"SERUM OSTEOPROTEGERIN - CAN IT PREDICT CHRONIC KIDNEY DISEASE AMONG HYPERTENSIVES?"

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TAMILNADU

MAY 2021

BONAFIDE CERTIFICATE

This is to certify that this dissertation titled **"SERUM OSTEOPROTEGERIN - CAN IT PREDICT CHRONIC KIDNEY DISEASE AMONG HYPERTENSIVES?"** is the original bonafide work done by **Dr.C.JANANI,** Post Graduate Student, Department of Biochemistry, Government Mohan Kumaramangalam Medical College, Salem during her post graduate course 2018 to 2021, under our direct supervision and guidance.

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DECLARATION

I, **Dr.C.JANANI**, Post Graduate, Department of Biochemistry, Government Mohan Kumaramangalam Medical College, solemnly declare that the dissertation titled "**SERUM OSTEOPROTEGERIN - CAN IT PREDICT CHRONIC KIDNEY DISEASE AMONG HYPERTENSIVES?"** was done by me at Department of Biochemistry, Government Mohan Kumaramangalam Medical College and Hospital under the expert guidance and supervision of my Professor and Head of the Department, **Dr. P. JOSEPHINE LATHA, M.D.** The dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for **M.D. Degree** in **BIOCHEMISTRY (BRANCH -XIII)**.

Place : Salem

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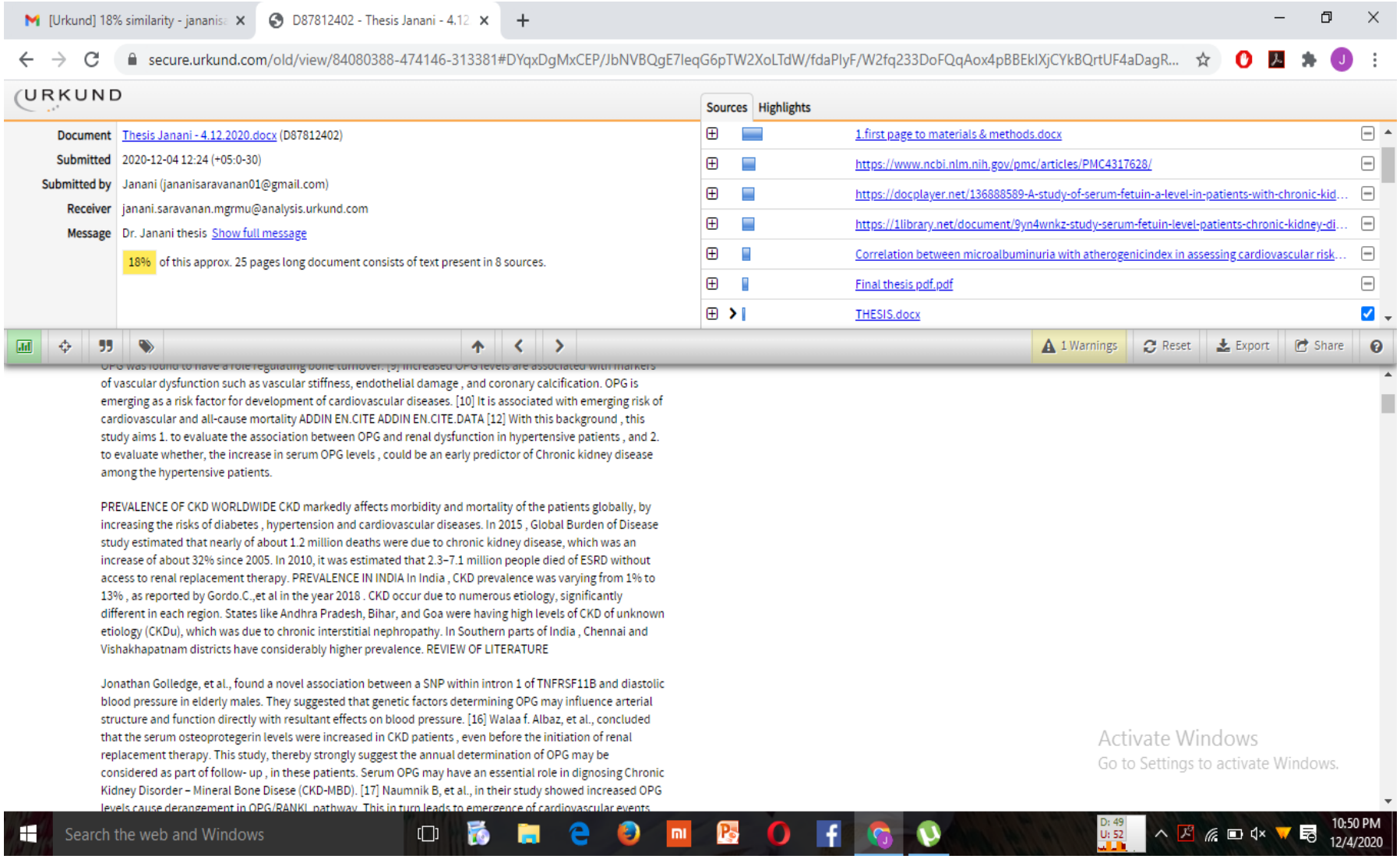

of vascular dysfunction such as vascular stiffness, endothelial damage , and coronary calcification. OPG is emerging as a risk factor for development of cardiovascular diseases. [10] It is associated with emerging risk of cardiovascular and all-cause mortality ADDIN EN.CITE ADDIN EN.CITE.DATA [12] With this background , this study aims 1. to evaluate the association between OPG and renal dysfunction in hypertensive patients , and 2. to evaluate whether, the increase in serum OPG levels , could be an early predictor of Chronic kidney disease among the hypertensive patients.

PREVALENCE OF CKD WORLDWIDE CKD markedly affects morbidity and mortality of the patients globally, by increasing the risks of diabetes , hypertension and cardiovascular diseases. In 2015 , Global Burden of Disease study estimated that nearly of about 1.2 million deaths were due to chronic kidney disease, which was an increase of about 32% since 2005. In 2010, it was estimated that 2.3–7.1 million people died of ESRD without access to renal replacement therapy. PREVALENCE IN INDIA In India , CKD prevalence was varying from 1% to 13% , as reported by Gordo.C.,et al in the year 2018 . CKD occur due to numerous etiology, significantly different in each region. States like Andhra Pradesh, Bihar, and Goa were having high levels of CKD of unknown etiology (CKDu), which was due to chronic interstitial nephropathy. In Southern parts of India , Chennai and Vishakhapatnam districts have considerably higher prevalence. REVIEW OF LITERATURE

Jonathan Golledge, et al., found a novel association between a SNP within intron 1 of TNFRSF11B and diastolic blood pressure in elderly males. They suggested that genetic factors determining OPG may influence arterial structure and function directly with resultant effects on blood pressure. [16] Walaa f. Albaz, et al., concluded that the serum osteoprotegerin levels were increased in CKD patients , even before the initiation of renal replacement therapy. This study, thereby strongly suggest the annual determination of OPG may be considered as part of follow- up , in these patients. Serum OPG may have an essential role in dignosing Chronic Kidney Disorder – Mineral Bone Disese (CKD-MBD). [17] Naumnik B, et al., in their study showed increased OPG levels cause derangement in OPG/RANKL pathway. This in turn leads to emergence of cardiovascular events

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SERUM OSTEOPROTEGERIN - CAN IT PREDICT CHRONIC KIDNEY DISEASE AMONG HYPERTENSIVES?

CONTENTS

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ABBREVIATION

ADMA	Asymmetric dimethylarginine
AER	Albumin Excretion Rate
BMD	Bone Mineral Density
CHF	Congestive Heart Failure
CKD	Chronic Kidney Disease
CKD-MBD	Chronic Kidney Disease-Mineral and Bone Disorder
CVD	Cardiovascular disease
CysC	Cystatin C
eGFR	Estimated Glomerular Filtration Rate
ECFV	Extracellular fluid Volume
EPO	Erythropoietin
ELISA	Enzyme Linked Immuno Sorbent Assay
ESRD	End Stage Renal Disease
FGF- 23	Fibroblast Growth Factor - 23
GLDH	Glutamate Dehydrogenase
GOD-POD	Glucose oxidase peroxidase
HDL	High Density Lipoprotein

KDIGO	Kidney Disease Improving Global Outcome
LDL	Low Density Lipoprotein
MCP-1	Monocyte Chemoattractant Protein - 1
NGAL	Neutrophil gelatinase – associated lipocalin
OPG	Osteoprotegerin
PAD	Peripheral Arterial Diseases
ROC Curve	Receiver Operating Characteristic Curve
SNP	Single Nucleotide Polymorphism
TNF	Tumor Necrosis Factor
TRAIL	TNF-related apoptosis-inducing ligand
TWEAK	TNF-like weaker inducer of apoptosis
uRBP4	Urine Retinol Binding Protein 4

INTRODUCTION

"SERUM OSTEOPROTEGERIN- Can it predict Chronic Kidney Disease among hypertensives?"

INTRODUCTION

Hypertension has been one of the major factors responsible for decline in renal function in patients with diabetic and non diabetic kidney disease. On the other hand, in patients with chronic kidney disease,[1] high blood pressure may develop at an early course of the disease and contribute to several adverse outcomes.[2] Thus, hypertension can be a cause or a consequence of CKD.[3] Blood pressure control is an integral component in the care of CKD patients, and is relevant at all stages of the disease, irrespective of the underlying cause.[4]

Chronic kidney disease [5] is the global health burden with a high economic cost to health systems. It is an independent risk factor for cardiovascular disease (CVD). All stages of CKD are associated with increased risks of cardiovascular complications, premature mortality and/or decreased quality of life. CKD is now recognised as having changed from a subspeciality issue to a global health concern, that demonstrates a need for national initiatives that will slow the progression to end stage renal disease and reduce CVD-related events in CKD patients.[4]

Chronic Kidney Disease is defined as damage to renal tissue or eGFR less than 60 ml/min/1.73 m^2 for minimum of 3 months duration. Renal damage is evidenced by pathological abnormalities or by markers of damage, such as abnormalities in blood or urine tests or imaging studies.[6] The most common causes of the CKD in India are diabetes and hypertension.

CKD has been classified into five stages, based on estimated GFR.[7]

Stages	eGFR (in ml/min/1.73 m^2)
Stage I	>90
Stage II	60–89
Stage IIIA	45–59
Stage IIIB	30–44
Stage IV	16-29
Stage V	< 15

Serum creatinine (sCr) is an important tool in assessing the renal function by estimated GFR. Though eGFR decreases at the earlier stages, creatinine concentration remains within the reference values. Mild renal insufficiency is therefore not sensitively detected by serum creatinine levels. This may prolong the diagnosis and management of renal disease. Hence, the detection of renal dysfunction at the initial stages is highly essential.[5]

Numerous biomarkers are now being explored in diagnosis, grading, and prognosis of renal disorders.

Osteoprotegerin (OPG), a cytokine decoy receptor of TNF(Tumour necrosis factor) family, is a glycoprotein molecule. OPG inhibits bone resorption, by antagonizing receptor activator for nuclear factor kB ligand (RANKL) and TNF-related apoptosis-inducing ligand.[8] OPG was found to have a role regulating bone turnover. [9]

Increased OPG levels are associated with markers of vascular dysfunction such as vascular stiffness, endothelial damage and coronary calcification. OPG is emerging as a risk factor for development of cardiovascular diseases.[10] It is associated with emerging risk of cardiovascular and all-cause mortality.[11] [12]

With this background, this study aims

1. to evaluate the association between OPG and renal dysfunction in hypertensive patients, and
2. to evaluate whether the increase in serum OPG levels, could be an early predictor of Chronic kidney disease among the hypertensive patients.

PREVALENCE OF CKD WORLDWIDE

CKD markedly affects morbidity and mortality of the patients globally [13] by increasing the risks of diabetes, hypertension and cardiovascular diseases. In 2015, Global Burden of Disease [14] study estimated that nearly of about 1.2 million deaths were due to chronic kidney disease, which was an increase of about 32% since 2005. In 2010, it was estimated that 2.3–7.1 million people died of ESRD without access to renal replacement therapy.[15]

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Naumnik B, et al., in their study showed increased OPG levels cause derangement in OPG/RANKL pathway. This in turn leads to emergence of cardiovascular events due to calcifications and sclerosis of blood vessels. Higher OPG levels in women on hemodialysis comparing to age matched HD men indicates the need for screening the presence of bone-mineral disorders and CVD in older female population.[18]

L.L. Yin et al., observed that the polymorphisms of A163G and G1181C loci on the OPG gene were correlated with the BMD of patients receiving hemodialysis. The genotype AA of A163G and the genotype CC of G1181C were identified as protective factors for BMD.[19]

Julia J. Scialla, et al., observed that levels of calcification biomarkers are associated with mortality in persons with ESRD strikingly in non diabetics wherein

elevated serum OPG were significantly involved in greater risk of cardiovascular morbidity and mortality.[20]

Chang Seong Kim,et al., found the association between serum OPG levels and bone loss in CKD patients, especially in females. Serum osteoprotegerin measurement might be a useful surrogate marker for determining bone loss in female patients with chronic kidney disease.[21]

My Svensson et al., in their study found an independent association of OPG with renal, cardiovascular complications and mortality in patients undergoing renal replacement therapy.[22]

Pinar Demir**,** et al., in their study found elevated serum OPG levels among dialysis patients, when compared to pre-dialysis, renal transplant and control groups. Serum levels of OPG were grossly reduced after renal transplant in patients with uremia. This favours the decline of previously elevated serum OPG levels after renal replacement therapy. Vascular calcifications, frequently occurring in renal disease, is considered as risk factor for coronary artery disease. It has a predictive role in CV morbidity and mortality.[23]

Raelene E. et. al., in their study showed the various mechanisms involved in pathogenesis of vascular dysfunction in CKD. Most commonly, uremia, mineral metabolic derangements, increased cytokines, and calcium-phosphate flux due to dialysis have significant role. Various bone matrix proteins such as osteoprotegerin, osteopontin and RANKL are released from damaged renal tissues. They are recognised as key regulators in vascular pathology and risk markers of arteriosclerosis.[24]

Steubl et al. observed a positive association of OPG with eGFR, while creatinine and cystatin C showed hyperbolic correlation to eGFR. OPG was correlated significantly with proteinuria, which is a strong predictor of CKD progression.[25]

Junichiro J. Kazama, et.al., found that OPG increases in CKD. Osteoclastogenesis inhibitory factor (OCIF) accumulates in serum of patients with renal failure. It is associated with development of secondary hyperparathyroidism in later stages of renal dysfunction. This showed an increased interest in evaluating OPG, as a biomarker in CKD patients.[26]

Haas, M.,et. al., showed elevated levels of osteoprotegerin and parathyroid hormone in patients undergoing renal replacement therapy.[27]

Mohamed and colleagues found that serum OPG levels were significantly lower in nephrotic syndrome when compared with normal individuals. This might have been due to either steroid effect or protein loss which are typical for this condition.[28]

Lewis J.R et.al., found high levels of serum OPG in association with risk of kidney dysfunction, and mortality among elderly females.[29]

Gupta et al., showed an association between urinary OPG and renal dysfunction. Urinary levels of OPG was increased in patients showing poor response to treatment, disease relapse or CKD development.[30]

Kiani, A.N. et. al., have reported a correlation between urinary OPG with disease status and prognosis of patients with lupus nephritis. In addition to circulating OPG, urinary OPG has been a potential biomarker of interest in renal disorders.[31]

Bernardi, S., Toffoli, et.al., in their study demonstrated that there is a significant inverse relation between OPG and renal function. Oxidative and inflammatory changes in renal tissue are anticipated following OPG administration. This led to a hypothesis of OPG, being a risk marker as well as a risk factor for renal dysfunction.[13]

Montanez-Barragan, A.,et al., showed that increased circulating OPG in predialysis, dialysis and transplant patients, may predict progression of vascular calcification and patient survival. OPG could be considered as a biomarker for development and progression of CKD. For this reason, several ongoing clinical studies have included OPG measurement for monitoring CKD progression and its complications.[32]

From various studies, [33] [34] [35]it is found that serum creatinine would show an insensitive response in early stages of renal damage. Serum creatinine levels remained in the normal range, even after 50% decline in eGFR, which necessitates the evaluation for early biomarker for demonostrating the decline in renal function.

Thus, multitude of studies demonstrate the cardiovascular arteriosclerotic changes in long standing hypertensive patients, that leads to multi-organ dysfunction especially kidneys. Hence the need for establishing renal dysfunction in hypertensive patients may be warranted. This study aims to elucidate a biomarker that predicts renal damage earlier than serum creatinine i.e., role of serum osteoprotegerin predicting the renal dysfunction in hypertensive patients.

HYPERTENSION

HYPERTENSION

Hypertension has been one of the leading causes of the global burden of disease. Hypertension doubles the risk of cardiovascular diseases, including coronary heart disease,[36] congestive heart failure (CHF), ischemic and hemorrhagic stroke, renal failure and peripheral arterial disease. Although there is reduction of the risks of cardiovascular and renal disease by antihypertensive therapy, large segments of the hypertensive population are either untreated or inadequately treated.

Clinically, hypertension is defined as that level of blood pressure at which the initiation of therapy reduces blood pressure–related morbidity and mortality.

EPIDEMIOLOGY

Blood pressure levels, the rate of age-related increases in blood pressure and the prevalence of hypertension may vary among countries and among subpopulations within a country. The likelihood of hypertension increases with age, and among individuals age ≥60 years, the prevalence is 65.4%.

Both environmental and genetic factors may contribute to regional and racial variations in hypertension prevalence.

ENVIRONMENTAL FACTORS

1. Obesity and weight gain (60% of hypertensives are >20% overweight)
2. Dietary NaCl intake (age-related increase in BP)
3. Low dietary intakes of calcium and potassium
4. Alcohol consumption
5. Psychosocial stress
6. Low levels of physical activity

GENETIC FACTORS

Hypertension has been a polygenic disorder wherein, a combination of genes acts with environmental exposures and makes a modest contribution to blood pressure. Genes that encode components of the renin-angiotensin-aldosterone system, along with angiotensinogen and angiotensin-converting enzyme [37] polymorphisms, may be related to hypertension and to blood pressure sensitivity to dietary NaCl. The alpha-adducin gene is associated with increased renal tubular absorption of sodium.

MECHANISMS OF HYPERTENSION

Cardiac output and peripheral resistance are the two determinants of arterial pressure. Cardiac output is determined by stroke volume and heart rate; stroke volume is related to myocardial contractility and to the size of the vascular compartment. Peripheral resistance is determined by functional and anatomic changes in small arteries (lumen diameter 100–400 μm) and arterioles.

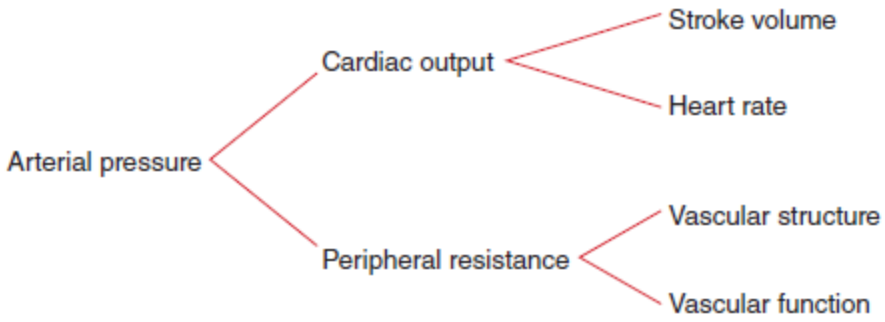


Figure no 1 : Determinants of hypertension

Integrated, Multifaceted System for Arterial Pressure Regulation

The arterial pressure is regulated by several interrelated systems, each of which performs a specific function. Regulatory systems are mainly concerned to return the arterial pressure immediately to the level that the person can get through the acute

episode and to return the blood volume eventually to its normal level so that the circulatory system can re-establish full normality.

Mechanisms can be divided into three groups:

- those react rapidly (within seconds or minutes)
- those respond over an intermediate time period(minutes or hours) and
- those provide long-term arterial pressure regulation (days, months, and years)

Rapidly Acting Pressure Control Mechanisms, Acting Within Seconds or Minutes.

They are (1) the baroreceptor feedback mechanism,

(2) the central nervous system ischemic mechanism and

(3) the chemoreceptor mechanism.[38]

Pressure Control Mechanisms That Act After Many Minutes.

(1) the renin-angiotensin vasoconstrictor mechanism,

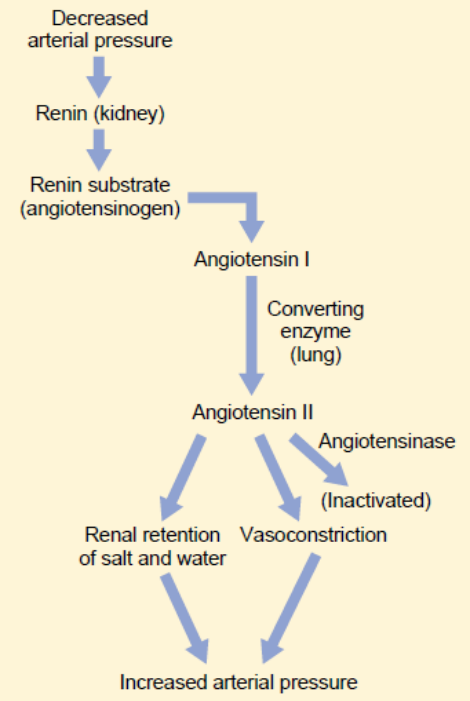


Figure no 2 : Renin angiotensin mechanism of Hypertension control

(2) stress-relaxation of the vasculature, and

(3) shift of fluid through the tissue capillary walls in and out of the circulation to re-adjust the blood volume as needed.[38]

Role of angiotensin II in renal pathology

Angiotensin II is a cytokine with many effects on the kidney, clearly beyond its classical function as a hemodynamic mediator.

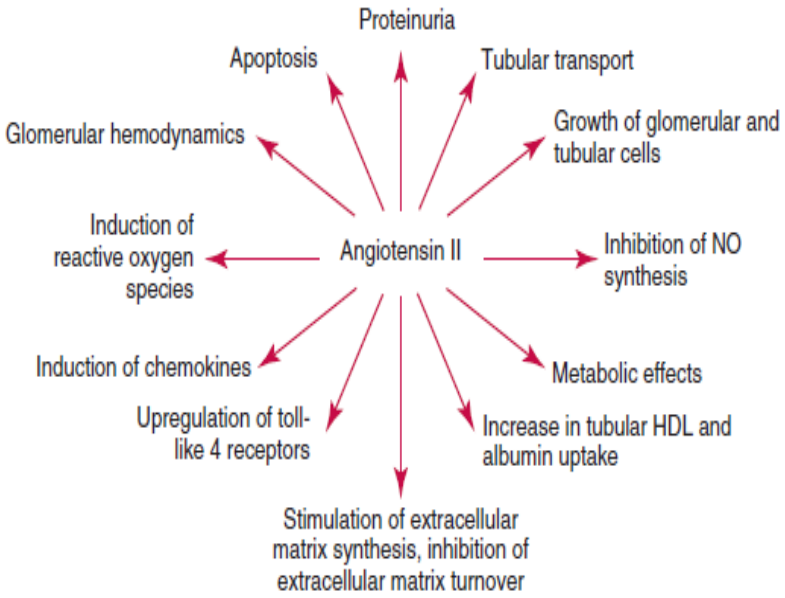


Figure no 3: Role of Angiotensin II

Long-Term Mechanisms for Arterial Pressure Regulation

Kidneys play a dominant role in long-term regulation of arterial pressure and hypertension. Sequential steps by which increased extracellular fluid volume increases the arterial pressure is illustrated in (fig no 4). Increased cardiac output has both a *direct effect* to increase arterial pressure and an *indirect effect* by first increasing the total peripheral resistance.

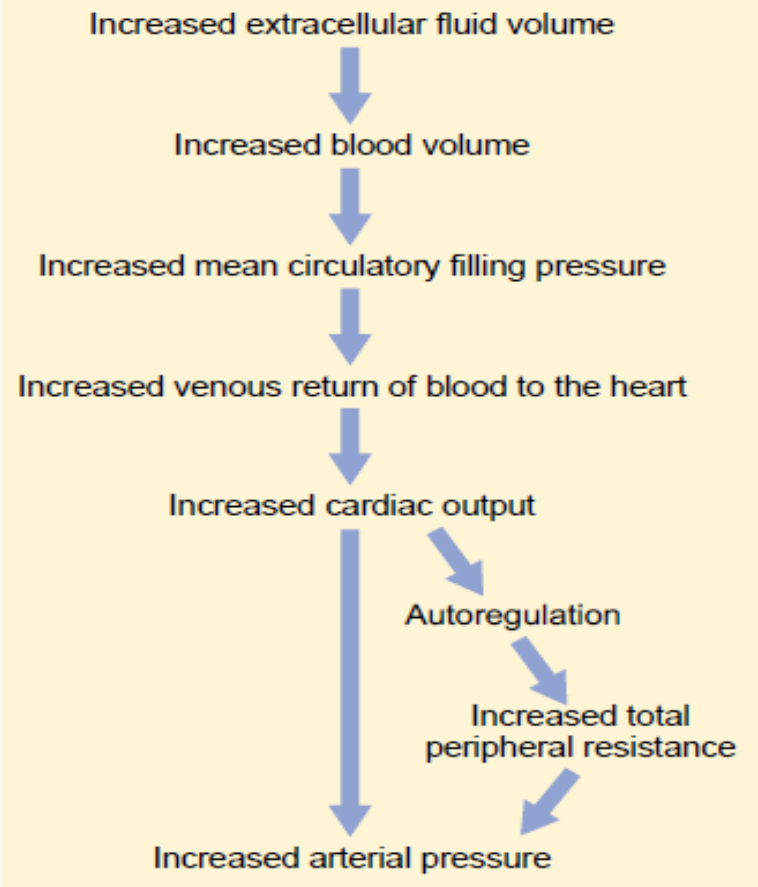


Figure no 4 : The Integrated System for Pressure Control

PATHOLOGIC CONSEQUENCES OF HYPERTENSION

Hypertension is an independent predisposing factor for heart failure, coronary artery disease, stroke, renal disease, and peripheral arterial disease (PAD).

HEART

Heart disease is the most common cause of death in hypertensive patients. Hypertensive heart disease is the result of structural and functional adaptations leading to left ventricular hypertrophy, CHF, abnormalities of blood flow due to atherosclerotic coronary artery disease and microvascular disease and cardiac arrhythmias.

BRAIN

Stroke is the second most frequent cause of death in the world; it accounts for 5 million deaths each year. Elevated blood pressure is the strongest risk factor for stroke. The incidence of stroke rises progressively with increasing blood pressure levels, particularly systolic blood pressure in individuals >65 years. Treatment of hypertension decreases the incidence of both ischemic and hemorrhagic strokes.

PERIPHERAL ARTERIES

Hypertensive patients with arterial disease of the lower extremities are at increased risk for future cardiovascular disease. The ankle-brachial index is a useful approach for evaluating PAD and is defined as the ratio of non-invasively assessed ankle to brachial (arm) systolic blood pressure. An ankle-brachial index <0.90 is considered diagnostic of PAD and is associated with >50% stenosis in at least one major lower limb vessel. An ankle-brachial index <0.80 is associated with elevated blood pressure, particularly systolic blood pressure.

KIDNEY

The kidney is both a target and a cause of hypertension. Primary renal disease is the most common etiology of secondary hypertension. Mechanisms of kidney-related hypertension include a diminished capacity to excrete sodium, excessive renin secretion in relation to volume status and sympathetic nervous system overactivity. Conversely, hypertension is a risk factor for renal injury and ESRD. The increased risk associated with high blood pressure is graded, continuous and present throughout the distribution of blood pressure above optimal pressure.

Renal risk is more closely related to systolic than to diastolic blood pressure. Atherosclerotic, hypertension-related vascular lesions in the kidney primarily affect preglomerular arterioles, resulting in ischemic changes in the glomeruli and postglomerular structures. Glomerular injury may be a consequence of direct damage to the glomerular capillaries due to glomerular hyperperfusion.

Studies related to hypertension induced renal damage, suggest that loss of autoregulation of renal blood flow at the afferent arterioles results in transmission of elevated pressures to an unprotected glomerulus with ensuing hyperfiltration, hypertrophy, and results in focal segmental glomerular sclerosis. With progressive renal injury, autoregulation of renal blood flow and glomerular filtration rate is lost which results in a lowering of blood pressure threshold for renal damage. This results in a vicious cycle of renal damage and nephron loss leading to more severe hypertension, glomerular hyperfiltration and further renal damage.

Glomerular pathology progresses to glomerulosclerosis, and eventually the renal tubules may also become ischemic and gradually atrophic. The renal lesion associated with malignant hypertension consists of fibrinoid necrosis of the afferent arterioles, sometimes extending into the glomerulus, and may result in focal necrosis of the glomerular tuft.

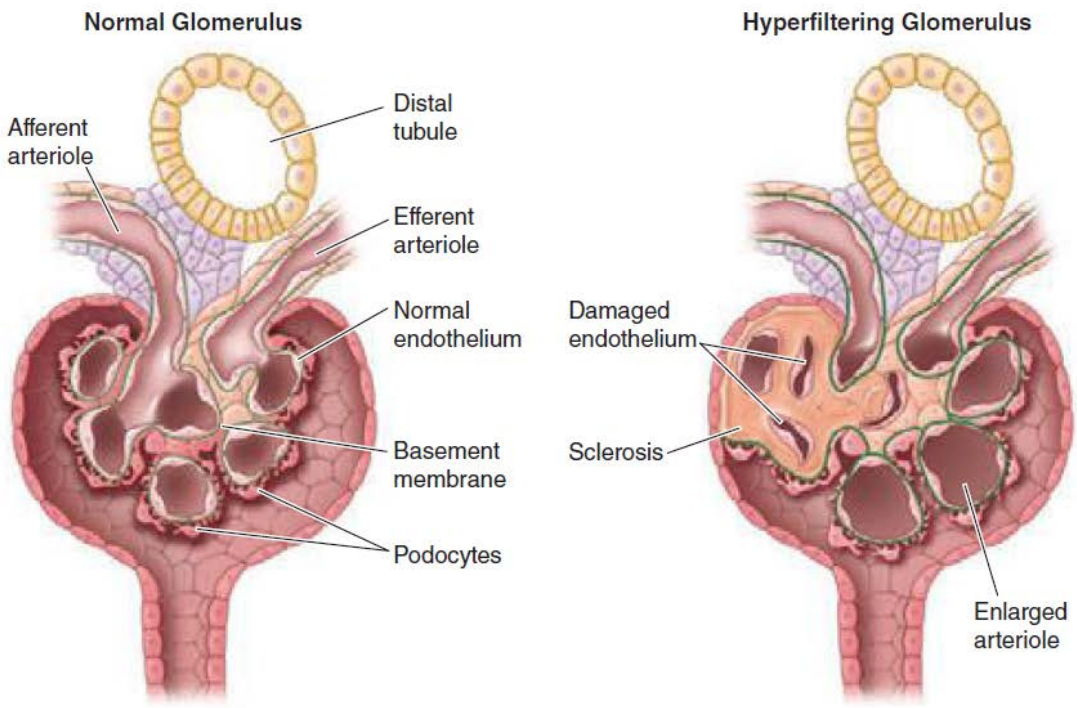


Figure no 5 : Normal and hyperfiltrating glomerulus

Left: Schema of the normal glomerulus *Right:* Secondary glomerular changes in hypertension

CHRONIC KIDNEY DISEASE

CHRONIC KIDNEY DISEASE

In the 2012 KDIGO system, CKD is *defined* as structural and functional abnormalities of renal tissue, for minimum of 3 months duration, with health implications.[14]

Leading Categories of Etiologies of CKD

- Diabetic nephropathy
- Glomerulonephritis
- Hypertension
- Cystic and tubulointerstitial nephropathy
- Autosomal dominant polycystic kidney disease

All the above said causes, accounts for greater than 90% of the CKD disease burden worldwide.

The commonest cause for CKD, is type 2 diabetes mellitus, leading to nephropathy. Patients with newly diagnosed CKD may also present with hypertension.

The increasing incidence of CKD in the elderly has been ascribed, in part, to decreased mortality rate from the cardiac and cerebral complications of atherosclerotic vascular disease, enabling a greater segment of the population to eventually manifest the renal component of generalized vascular disease. The vast majority of such patients with early stages of CKD will progress to cardiovascular and cerebrovascular consequences of the vascular disease before they can progress to the most advanced stages of CKD. Even a minor decrement in GFR or the presence of albuminuria is now recognized as a major risk factor for cardiovascular disease.

PATHOPHYSIOLOGY AND BIOCHEMISTRY OF UREMIA

Although serum urea and creatinine concentrations are used to measure the excretory capacity of the kidneys, accumulation of these two molecules themselves does not account for the many symptoms and signs that characterize the uremic syndrome in advanced renal failure. Hundreds of toxins that accumulate in renal failure have been implicated in the uremic syndrome. These include water-soluble, hydrophobic, protein-bound, charged and uncharged compounds. Additional categories of nitrogenous excretory products include guanidino compounds, urates and hippurates, products of nucleic acid metabolism, polyamines, myoinositol, phenols, benzoates and indoles. Serum concentrations of urea and creatinine are readily measured and represents the uremic state. Monitoring the levels of urea and creatinine in patients with impaired kidney function is currently an incomplete renal evaluation.

The uremic syndrome and the disease state associated with advanced renal impairment involve more than renal excretory failure. Various metabolic and endocrine functions are impaired or suppressed, which results in anemia, malnutrition, and abnormal metabolism of carbohydrates, fats and proteins. Plasma levels of many hormones, including PTH, FGF- 23, insulin, glucagon, steroid hormones including vitamin D and sex hormones, and prolactin, change with CKD as a result of reduced excretion, decreased degradation or abnormal regulation.

Finally, CKD is associated with worsening systemic inflammation. Elevated levels of C-reactive protein are detected along with other acute-phase reactants, whereas levels of negative acute-phase reactants, such as albumin and fetuin, decline with progressive reduction in GFR. Thus, the inflammation associated with CKD is

important in the *malnutrition-inflammation-atherosclerosis/calcification syndrome*, which contributes to the acceleration of vascular disease and comorbidity associated with advanced kidney disease.

The pathophysiology of the uremic syndrome can be divided into manifestations in three spheres of dysfunction:

1. Those consequent to the accumulation of toxins that normally undergo renal excretion, including products of protein metabolism;
2. Those consequent to the loss of other kidney functions, such as fluid and electrolyte homeostasis and hormone regulation; and
3. Progressive systemic inflammation and its vascular and nutritional consequences.

COMPLICATIONS OF UREMIA IN CKD

Uremia affects the functioning of each and every organ system.

FLUID, ELECTROLYTE, AND ACID-BASE DISORDERS

A) Sodium and Water Homeostasis

In initial stages of CKD, the sodium and water content of the body is elevated mildly, that is not evident clinically. Reabsorption of sodium and water at renal tubules is maintained at early stages, to balance sodium intake with the urinary excretion. This balance is disrupted by renal disorders like glomerulonephritis . As a result, dietary intake of sodium exceeds urinary excretion, causing retention of sodium and increase in extracellular fluid volume (ECFV). This leads to hypertension, which may accelerate the injury to nephron.

B) Potassium Homeostasis

CKD can be associated with accelerated and more severe disruption to potassium-secretion at the distal renal tubules, with decrease in GFR. It is due to the conditions causing hyporeninemic hypoaldosteronism.

C) Acid base disturbance

Metabolic acidosis is the commonest manifestation in advanced CKD. The combined presentation of hyperkalemia and hyperchloremia is present at the initial stages of CKD. With the declining renal function, the total acid excretion in urine is restricted to 30–45 mmol/day. An anion-gap metabolic acidosis develops from non-anion-gap metabolic acidosis with the accumulation of organic acids.

DISORDERS OF CALCIUM AND PHOSPHATE METABOLISM

Derangements of calcium and phosphate metabolism in CKD occur in the skeleton and the vascular bed, with the involvement of extra-osseous soft tissues at later stages.

A) Skeletal Manifestations of CKD

The major bone disorders in CKD are of two types

- high bone turnover with raised PTH levels
- low bone turnover with normal or low PTH levels

Sequential events in CKD leading to secondary hyperparathyroidism are as follows :[38]

Decrease in eGFR in kidney disese

↓

reduced excretion of phosphate

↓

retained phosphate stimulates osteocytes

↓

increased synthesis of FGF-23

↙ ↘

suppression of calcitriol production stimulate PTH production

↓

Low calcitriol levels

↓

Hyperparathyroidism

These changes are evident when the GFR falls below to 60 mL/min.

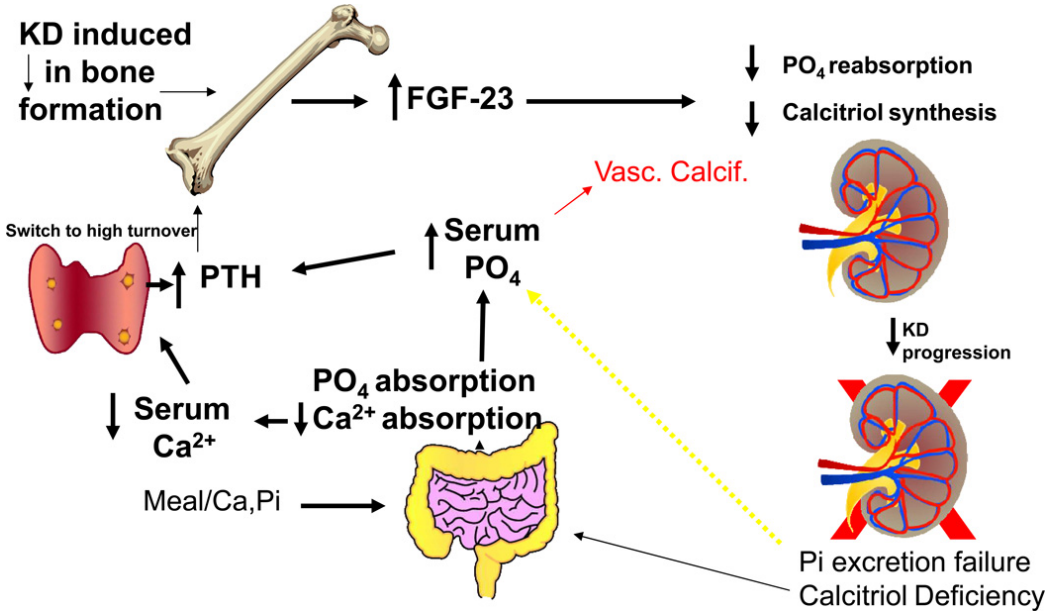


Figure no 6 : secondary changes due to hyperparathyroidism in CKD

B) Calcium and Phosphorus metabolism in CKD

Hyperphosphatemia and hypercalcemia are related to increased vascular calcification. The vascular calcification increases with advancing age and high phosphate levels. It is associated with decrease in PTH levels and low bone turnover. In advanced CKD, calcium ingested gets deposited at extraosseous sites like soft tissues and vascular bed. High levels of phosphate leads to vascular calcification and ossification, inducing changes in genetic expression of vascular cells.

Calciphylaxis or calcific uremic arteriolopathy is seen in ESRD. Pathologically, vascular occlusion is well evidenced in association with severe vascular and soft tissue calcification. More common in dialysis patients.

CARDIOVASCULAR COMPLICATIONS

At all stages of CKD, Cardiovascular disorders are the common cause for morbidity and mortality.

A) Ischemic Vascular Disease

CKD is the major risk factor for the development of ischemia in heart, brain and peripheral vasculature . The prevalence of vascular disease in CKD are increased due to hypertension, hypervolemia, increased sympathetic activity, lipid disorders, hyperhomocysteinemia, anemia, high PTH levels, high PO_4, increased FGF-23 and generalized inflammation.

Acute-phase reactants like cytokines and C-reactive protein are increased , with decrease in serum albumin and fetuin levels. The vascular occlusive disease are due to underlying inflammatory pathology, leading to rapid vascular calcification, especially in hyperphosphatemia.

These abnormalities augment myocardial ischemia, left ventricular hypertrophy and microvascular disease in CKD patients. Also, hemodialysis, with its associated complications of hypotension and hypovolemia, further aggravates coronary ischemia.

B) Cardiac Failure

Cardiac failure in CKD is mainly due to salt and water retention that leads to pulmonary edema. Cardiac failure may be systolic or diastolic dysfunction or both. In advanced CKD, pulmonary edema presents as breathlessness and a “bat wing” pattern of alveolar edema in chest x-ray.

C) Hypertension and LVH

In CKD, Hypertension is the commonest manifestation and complication as well. It develops at early stages of renal impairment and leads to progressive hypertrophy of left ventricles. Many studies have shown an association between the level of blood pressure and rate of progression of kidney disease. The strongest risk factors for the development of cardiovascular morbidity and mortality in CKD patients, are LVH and dilated cardiomyopathy. They are due to prolonged hypertension and extracellular fluid volume expansion. In addition, anemia and the placement of an arteriovenous fistula for hemodialysis can generate a high cardiac output state and consequent heart failure.

D) Pericardial Disease

Pericarditis, seen in advanced uremia, is characterized by chest pain with respiratory acceleration and friction rub. ECG changes in pericarditis are PR-interval depression and ST-segment elevation. With the advent of early initiation of dialysis,

it's incidence has been reduced. It is now more often observed in underdialyzed, nonadherent patients than in those starting dialysis.

HEMATOLOGIC ABNORMALITIES

A) Anemia

A normocytic, normochromic anemia is observed in stage 3 and 4 of CKD. It is due to decrease in production of erythropoietin (EPO) by the diseased kidneys.[39]

Causes for anemia in CKD

1. Relative deficiency of erythropoietin
2. Bleeding disorders
3. Decreased red blood cell survival
4. Iron deficiency
5. Folate or vitamin B12 deficiency
6. Hemoglobinopathy
7. Hyperparathyroidism
8. Bone marrow fibrosis
9. Chronic inflammation

B) Abnormal coagulation

Patients with advanced CKD may have prolongation in bleeding time, decrease in activity of platelet factor III, abnormal platelet aggregation and impaired prothrombin consumption. CKD patients are highly susceptible to thromboembolism, especially in nephrotic-range of proteinuria. The loss of anticoagulant factors due to proteinuria leads to thromboembolic manifestations.[40]

NEUROMUSCULAR COMPLICATIONS

Common neurological complications in CKD are peripheral neuropathy, autonomic neuropathy and muscular weakness. Sleep disturbance and impaired memory are seen in early stages. In advanced renal failure, twitches, myoclonus, nystagmus and loss of consciousness may be seen.

Peripheral neuropathy usually becomes clinically evident after the patient reaches stage 4 CKD. The “restless leg syndrome” is characterized by ill-defined sensations of sometimes debilitating discomfort in the legs and feet relieved by frequent leg movement. Many of the complications described above will resolve with dialysis.

GASTROINTESTINAL AND NUTRITIONAL ABNORMALITIES

Uremic fetor, is associated with an unpleasant metallic taste (dysgeusia). It is due to release of ammonia from urea degradation in saliva. GI bleeding can occur in advanced renal failure due to gastric mucosal ulcerations.

Due to low calorie and protein intake, protein-energy malnutrition is common in advanced CKD. Metabolic acidosis in association with the activation of inflammatory cytokines will promote catabolism of proteins.

ENDOCRINE-METABOLIC DISTURBANCES

Impairment of glucose metabolism in CKD, is manifested by delay in glucose utilization following glucose intake. Blood glucose levels are normal or mildly raised in fasting state. Since, insulin removal by uremic kidneys are impaired, plasma insulin levels are raised. Patients on insulin therapy, hence require dose reduction , in view of their worsening renal function.

DERMATOLOGIC ABNORMALITIES

The most troublesome dermatological manifestations of the uremic syndrome is pruritis. Skin discoloration in patients with advanced CKD, is because of the deposition of pigmented metabolic products or *urochromes*. *Nephrogenic fibrosing dermopathy* is the progressive induration of soft subcutaneous tissues, commonly found in arms and legs. This is more specific finding among advanced CKD patients.

STAGING OF CKD

The KDIGO guideline[41] stratifies GFR from category G1 (more than 90 mL/min/1.73 m^2) through to G5 (GFR less than 15 mL/min/1.73 m^2). A GFR of less than 60 mL/ min/1.73 m^2 (G3 to G5) is considered as decreased levels.

Category G3 is further subdivided into G3A (GFR 45–59 mL/min/1.73 m^2) and G3B (GFR 30–44 mL/min/1.73 m^2), based on their differing epidemiologic and prognostic significances.

Prognosis of CKD by GFR and albuminuria categories: KDIGO 2012				Persistent albuminuria categories description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73 m^2) description and range	G1	Normal or high	≥90			
	G2	Mildly decreased	60–89			
	G3a	Mildly to moderately decreased	45–59			
	G3b	Moderately to severely decreased	30–44			
	G4	Severely decreased	15–29			
	G5	Kidney failure	<15			

Figure no 7: KDIGO grading of CKD

BIOMARKERS OF RENAL AND TUBULAR FUNCTION

Biomarkers are the signal molecules in diseased state. They are generally protein in nature. They are altered in physiological processes, pathological consequences and in response to therapy. An ideal biomarker is that which is easily measured with good sensitivity and specificity from readily available body fluids and/or tissues. It aids in early detection of a disease and treatment monitoring.

1. Creatinine:

Creatinine is a degraded product of creatine ,of molecular weight of 113 Da. It is freely filtered but neither reabsorbed nor metabolized by renal tubules. The excreted creatinine in urine is mainly secreted from proximal tubular epithelial cells. [42]

2. Cystatin C :

CysC is released by all nucleated cells. It is a non glycosylated protein . It is freely filtered, reabsorbed and completely metabolized in tubular epithelial cells. Unlike creatinine, CysC is not secreted by renal tubules, not influenced by muscle mass and has less intra-individual variability. [43]

3. Albuminuria :

Albumin excretion rate (AER) are seen in spot urine samples or 24 hours urine collection. Increases should be confirmed in at least two of three samples, within a period from 3 to 6 months.

Microalbuminuria is defined as by, AER > 30 mg/24 h or an albumin-creatinine ratio (ACR) of 30-300 mg/g (0.3-3 mg/mmol). Higher values indicate macroalbuminuria, also called clinical nephropathy.

In high risk patients for CKD, the ACR > 30 mg/g is demonstrated to be a risk factor for cardiovascular (CV) death and all cause mortality, progression of kidney disease. But this may not necessarily reflect kidney damage and may be a marker of endothelial dysfunction.

New biomarkers for kidney damage:[44]

Although albuminuria is a powerful biomarker, it may occur after the damage has occurred or may not be present in other types of kidney damage such as tubulointerstitial disease and hypertensive kidney disease. This has led to the search for new biomarkers that are also non-invasive and could better correlate with the etiology of the kidney disease. Early identification of patients with CKD could allow implementing early interventions to reduce CVD or CKD progression.

Few of new markers are NGAL, KIM1, FGF-23, MCP-1, URBP4, ADMA

1. NGAL is an iron-carrying protein of 25 kDa belongs to lipocalins family. It is expressed by tubular renal epithelial cells following tubulointerstitial injury. It is an established marker for acute kidney injury however its role in CKD is less studied. Urinary NGAL to creatinine ratio was associated with CV risk factors and mortality in CKD.
2. KIM1 is a transmembrane protein,whose expression has been upregulated after kidney injury. It is an early biomarker for proximal tubular damage since it is expressed in the urine during the first 12 h of the tubular injury.
3. FGF23 is a 32-kDa phosphaturic protein secreted by bone osteocytes. It promotes phosphate excretion, decrease calcitriol and parathyroid hormone. In

CKD the increase of FGF-23 level precedes the decline in vitamin 1,25-(OH)2 vitamin D3 and the increase of PTH level. [45]

4. MCP-1 inflammatory chemokines - Expression of MCP-1 is up regulated in kidney diseases due to sustained inflammatory response, such as diabetic nephropathy and lupus nephritis. Glomerular and tubular kidney cells release MCP-1 in high glucose levels. MCP-1 levels are elevated in diabetic nephropathy. [46]
5. uRBP4 is a 21 KDa protein, derived from plasma RBP4 (pRBP4). It is produced mainly in the liver but also in the adipose tissue where it performs as an adipokine. uRBP4 is currently the most sensitive functional biomarker of proximal tubule. pRBP4 is filtered at the glomerulus and completely reabsorbed in the proximal tubule.[47]
6. ADMA is 202 Da, normally synthesized intracellularly and eliminated through the urine. In the diabetic and non diabetic population, ADMA levels are higher as GFR declines and are associated with rapid kidney function decline.[37]

TRAIL, OPG, and TWEAK biology

Members of TNF family are involved in renal pathophysiology. The frequently involved biomolecules are TNF-related apoptosis-inducing ligand(TRAIL), osteoprotegerin (OPG), and TNF-like weaker inducer of apoptosis (TWEAK). They are involved in activating inflammatory pathways leading to development and

progression of kidney disease. They are also involved in regulating cell proliferation, differentiation, inflammation, necrosis, apoptosis, angiogenesis and fibrosis.

TRAIL (Apo-2 Ligand, CD253, or TNF-SF10)

It is a pleiotropic cytokine, type II transmembrane protein made of 281 amino acids. The biological feature of TRAIL is the ability to induce apoptosis only in transformed /cancer cells and sparing the normal cells. TRAIL activates necrosis, apoptosis, and autophagy, proliferative and proinflammatory pathways. TRAIL is expressed only by the renal tubules, not by the glomeruli in healthy conditions.

OPG

A glycoprotein involved in bone resorption regulation, is released by vascular smooth muscle cells, endothelial cells, osteoblasts and immune cells. It exerts various effects on tissues by binding to RANKL and/or TRAIL. OPG is involved in bone remodeling. OPG circulating levels change in several diseases. OPG levels were associated with markers of vascular damage. Studies have shown that OPG is an independent risk factor for cardiovascular (CV) morbidity and mortality.

TWEAK (also known as TNFSF12 or Apo3L)

It is a type II transmembrane protein with 249 amino acids. It is cleaved by furin endoprotease, producing a 18 kDa soluble protein with 156 amino acids. Soluble TWEAK is expressed by monocytes, natural killer cells, and dendritic cells. It was known to promote the transformation from innate to adaptive immunity responses. Biological effects of TWEAK includes cell survival, proliferation, migration, apoptosis, and angiogenesis.

Benito-Martin et.al., found that cultured epithelial cells of proximal tubules constitutively secreted exosome-like vesicles that contained OPG. This suggests that tubular cell exosomes are involved in regulatory roles such as matrix deposition, inflammation, and apoptosis. TRAIL or TWEAK were not found in exosomes. TWEAK is constitutively expressed by tubular and glomerular cells like mesangial cells and podocytes.

OSTEOPROTEGERIN

OSTEOPROTEGERIN

DISCOVERY AND HISTORY

Osteoprotegerin (OPG) was first identified in 1997 simultaneously by two different research groups. Simonet et al. (1997) - in a foetal rat intestine complementary deoxyribonucleic acid- (cDNA-) sequencing project discovered a new possible member of the tumour necrosis factor (TNF) receptor superfamily.[8] Another research group, Tsuda et al., found a novel binding protein with no homology to known proteins in the conditioned medium of human embryonic lung fibroblasts which inhibited osteoclastogenesis. They termed this protein osteoclastogenesis inhibitory factor (OCIF). A year later, Yasuda et al. published that these independent findings referred to the same molecule.

The OPG gene was identified and cloned in 1997

NOMENCLATURE

The American Society of Bone and Mineral Research Committee has decided to use the term OPG as it implies its bone protective characteristics (Latin: "*os*" bone and "*protegere*" to protect). Otherwise called as TNF receptor superfamily member 11B and Osteoclastogenesis inhibitory factor (OCIF) . It is considered as the negative regulator of osteoclastic bone resorption.

GENETICS

The gene for OPG is located on the chromosome 8 (8q24). It has five exons over 29 kilo bases (Kb). Northern blot analysis using a full-length cDNA probe produced three messenger ribonucleic acid (mRNA) transcripts of 2.4, 4.2, and 5.6 Kb. The band at 2.4Kb constituting the major transcript and the two other transcripts represent alternatively spliced forms containing all or a portion of the second intron that encodes for a soluble molecule.[48]

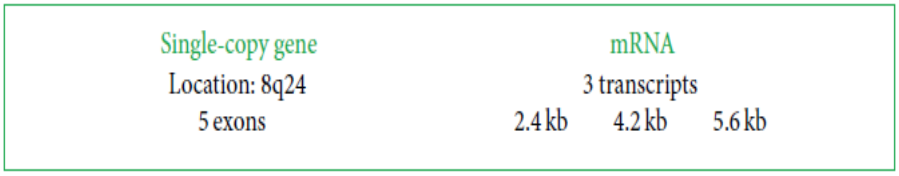


Figure no 8 : Genetics of OPG

SOURCE

OPG mRNA has been found in various tissues like kidney, bone, liver, stomach, lung, heart, intestine, thyroid gland, brain and spinal cord. OPG is produced from endothelial cells and vascular smooth muscle cells. Normally, it circulates in the blood at lower levels than in the tissues.

BIOCHEMICAL STRUCTURE

OPG is a secretory glycoprotein (molecular wt 60 kDa). It is synthesized as a propeptide with 401 aminoacids. Cleavage of signal peptide from it, generates the mature peptide with 380 amino acids. It exists in either monomeric or dimeric forms, circulates mainly as a monomer. OPG lacks transmembrane and cytoplasmic domains.[48]

STRUCTURAL DOMAINS OF OPG

It has seven structural domains

(i) Domains 1–4: *four cysteine rich pseudorepeats* structurally related to the TNF receptor family located in the N-terminal that is essential for the inhibition of osteoclastogenesis

(ii) Domains 5-6: *two death domains* at the carboxyterminal end of the protein contain apoptosis mediating death domain homologous regions

(iii) Domain 7: a *heparin binding site* at C terminal region interacts with numerous proteoglycans as well as a free cysteine residue required for disulphide bond formation and dimerization.

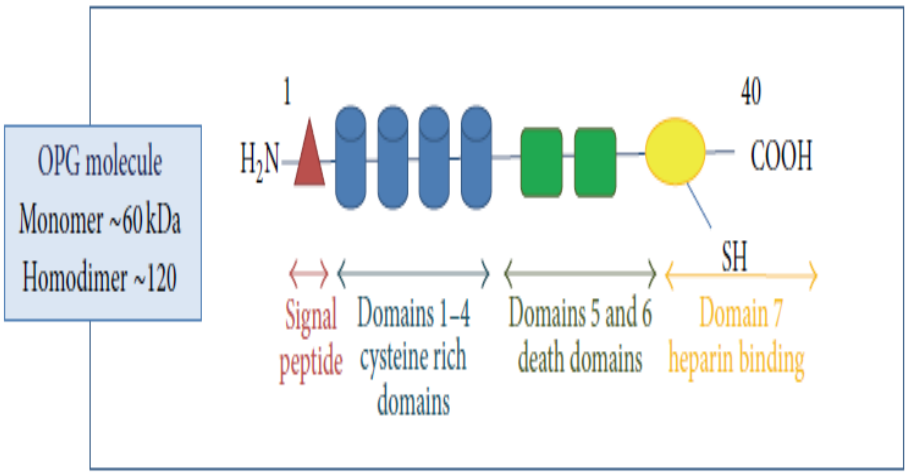


Figure no 8 : Structural domains of OPG

MECHANISM OF ACTION

OPG is released from numerous cell types such as osteoblasts, kidney, liver, spleen, bone marrow, lungs and skin. It is a decoy receptor, prevents its interaction with RANK by inhbiting RANKL.

OPG/RANKL PATHWAY

RANKL

↓

Binds to receptor RANK

↓

Activates the inflammatory cascade within the cells

↓

Exerts profound effect on various cell functions

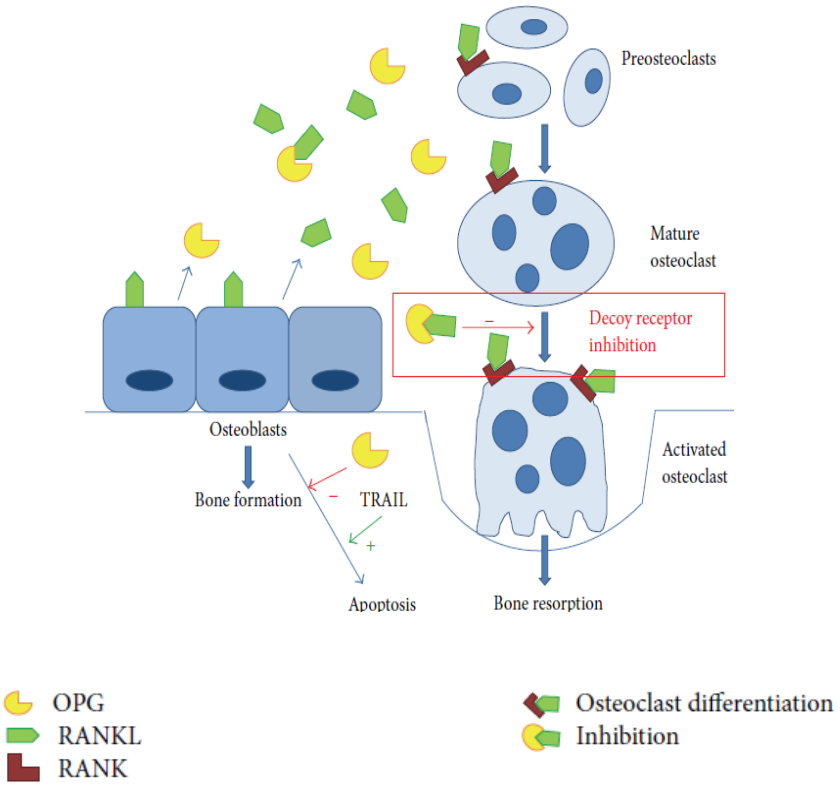


Figure no 9 : Mechanism of action of OPG

Hormones and cytokines are known to regulate OPG production. Mainly steroid hormone estrogen increase OPG levels, while androgens decrease it.[49]

PATHOLOGICAL ROLE

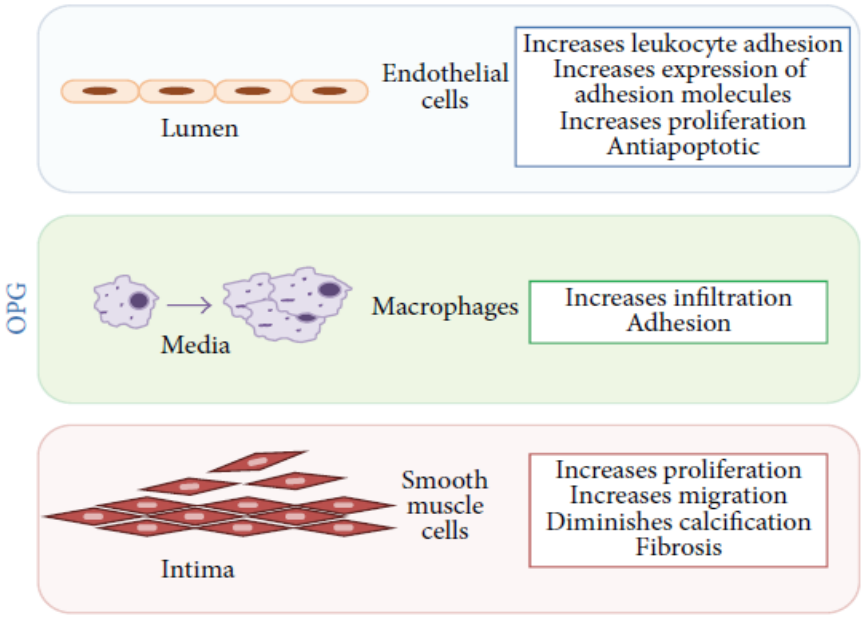


Figure no 10 : Role of OPG at various sites

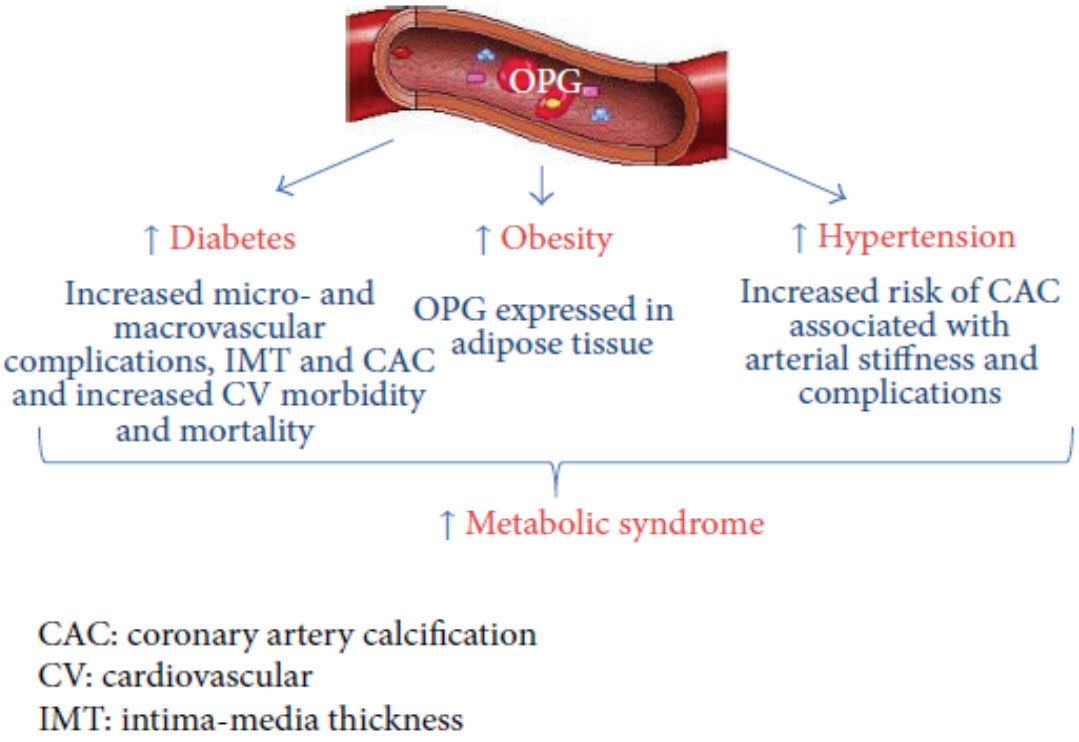


Figure no 11 : Pathological Role of OPG

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY METHODOLOGY

A case control study was conducted at Government Mohan Kumaramangalam Medical College Hospital(GMKMCH), Salem, during December 2018 –November 2019. Informed consent was obtained from all study participants. All procedures concerned with patients were performed after obtaining ethical clearance from the Institutional Ethical Committee.

STUDY POPULATION

The study consisted of 65 patients with hypertension taken as cases. 35 patients of CKD from stages 1-3, attending the outpatient clinic at Nephrology department, GMKMCH, Salem were included as controls. 4 & 5 stages were not included.

The cases were divided into two groups of patients with hypertension less than 5 years duration and of patients with hypertension of 5- 10 years duration. The controls were classified into 5 CKD stages as per KDOQI based on eGFR. CKD EPI formula was used for calculating eGFR.

The study was started after approval from the institutional ethical committee. The study protocol was clearly explained to all the patients enrolled in study.

Patients were subjected for detailed evaluation followed by specimen collection. Controls were chronic kidney disease individuals based on their clinical manifestations and routine investigations with elevated renal parameters and estimated GFR between 45 - 90 ml/min/1.73m^2.

INCLUSION CRITERIA

i. Age 30 to 65 years

ii. Hypertensive patients on treatment

iii. Chronic kidney disease (stages 1- 3)

EXCLUSION CRITERIA

i. Age <30 &>65

ii. Diabetic patients

iii. On irregular antihypertensive medications

iv. Any systemic illness causing renal damage

v. Congenital/intrinsic renal damage

vi. Stage 4 & 5 CKD

SPECIMEN COLLECTION

Venous blood of five ml was collected in a clot activator tube, from all the participants. Immediately, serum was separated by centrifuging at 3000 rpm for 10 -15 minutes. It was aliquoted and stored into an eppendorf tube at -20°C until analysis for OPG. Routine investigations like glucose, urea, creatinine, lipid profile, total protein, albumin and calcium were performed on the day of sample collection in CHEM5 semi auto analyser.

METHODS

The measurement of serum osteoprotegerin levels was done by ELISA with commercial reagent kits from Elabscience. Osteoprotegerin ELISA assay was Sandwich type, with a biotin labelled antibody.

Sensitivity: 0.10 ng/mL

Detection Range: 0.16-10 ng/mL

Repeatability: Coefficient of variation is < 10%

Estimation of the various analytes was done by following methods

Blood Sugar	-	GOD POD method
Blood Urea	-	GLDH -urease method
Serum Creatinine	-	Modified Jaffe's Method
Serum Total Cholesterol	-	CHOD-PAP method
Serum Triglycerides	-	Glycerol 3 Phosphate oxidase method
Serum HDL	-	Phosphotungstic Acid method
Serum Calcium	-	Arsenazo method
Total protein	-	Biuret method
Serum Albumin	-	Bromocresol green method

CALCULATED PARAMETRES

1. eGFR – CKD EPI equation
2. LDL – Friedewald formula

ESTIMATION OF OSTEOPROTEGERIN

Method : Sandwich-ELISA principle

Instruments used - ROBONIK ELISA reader and washer

Test principle

- The microtitre 96 well ELISA plate is pre-coated with an antibody that is specific to Human OPG.
- Standards and/or samples are added to the microtitre wells . It combines with coated antibody in the wells.
- Then a biotinylated antibody unique to Human OPG is added. Followed by it, is addition of Horseradish Peroxidase (HRP) conjugate to micro plate well.
- After incubation for prescribed duration, washing steps are performed to remove the unbound components.
- The substrate solution is added , without being exposed to light..
- Blue colour is formed in wells indicating the presence of OPG, biotinylated antibody and HRP conjugate.
- The colour turns yellow on adding stop solution, indicating the reaction termination.
- The optical density (OD) is measured using microplate reader at 450 nm.
- The concentration of Human OPG is calculated by proportionating to the optical density.
- The OPG concentration in samples are calculated from OD values of the standard curve.

Preparation of reagents

1. All the assay reagents were brought to room temperature (18~25℃) before using. Microplate reader was set and preheated for 15 min before OD measurement.
2. **Wash Buffer:** 750 mL of Wash Buffer is prepared by diluting 30 mL of concentrated wash buffer with 720 mL of deionized water.
3. **Standard working solution:**
    - The given standard was centrifuged at 10,000g for 1 min.
    - 1 mL of Sample Diluent was added.
    - Allowed to stand for 10 min.
    - It was inverted several times gently for thorough mixing.
    - This reconstitution provides a working solution of 10 ng/mL.
    - Then serial dilutions were made as required.
    - The recommended dilution gradient was made as given :

      10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0 ng/mL.

Dilution method:

- Seven eppendorf tubes were taken.
- 500uL of given Standard & Sample Diluent were added in each tube.
- 500uL of the 10 ng/mL working solution was pipetted into the first tube and mixed thoroughly to provide 5 ng/mL working solution.
- Then, 500uL of the solution was pipetted from former tube into the latter one as shown below .

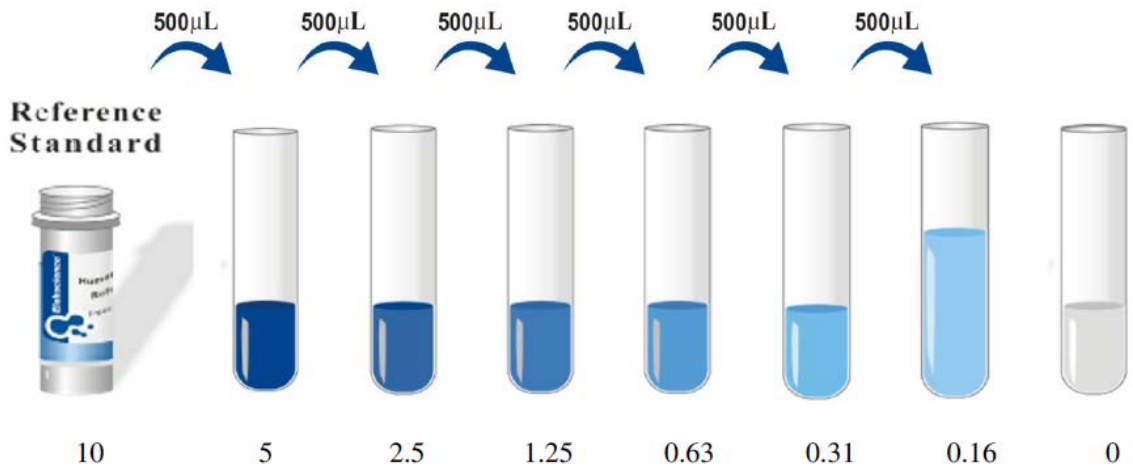


Figure no 12 : Dilution of OPG standard

1. **Biotinylated Detection Ab working solution:**

The required amount was calculated before the experiment (100 µL/well). The stock tube was centrifuged before use, and the 100× Concentrated Biotinylated Detection Ab was diluted to 1×working solution with Biotinylated Detection Ab Diluent.

2. **Concentrated HRP Conjugate working solution:**

The required amount was calculated before the experiment (100 µL/well). The 100× Concentrated HRP Conjugate was diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Procedure

As per the procedure provided in the kit, reagents and standards were prepared meticulously.

- 100 µL of working standard or sample was added to each well. The plate was incubated for 90 min at 37℃.

- The liquid was removed out of each well.
- 100 µL of **Biotinylated Detection Ab working solution** was added to each well. It was then incubated for 1 hour at 37°C.
- Solution was aspirated from each well, and washed with 350 µL of **wash** 3 times.
- 100 µL of **HRP Conjugate solution** was added to each well. It was then incubated for 30 min at 37°C.
- Solution from each well was aspirated or decanted, and the wash process was repeated for five times.
- 90 µL of **Substrate Reagent** was added to each well followed by incubation for 15 min at 37°C.
- 50 µL of **Stop Solution** was finally added to each well.
- The optical density was determined for 96 wells with a micro-plate reader that is set to 450nm.

ESTIMATION OF GLUCOSE

Glucose oxidase peroxidase (GOD/POD) method , End Point method

Principle

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

$$H_2O_2 + \text{Phenol} + \text{4AminoAntipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinonemine dye} + H_2O$$

Pink colour of Quinonemine , formed is proportional to glucose concentration measured at 450nm.

Composition of reagent	
GOD	≥ 20000U/l
POD	≥ 2000U/l
Buffer (Phosphate buffer)	200 mmol/l
Phenol	10 mmol/l

Concentration of glucose standard used is 100 mg/dl

Procedure

	Blank	**Standard**	**Test**
Reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard	-	10 µl	-

The contents were mixed well and incubated for 15min at 37°C.The absorbance of the standard and test were measured against reagent blank at 505nm.

Reference interval

Plasma glucose: 70 –100 mg/dl (fasting)

ESTIMATION OF BLOOD UREA

GLDH - urease method (kinetic assay)

Principle

Urea is hydrolysed to produce ammonia and carbon dioxide by urease.

The ammonia combines with alpha-oxoglutarate and NADH in the presence of glutamate dehydrogenase. It produces glutamate and NAD^+.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} \text{Ammonia} + CO_2$$

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

The decrease in absorbance is directly proportional to the concentration of urea in the sample, which is measured at 340nm.

Composition of reagent

Reagent I : Buffer reagent

Reagent II : Enzyme Reagent

Standard concentration of urea used was 50mg/dl

Preparation of reagent

4 parts of reagent I mixed with one part of reagent II

Procedure

	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

The contents are mixed well and absorbance read at 340nm.

Reference Interval

Plasma/ Serum Urea : 15- 40 mg/dl

ESTIMATION OF CREATININE

Modified Jaffe's Method, two point kinetic assay

Principle

Creatinine reacts with alkaline picrate and orange yellow coloured compound. The absorbance of the orange-yellow colour is measured at 505nm. It is directly proportional to the creatinine concentration in the sample.

Composition of reagent

Reagent I

Picric acid reagent (25.8 mmol/l)

Reagent II

Sodium hydroxide reagent (95 mmol/l)

Creatinine standard: 2 mg/dl

Preparation of reagent

Equal volume of R1 & R2 reagents were mixed.

Procedure

	Blank	**Standard**	**Test**
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	100 µl	-
Test	-	-	100 µl

The contents are mixed well and absorbance at 505 nm

Reference interval

Male : 0.7 – 1.4 mg/dl

Female : 0.6 – 1.2 mg/dl

Children : 0.5- 1mg/dl

ESTIMATION OF CHOLESTEROL

Cholesterol oxidase – PAP method , end point assay

Principle

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3one} + \text{Peroxide}$$

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

Absorbance of quinoneimine dye produced is directly proportional to concentration of cholesterol in the sample.

Composition of reagent

Good‘s Buffer (of pH-6.4)	100 mmol/l
Phenol	5 mmol/l
4-AAP	0.3 mmol/l
Cholesterol esterase	>200 U/l
Cholesterol oxidase	> 100 U/l
Peroxidase	>3000U/l

Procedure

	Blank	**Standard**	**Test**
Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

The contents were mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read at wavelength of 505 nm.

Calculation

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

Reference interval

Plasma/ Serum

Age	**mg/dl**
2-12 months	60-190
1 – 20 years	110-230
Adults	< 200

Interference

Hb upto 200mg/dl, bilirubin upto 10mg/dl and TGL upto 700 mg/dl will not interfere the assay.

ESTIMATION OF TRIGLYCERIDES

GPO-PAP method, endpoint assay

Principle

$$\text{Triglycerides + water} \xrightarrow{\text{Lipoprotein Lipase}} \text{Free fatty acid + Glycerol}$$

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

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{Glycerol-3-Phosphate Oxidase}} \text{DHAP + } H_2O_2$$

$$H_2O_2 + \text{3,5-DHBS + 4AAP} \xrightarrow{\text{Peroxidase}} \text{Quinonemine + } 2H_2O$$

The colour intensity of Quinoemine formed is proportional to concentration of the triglyceride in the sample. It is measured at 505nm .

Triglyceride standard concentration used was 200 mg/dl

Composition of reagent

Reagent 1

Lipoprotein Lipase	4000 U/l
4- Amino antipyrine	0.4 mmol/l
Peroxidase	2200 U/l
Glycerol Phosphate Oxidase	4000 U/l
ATP	2mmol/l
Glycerol kinase	1500 U/l

Reagent 2

Pipes buffer(pH-7.0) : 40 mmol/l

Magnesium salt : 2.5 mmol/l

DHBS : 0.2mmol/l

Reagent preparation

Reagent was prepared by mixing 4 parts of R1 with 1 part of R2

It is stable for 3months at 2-8 ° C

Sample : fasting serum. Hemolysed samples are avoided

Procedure

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

The contents were mixed and incubated for 10 min. Absorbance for test and standard were measured at 505nm.

Calculation

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

Reference interval

Plasma/ Serum	mg/dl
Fasting level	25-160

Linearity - upto 1000 mg/dl

Sensitivity - 2 mg/dl

Interference

Hb upto 300 mg/dl, ascorbate upto 3 mg/dl, bilirubin upto 20 mg/dl will not interfere the assay.

ESTIMATION OF HDL

Phosphotungstic acid method, end point assay

Principle

Chylomicrons, LDL and VLDL are precipitated by phosphotungstate in the presence of divalent cations like magnesium. The HDL cholesterol remnants are found in the supernatant.

$$\text{Serum/plasma} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \text{HDL} + (\text{LDL} + \text{VLDL} + \text{Chylomicrons})$$

Composition of reagent

Reagent 1 : Precipitating reagent

Magnesium chloride	40 mmol/l
Phosphotunstic acid	2.4 mmol/l

HDL cholesterol standard of 25 mg/dl was used

Precipitation of VLDL, LDL and chylomicrons done as follows

Precipitating reagent	500 µl
Test	250 µl

The contents were mixed well and allowed to stand for 10min at room temperature. Then it was centrifuged at 4000 rpm for 10 min. Clear supernatant was obtained. The supernatant was used to determine the HDL concentration in the sample.

Procedure

Reagents	Blank	Standard	Test
Cholesterol reagent	1000 µl	1000 µl	1000 µl
Distilled H2O	50 µl	-	-
HDL Standard	-	50 µl	-
Supernatant liquid	-	-	50 µl

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

Calculation

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{conc. of std} \times \text{dilution factor}$$

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

Linearity - upto 125 mg/dl

Reference Interval

Male - 30-65 mg/dl

Female - 35-80 mg/dl

Interference

High TGL levels more than 300mg /dl interferes with the test. At high concentrations, bilirubin and ascorbate will interfere the precipitation reaction.

ESTIMATION OF TOTAL PROTEIN

Biuret Method, End point assay

Principle

Serum protein reacts with copper ions of biuret reagent , in alkaline medium to form purple coloured complex. The colour formed is directly proportional to the protein concentration in the sample. It is measured at 545nm.

Composition of reagent

Biuret Reagent:

i. Copper acetate

ii. Sodium hydroxide

iii. Potassium iodide

Protein Standard of concentration 10 g/dl was used.

Procedure

20µl of serum was added to 1ml of reagent. It was mixed well and incubated for 10minutes at room temperature. The absorbance of standard and sample read at 545 nm.

Calculation

$$\text{Serum Total protein in g/dl} = \frac{(\text{Abs.T}) - (\text{Abs.B})}{(\text{Abs.S}) - (\text{Abs.B})} \times 10$$

Reference interval

Serum protein 6.4-8.3 g/dl

Linearity : upto 15g/dl

ESTIMATION OF ALBUMIN

Bromocresol green (BCG) Method, End point assay

Principle

In acidic medium, albumin in serum reacts with bromocresol green dye in reagent to form coloured complex. The colour produced is directly proportional to albumin concentration in the sample. It is measured at 630nm.

Composition of reagent

i. Bromocresol green

ii. Acetate buffer

iii. Detergent with of pH 4.1

Albumin standard of concentration 2 g/dl was used

Procedure

10µl of serum was added to 1ml of reagent. The contents were mixed and incubated for 1minute at room temperature. The absorbance of standard and sample read at 630 nm.

Calculation

$$\text{Serum Albumin in g/dl} = \frac{(\text{Abs.T})-(\text{Abs.B})}{(\text{Abs.S})-(\text{Abs.B})} \times 2$$

Reference interval

Serum albumin 3.5-5.0 g/dl

Linearity : till 7 g/dl

ESTIMATION OF CALCIUM

Arsenazo method, End point assay

Principle

Arsenazo combines with calcium ions at pH 6.5 to form a coloured complex. The colour produced is directly proportional to the calcium concentration in the sample. It is measured at 650nm.

Reagent

Arsenazo III Buffer

Calcium standard of concentration 10 mg/dl was used

Procedure

10µl of serum was added to 1ml of reagent. The contents were mixed and incubated for 10 minute at room temperature. The absorbance of standard and sample read at 650 nm.

Calculation

$$\text{Serum Calcium mg/dl} = \frac{(\text{Abs.T})-(\text{Abs.B})}{(\text{Abs.S})-(\text{Abs.B})} \times 10$$

Reference interval

Serum calcium levels: 8.6 - 10.2 mg/dl

Linearity is upto 16 mg/dl

CALCULATED PARAMETERS :

1. eGFR values were calculated using CKD EPI formula.

   GFR = 141 × min(S_{cr}/κ, 1)α × max(S_{cr}/κ, 1)$^{-1.209}$ × 0.993^{Age} × 1.018

   [if female] ×1.159 [if black]

   S_{cr} - serum creatinine in mg/dL,

   κ value is 0.7 for females and 0.9 for males,

   α value is -0.329 for females and -0.411 for males,

   min denotes the minimum value of S_{cr}/κ or 1, and

   max denotes the maximum value of S_{cr}/κ or 1

2. LDL Cholesterol calculated using Friedewald's equation

   Friedewald Formula

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

Where all concentrations are given in mg/dL (triglyceride/2.22 is used when units are expressed in mmol/L). The factor (triglyceride)/5 is an estimate of VLDL-C and is based on the average ratio of triglyceride to cholesterol in VLDL. The calculation is precluded in samples with TGL concentrations above 400 mg/dL or in those with increased quantities of chylomicrons (non fasting specimens). The Friedewald equation has been found to be most accurate in samples with triglyceride concentrations below 200 mg/ dL.

STATISTICS AND RESULTS

STATISTICS AND RESULTS

STATISTICS

Graph Pad Prism 8.2.1 was used in statistical analysis. p value of less than 0.05 was considered as significant.

Mean and Standard Deviation was used to express the continuously distributed data.

The mean value of various biochemical parameters between controls and cases were compared using unpaired t test.

Pearson Correlation analysis was used to demonstrate the correlation of serum OPG levels with serum creatinine, protein, albumin, calcium and eGFR.

Linear regression analysis was performed to evaluate the predictive role of the osteoprotegerin in renal dysfunction among hypertensive patients.

The diagnostic accuracy of serum osteoprotegerin levels between hypertensive patients [39] and CKD patients was found using ROC curve.

RESULTS

Serum osteoprotegerin, blood sugar, blood urea, serum creatinine, serum total protein, serum albumin, serum lipid profile, serum calcium, serum uric acid, serum total protein and albumin levels were estimated in controls and cases.

eGFR values were calculated by CKD EPI formula.

TABLE NO : 1 DESCRIPTIVE DATA OF CASES AND CONTROLS

S.No	Parameters	Cases (n=65)	Controls (n=35)
		mean ±SD	mean ±SD
1.	Age (years)	55.5±8.7	53.6±7.1
2.	Hypertension duration (years)	4.3±3.7	7.9 ±3.0
3.	Blood sugar (mg/dl)	106.0±26.17	93.8±13.83
4.	Blood urea (mg/dl)	25.75±5.7	51.86±8.97
5.	Sr. Creatinine (mg/dl)	1.11±0.24	1.74±0.53
6.	Total cholesterol (mg/dl)	205.1±34.52	263.4±50.81
7.	Triglycerides (mg/dl)	218.9±84.54	191.4±44.44
8.	HDL(mg/dl)	46.82±8.74	40.49±4.80
9.	LDL(mg/dl)	114.5±33.91	184.7±47.02
10.	Sr. Calcium(mg/dl)	8.52±1.42	7.90±0.95
11.	Sr.Uric acid (mg/dl)	4.50±1.08	4.89±0.77
12.	Total protein(g/dl)	6.59±0.78	6.81±0.53
13.	Albumin(g/dl)	3.47±0.48	3.66±0.48
14.	eGFR	66.01±16.13	44.16±13.09
15.	OPG (ng/dl)	0.73±1.01	3.63±1.99

TABLE NO 2 : SEX DISTRIBUTION IN CASES AND CONTROLS

	NO OF MALE PAIENTS	NO OF FEMALE PATIENTS
CONTROLS	24	11
CASES	28	37

CHART NO 1 : SEX DISTRIBUTION IN CASES AND CONTROLS

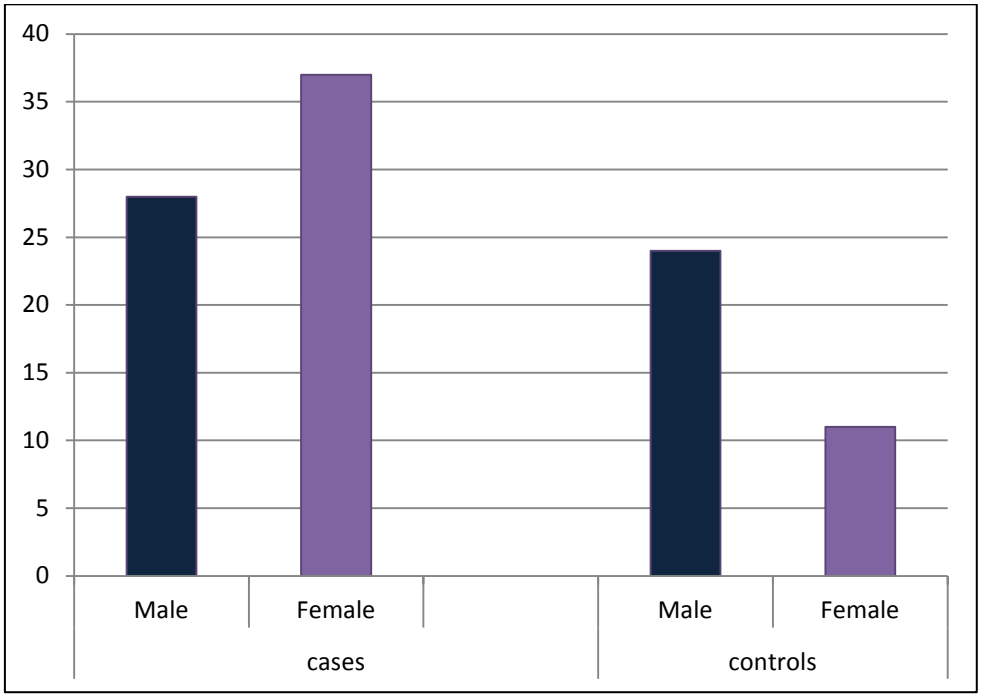


TABLE NO 3: STATISTICAL ANALYSIS OF SERUM OSTEOPROTEGERIN IN CONTROL AND CASES

OSTEOPROTEGERIN ng/ml	N	MEAN	SD	INFERENCE
CONTROLS	35	3.638	1.995	p= 0.0001 p<0.05
CASES	65	0.734	1.017	

CHART NO 2: MEAN OPG LEVELS IN CONTROLS AND CASES

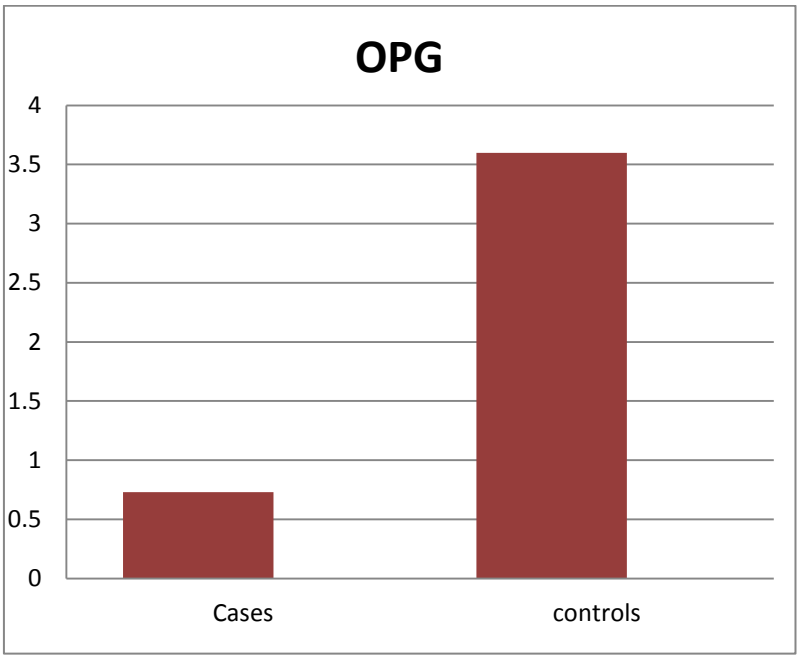


TABLE NO 4: STATISTICAL ANALYSIS OF SERUM CREATININE IN CONTROLS AND CASES

CREATININE mg/dl	N	MEAN	SD	INFERENCE
CONTROLS	35	1.747	0.5372	p = 0.0001
CASES	65	1.119	0.2475	p < 0.05

CHART NO 3 : MEAN CREATININE LEVELS IN CASES & CONTROLS

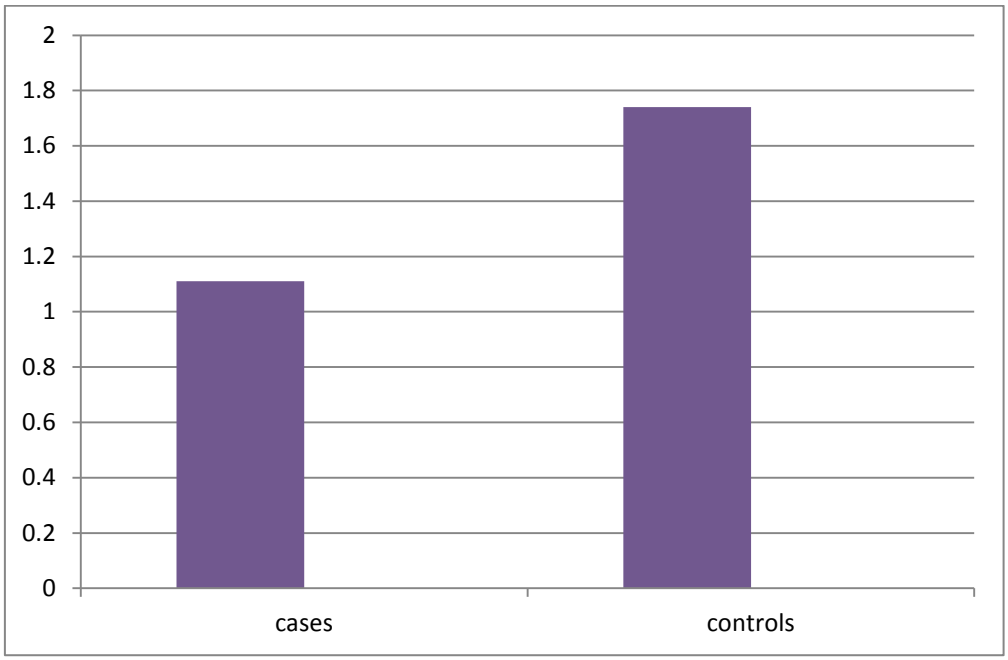


TABLE NO 5 : STATISTICAL ANALYSIS OF TOTAL CHOLESTEROL LEVELS IN CONTROLS AND CASES

T.CHOLESTEROL mg/dl	N	MEAN	SD	INFERENCE
CONTROLS	35	263.4	50.81	p = 0.0001 p < 0.05
CASES	65	205.1	34.52	

CHART NO 4 : MEAN TOTAL CHOLESTEROL LEVELS IN CASES & CONTROLS

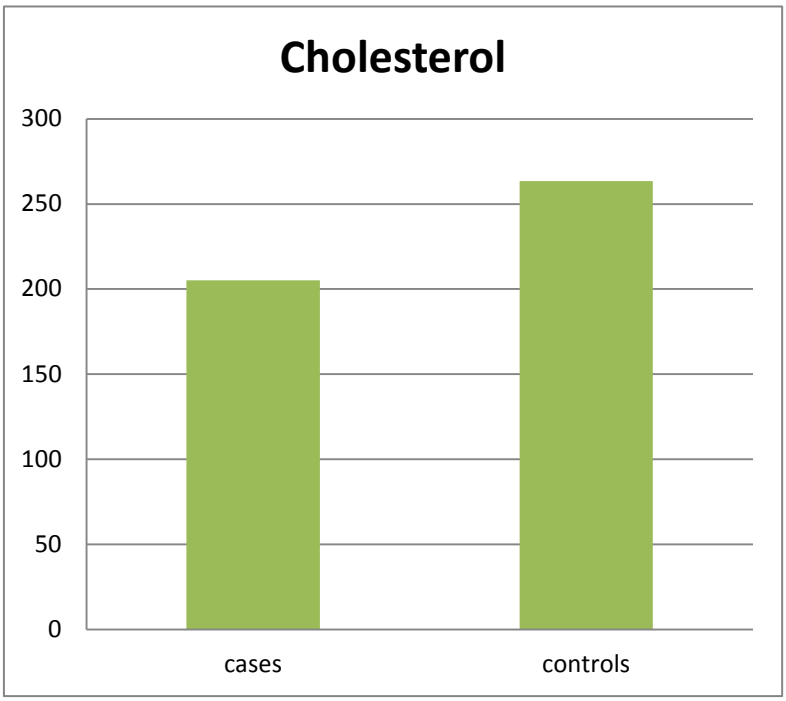


TABLE NO 6 : STATISTICAL ANALYSIS OF SERUM TRIGLYCERIDE LEVELS IN CONTROLS AND CASES

TGL (mg/dl)	N	MEAN	SD	INFERENCE
CONTROLS	35	191.4	44.44	p = 0.0001 p < 0.05
CASES	65	218.9	84.54	

CHART NO 5 : MEAN TGL LEVELS IN CASES & CONTROLS

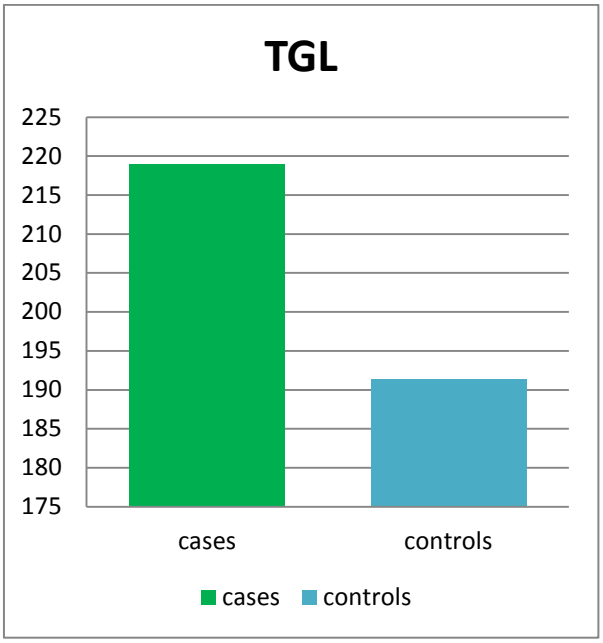


TABLE NO 7: STATISTICAL ANALYSIS OF SERUM HDL LEVELS IN CONTROLS AND CASES

HDL mg/dl	N	MEAN	SD	INFERENCE
CONTROLS	35	40.49	4.80	p= 0.0001
CASES	65	46.82	8.74	p < 0.05

CHART NO 6 : MEAN HDL LEVELS IN CASES & CONTROLS

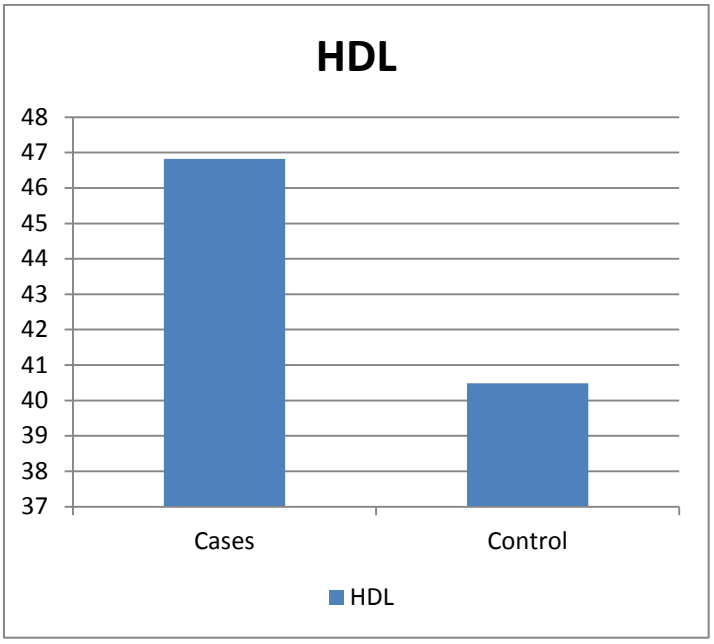


TABLE NO 8: STATISTICAL ANALYSIS OF LDL LEVELS IN CONTROLS AND CASES

LDL mg/dl	N	MEAN	SD	INFERENCE
CONTROLS	35	184.7	47.02	p= 0.0001
CASES	65	114.5	33.91	p < 0.05

CHART NO 7 : MEAN LDL LEVELS IN CASES & CONTROLS

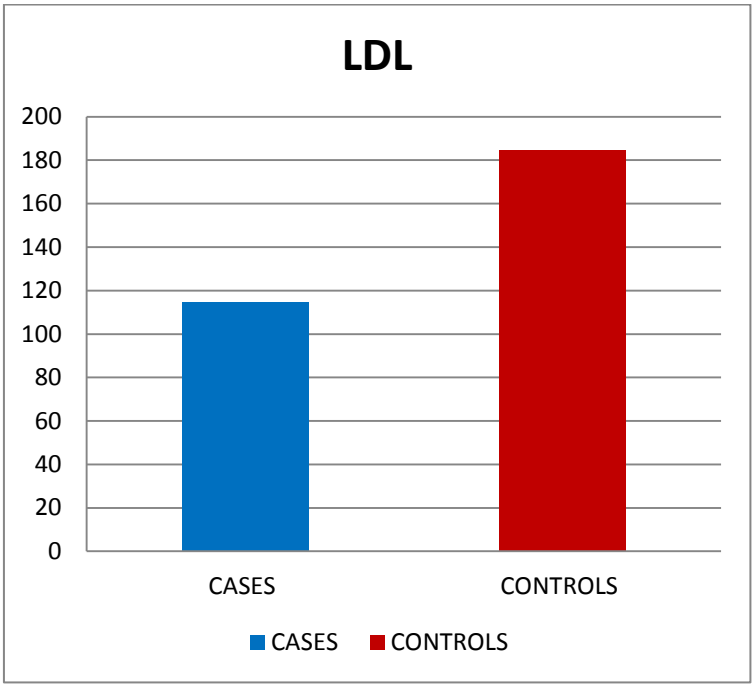


TABLE NO 9 : STATISTICAL ANALYSIS OF SERUM CALCIUM LEVELS IN CONTROLS AND CASES

CALCIUM mg/dl	N	MEAN	SD	INFERENCE
CONTROLS	35	7.909	0.951	p = 0.0001 p < 0.05
CASES	65	8.52	1.426	

CHART NO 8 : MEAN LEVELS OF CALCIUM IN CASES & CONTROLS

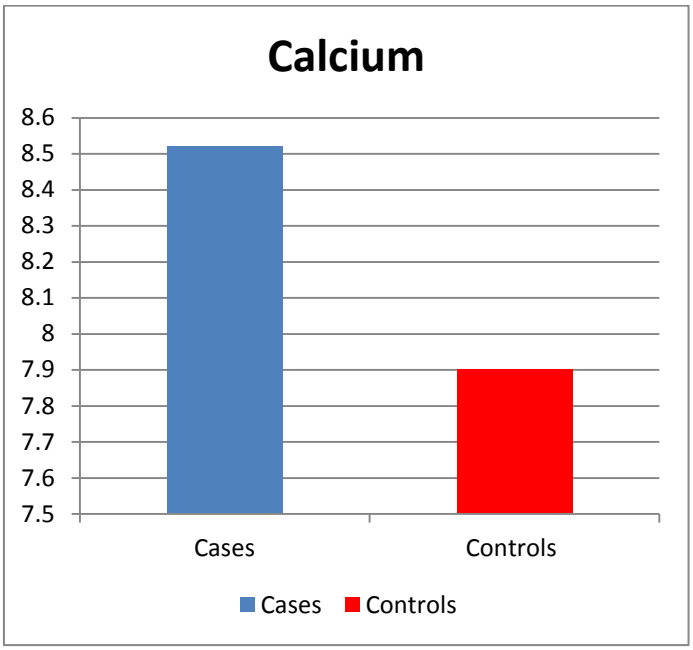


TABLE NO 10 : PEARSON'S CORRELATION ANALYSIS OF OPG AND eGFR IN CASES

Correlations

		OPG	eGFR
OPG	Pearson Correlation	1	-.754**
	Sig. (2-tailed)		.000
	N	65	65
eGFR	Pearson Correlation	-.754**	1
	Sig. (2-tailed)	.000	
	N	65	65

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE NO 11 : PEARSON'S CORRELATION ANALYSIS OF OPG AND BMI IN CASES

Correlations

		OPG	BMI
OPG	Pearson Correlation	1	.246*
	Sig. (2-tailed)		.049
	N	65	65
BMI	Pearson Correlation	.246*	1
	Sig. (2-tailed)	.049	
	N	65	65

*. Correlation is significant at the 0.05 level (2-tailed).

TABLE NO 12 : PEARSON'S CORRELATION ANALYSIS OF OPG AND CREATININE IN CASES

		OPG	CREATININE
OPG	Pearson Correlation	1	.709**
	Sig. (2-tailed)		.000
	N	65	65
CREATININE	Pearson Correlation	.709**	1
	Sig. (2-tailed)	.000	
	N	65	65

**. Correlation is significant at the 0.05 level (2-tailed).

TABLE NO 13 : PEARSON'S CORRELATION ANALYSIS OF OPG AND TOTAL CHOLESTEROL IN CASES

Correlations

		OPG	CHOLESTEROL
OPG	Pearson Correlation	1	.321**
	Sig. (2-tailed)		.009
	N	65	65
CHOLESTEROL	Pearson Correlation	.321**	1
	Sig. (2-tailed)	.009	
	N	65	65

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE NO 14 : PEARSON'S CORRELATION ANALYSIS OF OPG AND LDL IN CASES

Correlations

		OPG	LDL
OPG	Pearson Correlation	1	.340*
	Sig. (2-tailed)		.005
	N	65	65
LDL	Pearson Correlation	.340*	1
	Sig. (2-tailed)	.005	
	N	65	65

*. Correlation is significant at the 0.05 level (2-tailed).

BIVARIATE CORRELATION WITHIN CASES (HYPERTENSION) GROUP

Pearson correlation

	SERUM OPG
eGFR	-0.754
Creatinine	0.709
BMI	0.246
Total cholesterol	0.321
LDL	0.340

$p < 0.01$

Pearson Correlation Analysis in patients in cases group showed a significant positive correlation between serum OPG with Creatinine, BMI, total cholesterol and LDL ($p < 0.01$) as shown in tables 11, 12, 13, 14. Bivariate correlation analysis between serum OPG and eGFR is shown in Table 10. An inverse association was found among hypertensive patients, between serum OPG and eGFR ($r = -.754$, $P<0.01$).

TABLE NO 15 : PEARSON'S CORRELATION ANALYSIS OF OPG AND CREATININE IN CONTROLS

Correlations

		OPG	CREATININE
OPG	Pearson Correlation	1	.551**
	Sig. (2-tailed)		.001
	N	35	35
CREATININE	Pearson Correlation	.551**	1
	Sig. (2-tailed)	.001	
	N	35	35

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE NO 16 : PEARSON'S CORRELATION ANALYSIS OF OPG AND TOTAL CHOLESTEROL IN CONTROLS

Correlations

		OPG	CHOLESTEROL
OPG	Pearson Correlation	1	.566**
	Sig. (2-tailed)		.000
	N	35	35
CHOLESTEROL	Pearson Correlation	.566**	1
	Sig. (2-tailed)	.000	
	N	35	35

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE NO 17 : PEARSON'S CORRELATION ANALYSIS OF OPG AND TGL IN CONTROLS

Correlations

		OPG	TGL
OPG	Pearson Correlation	1	.661**
	Sig. (2-tailed)		.000
	N	35	35
TGL	Pearson Correlation	.661**	1
	Sig. (2-tailed)	.000	
	N	35	35

**. Correlation is significant at the 0.01 level (2tailed).

TABLE NO 18 : PEARSON'S CORRELATION ANALYSIS OF OPG AND eGFR IN CONTROLS

Correlations

		OPG	eGFR
OPG	Pearson Correlation	1	-.748**
	Sig. (2-tailed)		.000
	N	35	35
eGFR	Pearson Correlation	-.748**	1
	Sig. (2-tailed)	.000	
	N	35	35

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE NO 19 : PEARSON'S CORRELATION ANALYSIS OF OPG AND LDL IN CONTROLS

Correlations

		OPG	LDL
OPG	Pearson Correlation	1	.471*
	Sig. (2-tailed)		.004
	N	65	65
LDL	Pearson Correlation	.471*	1
	Sig. (2-tailed)	.004	
	N	65	65

*. Correlation is significant at the 0.05 level (2-tailed).

BIVARIATE CