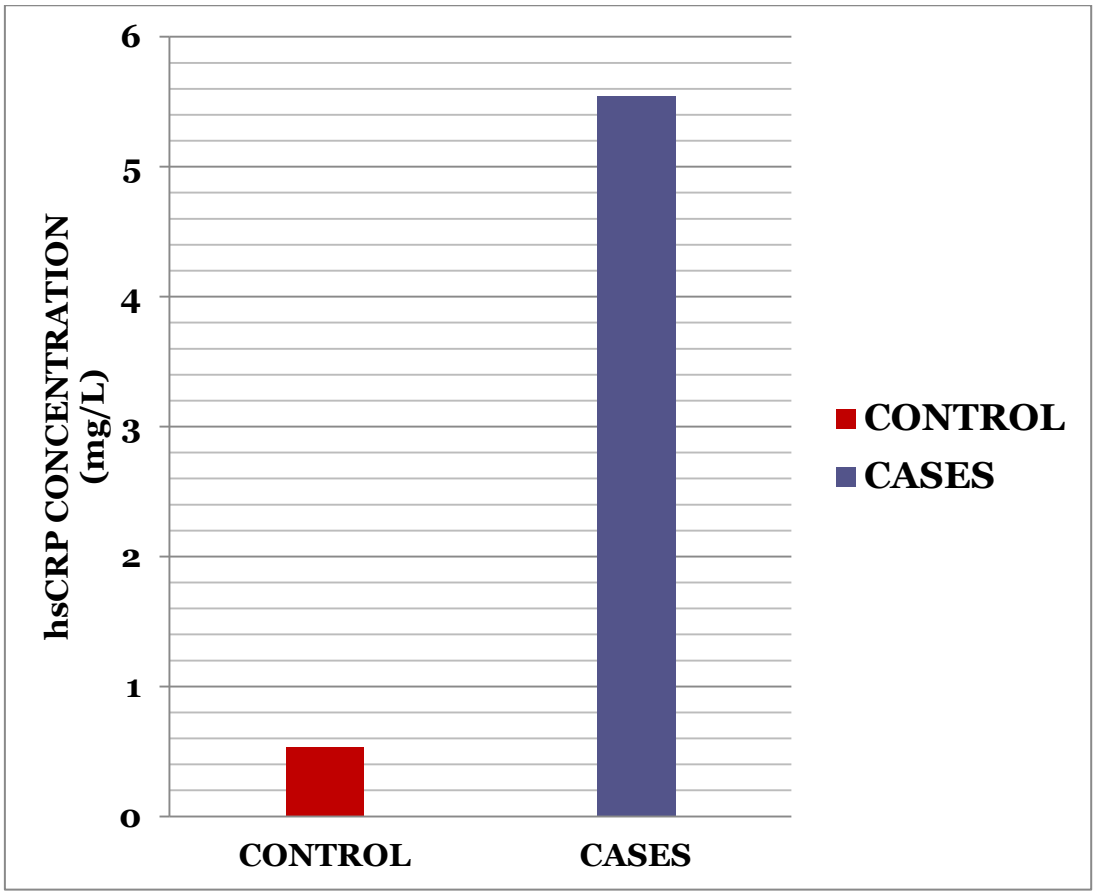

6
5
4
3
2
1
0
hsCRP CONCENTRATION (mg/L)
CONTROL
CASES
CONTROL
CASES

FIGURE 7

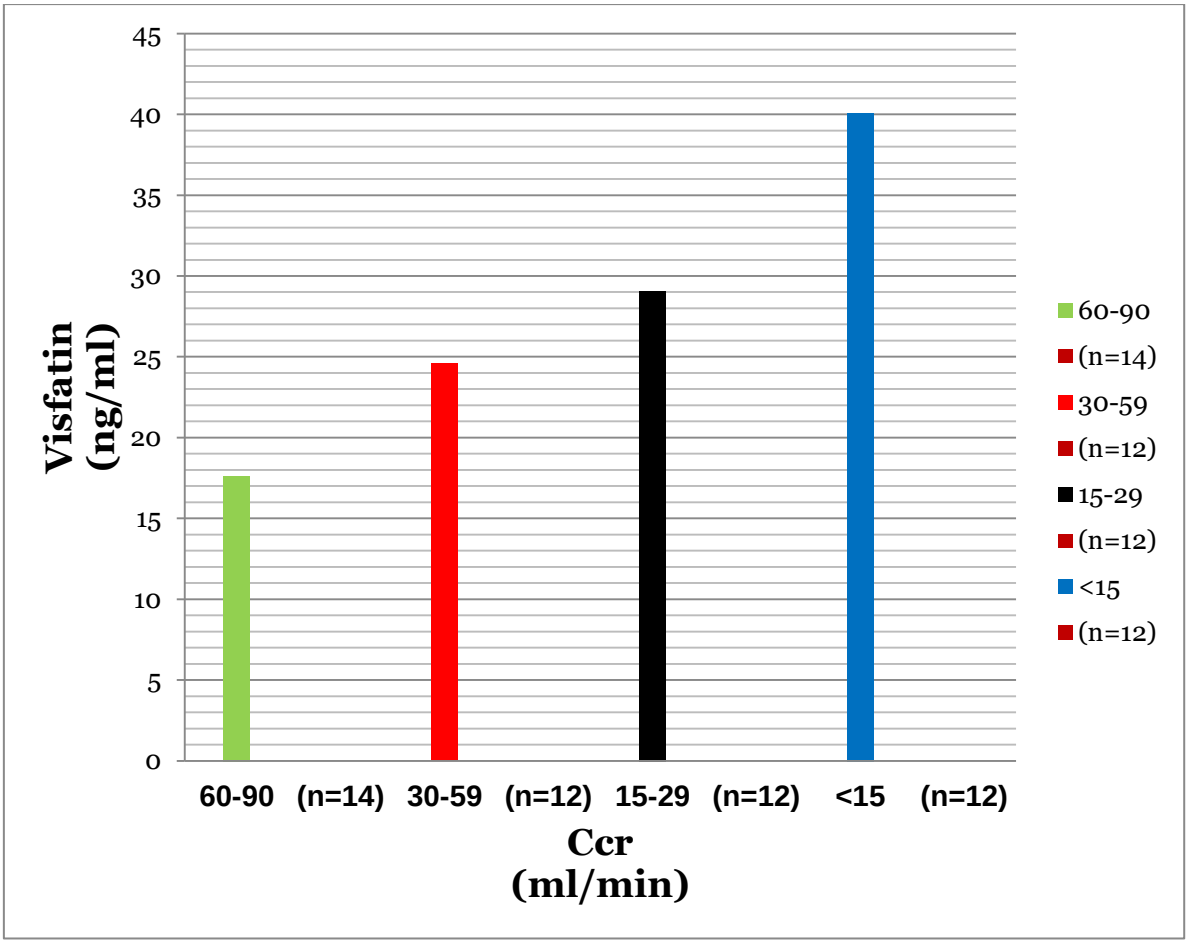

45
40
35
30
25
20
15
10
5
0
Visfatin (ng/ml)
60-90
(n=14)
30-59
(n=12)
15-29
(n=12)
<15
(n=12)
60-90 (n=14) 30-59 (n=12) 15-29 (n=12) <15 (n=12)
Ccr (ml/min)

FIGURE 8

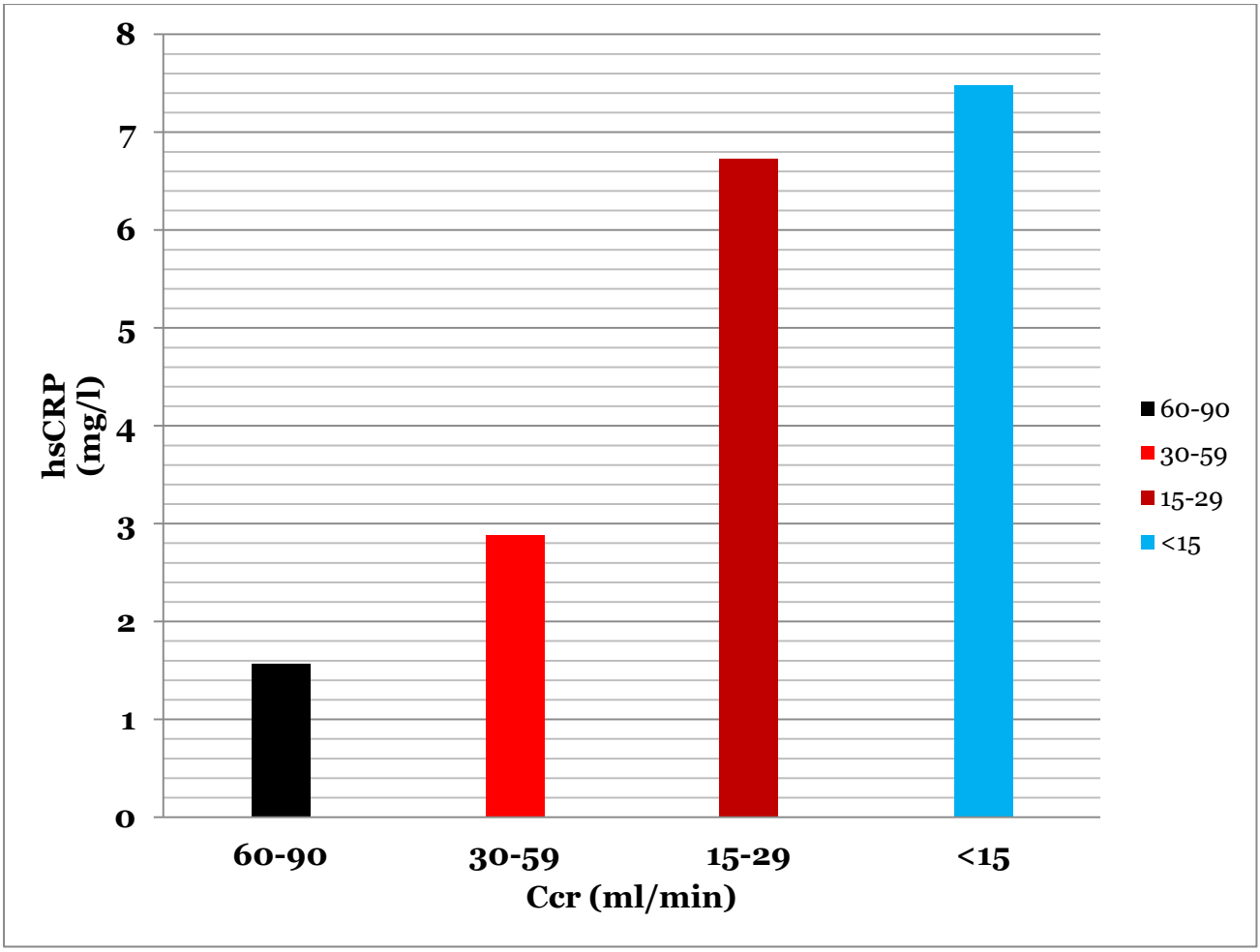

8
7
6
5
4
3
2
1
0
hsCRP (mg/l)
60-90
30-59
15-29
<15
Ccr (ml/min)
60-90
30-59
15-29
<15

FIGURE 9

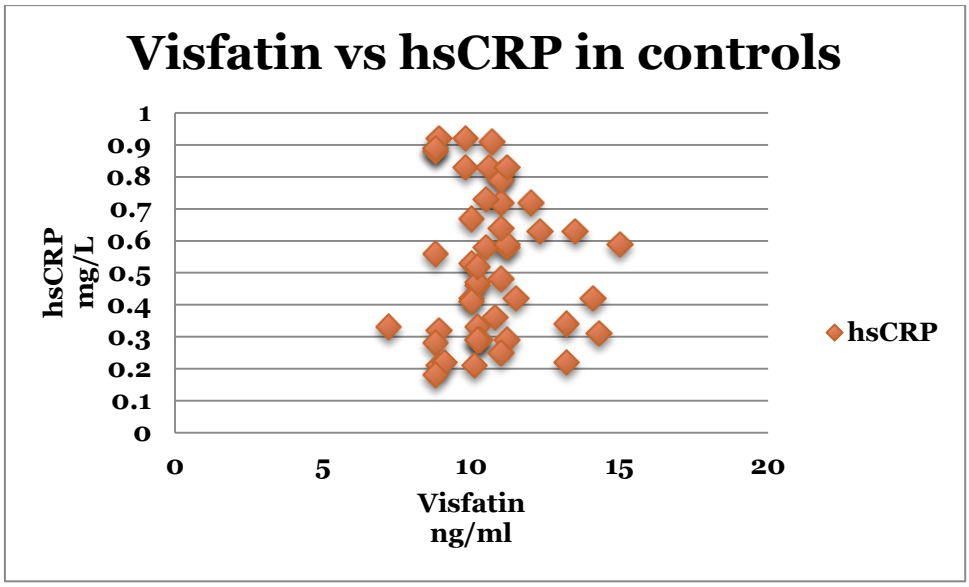


FIGURE 10

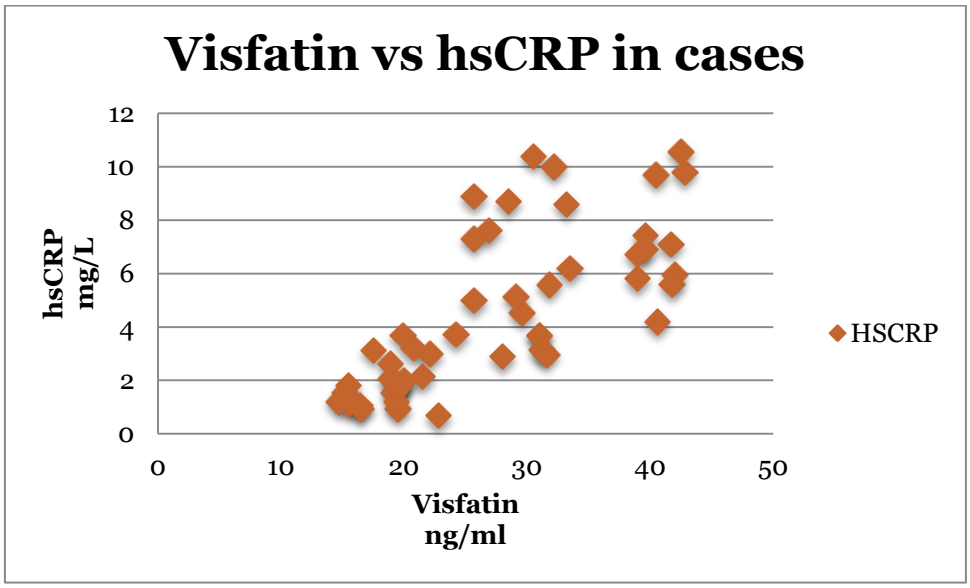


FIGURE 11

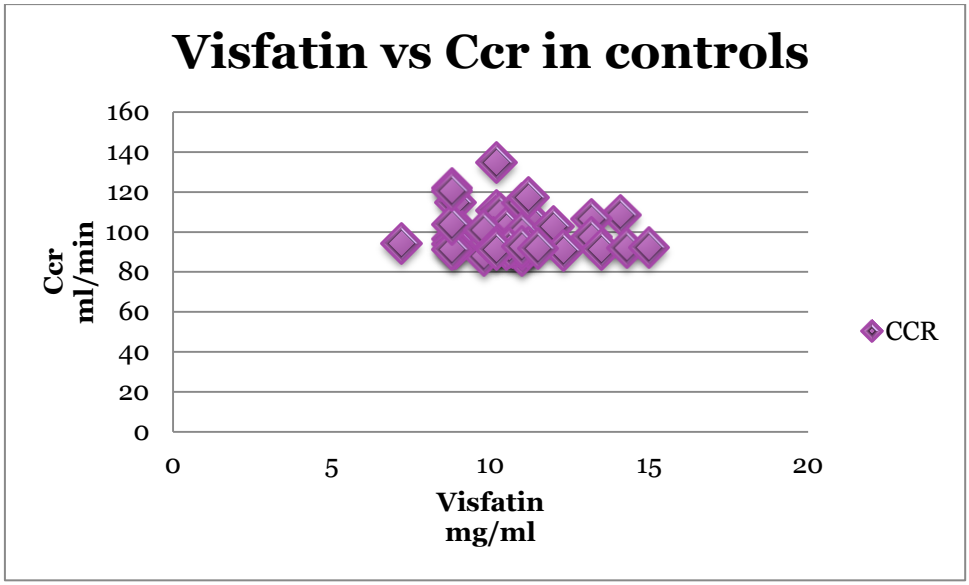
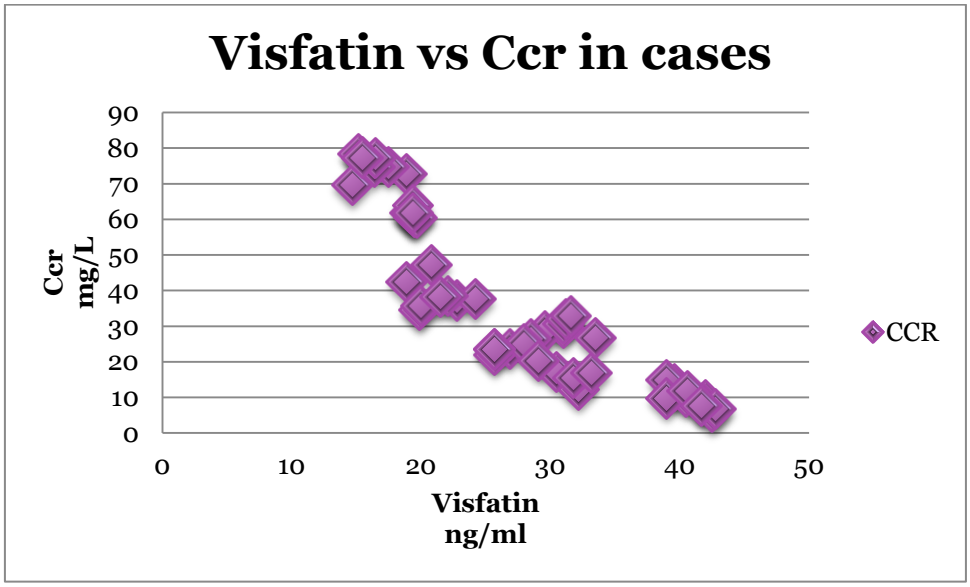


FIGURE 12



`

FIGURE 13

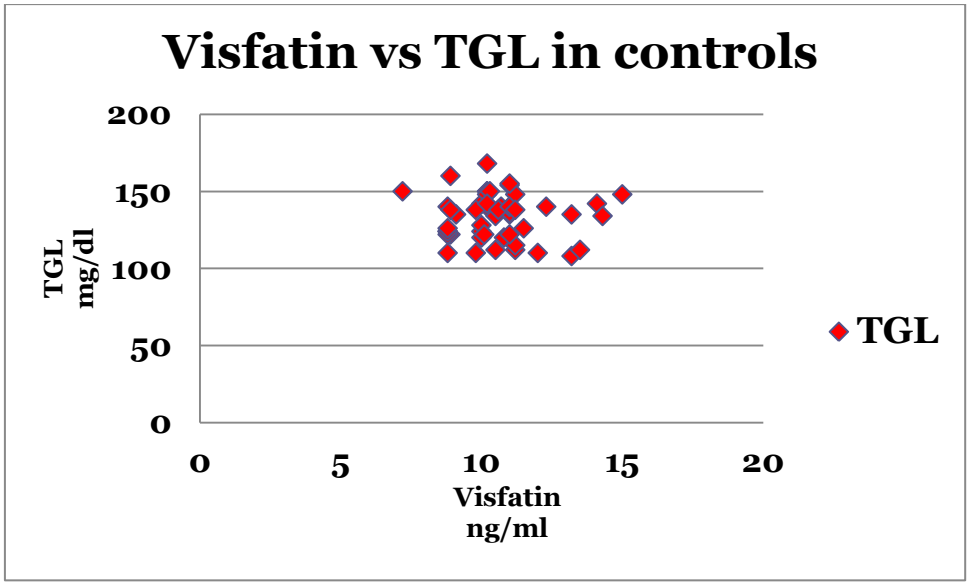


FIGURE 14

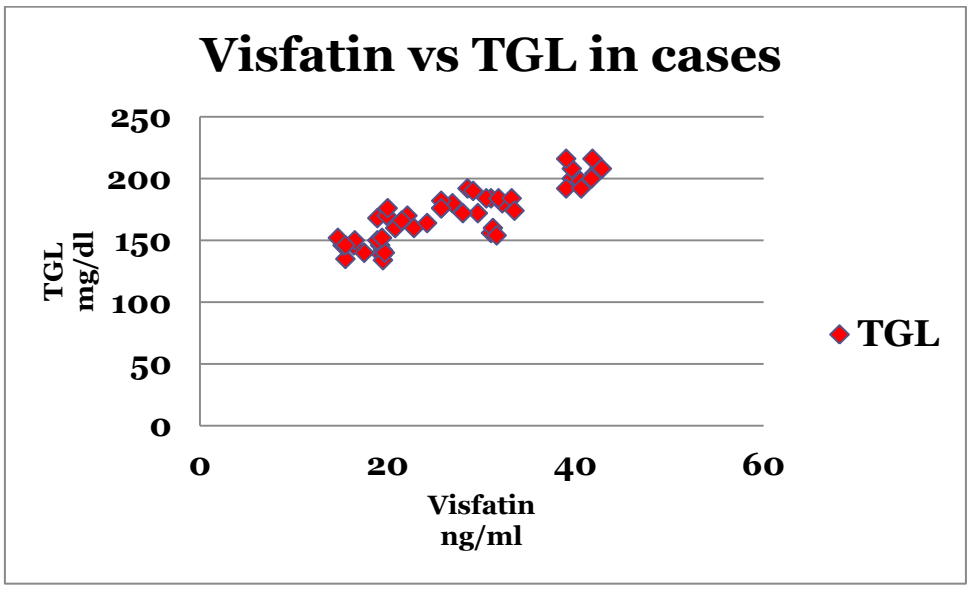


FIGURE 15

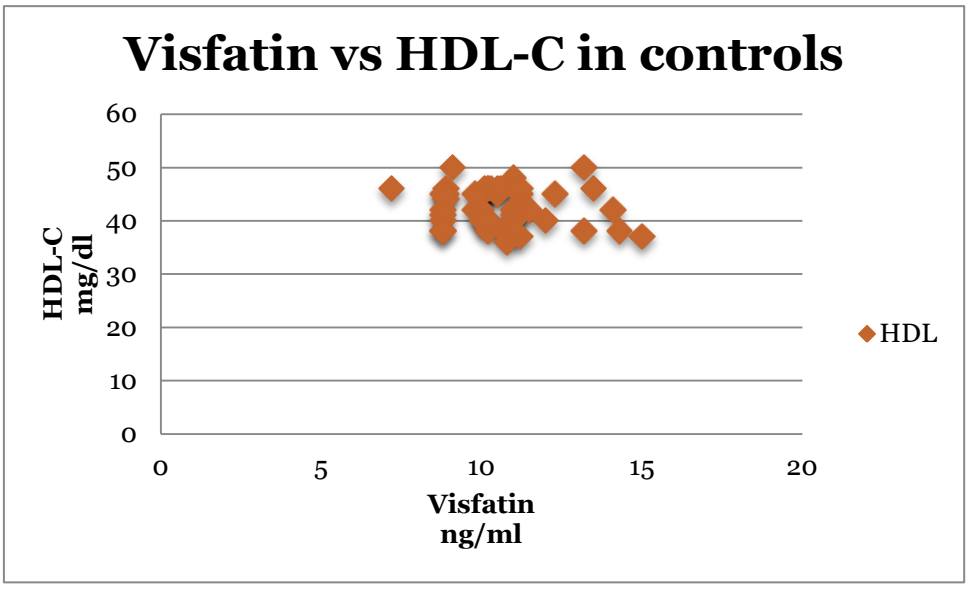
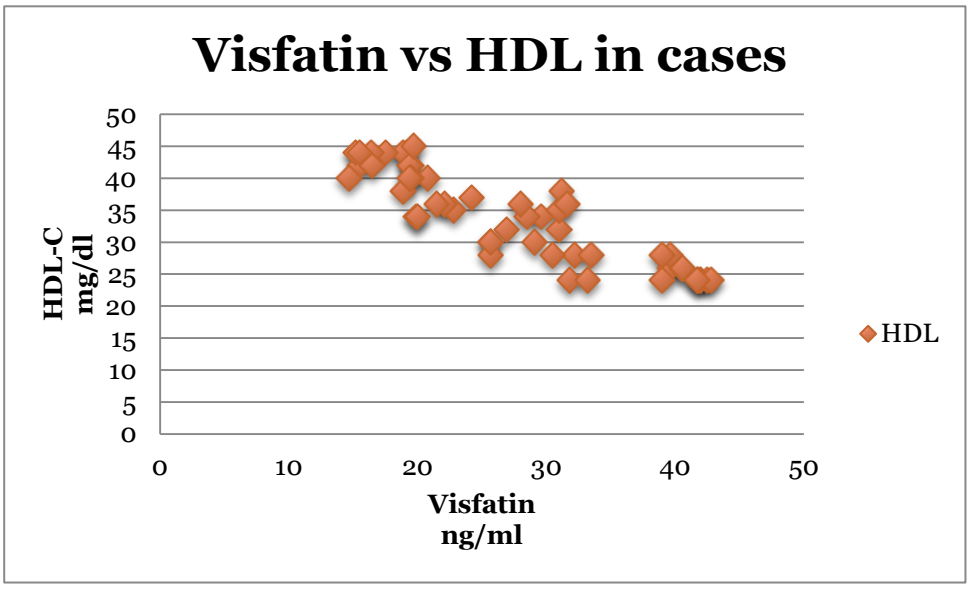


FIGURE 16



DISCUSSION

In the present study serum Visfatin concentrations were found to be significantly increased in patients with CKD (mean 27.42 ± 8.92) when compared to the control group (mean 10.62 ± 1.57).

When patients in different stages of CKD were compared, serum Visfatin levels were found to be progressively increased from stage 2 to stage 5 in comparison with the control group. This observation shows that increase in serum Visfatin develops relatively in the early stages of CKD, and is further increased with the progression of renal dysfunction and inversely correlated with Creatinine clearance ($r = -0.898$, $p < 0.01$ significant).

We also observed that serum Visfatin levels were significantly higher in all age groups and in both genders when compared to controls which indicates that age and gender does not have an impact on serum Visfatin levels. These findings are in accordance with the study of *Tang et al* which reported an increase in serum Visfatin levels in all stages of CKD[69].

CKD is a state of chronic persistent low grade subclinical inflammation in which there is a chronic systemic elevation of pro-inflammatory mediators and cytokines released from adipose tissue. Kidney plays an important role in the excretion of adipokines. The decreased renal function in CKD patients leads to altered handling of these adipokines causing its accumulation in the body. Hence serum Visfatin level is increased in CKD patients. Visfatin plays an

important role in innate immunity. It is secreted by activated lymphocytes, monocytes and neutrophils and stimulates IL-6 secretion via P_{38} Mitogen Activated Protein Kinase (MAPK) and MAPK Kinase 1 (MEK1) pathways. Visfatin also induces the expression of inflammatory mediators in human endothelial cells through the NF-κB pathway. Visfatin also stimulates the synthesis of CRP which is a positive acute phase reactant through IL-6. The prototypic biomarker of inflammation in the clinical setting is hsCRP and its higher level is associated with cardiovascular risk in CKD patients. Hence Visfatin could play an indirect role in CVD in CKD patients.

In the present study we observed a significantly higher levels of serum hsCRP in CKD cases when compared to controls [mean value: cases 4.5432 ± 2.9689; controls 0.5360 ± 0.2343, Pvalue < 0.05].As the renal function declined, we observed a progressive increase in the serum hsCRP levels. Further a strong positive correlation was found between serum Visfatin and hsCRP levels ($r=0.746$, $p<0.01$) which shows that increased serum Visfatin stimulates the synthesis of CRP from liver. These results of the present study are in accordance with that of the previous studies suggesting a global pro-atherogenic inflammatory activation occurs even in early stages of CKD leading onto upregulation of serum Visfatin.

Dyslipidemia, an atherogenic risk factor contributes to the initiation and progression of CKD partly by stimulating and amplifying the effect of

inflammatory mechanisms. In the present study we observed a significantly higher serum TGL and VLDL-C in cases than controls ($p < 0.05$). There is no significant difference of TC and LDL between cases and controls. Furthermore we observed a significant positive correlation of serum Visfatin with TGL ($r = 0.877$, $p<0.01$) which is an independent strong predictor of cardio vascular events. We also observed a strong negative correlation of serum Visfatin with the HDL-C ($r = - 0.899$, $p<0.01$) which is considered as an independent strong inverse predictor of cardio vascular events. Inflammation and dyslipidemia are well known risk factors of atherosclerosis. Visfatin plays a role in linking inflammation and lipid dysregulation to atherosclerosis.

Previous studies have been demonstrated that increased Visfatin could be considered as a marker of endothetial dysfunction to predict the incidence of cardiovascular disease in CKD[71]. *Kim et al* observed the effect of visfatin on vascular endothelium. It induces the inflammatory mediators in endothetial cells through NF-KB pathway. Visfatin belongs to middle molecule uremic retension substance family which induces the leucocyte adhesion to endothelial cells and aortic endothelium by induction of cell adhesion molecules such as ICAM -1 (Intra Cellular Adhesion Molecule -1) and VCAM-1 (Vascular Cell Adhesion Molecule -1). Furthermore Visfatin enhances the production of reactive oxygen species through NADPH dependent pathway which accelerates vascular diseases by causing endothelial dysfunction. Accumulated Visfatin in CKD

patients may directly affect the endothelium to cause endothelial dysfunction. In addition to that Visfatin enhances the vascular smooth muscle cell proliferation and maturation. Taken together Visfatin may be considered as a surrogate marker of endothelial dysfunction in CKD patients[72]. This is supported by *Axelsson et al* who observed a positive association between Visfatin and endothelial cell adhesion molecule, which is a marker of endothelial dysfunction. This finding is further supported by *Yilmaz et al* who observed improvement in endothelial dysfunction by assessing flow mediated vasodilatation of the brachial artery during the first month after Renal Transplantation and the degree of improvement was correlated with the decreasing Visfatin concentration in blood[73].

Taken together the results of the present study suggest that serum Visfatin is a novel marker of endothelial dysfunction in CKD patients. Both intra and extra cellular Visfatin act as regulator of vascular function. As an intracellular form it extents the life span of vascular smooth muscle cells by augmenting SIRT-1 mediated p53 degradation. As an extracellular isoform, working in NAD dependent fashion ,it enhances the vascular smooth muscle cell proliferation and maturation leading on to atherosclerotic changes. It also suggests that higher the level of Visfatin the higher the degree of severity of CKD. The higher level of Visfatin in CKD is due to either chronic inflammation which is associated with this disease (or) hypoxia as a result of tubulonecrosis,

anemia and decreased capillary flow. Elevated Visfatin is also associated with increased mortality in CKD patients. Hence serum Visfatin could be considered as a novel marker of cardiovascular disease in CKD patients to predict Premature Atherosclerosis and Death.

CONCLUSION

- The present study demonstrated that serum Visfatin levels are significantly increased in patients with CKD. This increase in serum Visfatin level is progressive from the early stages to the late stages of CKD .
- The chronic inflammatory state and hypoxia due to tubulonecrosis, anemia and decreased capillary flow could be responsible for the increased serum Visfatin level in CKD patients.
- Visfatin causes endothelial dysfunction either directly or indirectly suggesting the possible role in the development of coronary atherosclerosis.
- Visfatin may be considered as the novel marker of mortality predictor in CKD patients. Higher the visfatin level, higher the mortality of CKD patients.

LIMITATIONS OF THE STUDY

- Application of imaging techniques would have helped us to evaluate flow mediated dilatation for endothelial dysfunction and intimal thickness of Carotid artery for atherosclerosis.
- Estimation of other biochemical parameters of endothelial dysfunction like Endothelin -1, Thrombomodulin, NO and E-selectin would have helped us to assess the degree of endothelial dysfunction & atherosclerosis.

SCOPE FOR FUTURE STUDY

Inflammation and endothelial dysfunction which are common pathological events in Chronic Kidney Disease to develop cardiovascular decrease. Hence blocking this novel inflammatory adipokine may be helpful in preventing (or) at least delaying the progression of such complications in CKD.

FK 866 or APO 866 is an inhibitor of Visfatin , which is currently used as an anticancer drug. Novel therapeutic approaches targeting Visfatin is the goal of future research.

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PROFORMA

SERUM VISFATIN- A NOVEL MARKER IN CHRONIC KIDNEY DISEASE

NAME OF THE PATIENT :

AGE/SEX :

OCCUPATION :

ADDRESS :

COMPLAINTS :Oliguria/Nocturia/Dysuria/Pedaledema/anasarca/ Fever/ Weight loss

PAST HISTORY :HT/DM/Tuberculosis/Cerebrovascular disease/liver disease/Rheumatoid arthritis

PERSONAL HISTORY :Diet/Tobacco/Alcohol/Cigarette use

FAMILY HISTORY :HT/DM/Renal disease

DRUG HISTORY : Drugs (lipid lowering drugs, calcium/phosphate binders)/renal replacement therapy

GENERAL EXAMINATION:

Ht: Wt: BMI: BP: PR:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS :

1.BLOOD SUGAR(FASTING):

2.BLOOD UREA :

3.SERUM CREATININE:

4.CREATININE CLEARANCE:

5.SERUM VISFATIN:

6.SERUM hsCRP:

7.LIPID PROFILE:

TOTAL CHOLESTEROL:

TRIGLYCERIDES:

HDL:

LDL:

VLDL:

CONSENT FORM

Dr.S.SYED ALI FATHIMA, post graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing a Study On Serum Visfatin-a novel marker in chronic kidney disease. The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature :

Name:

Place: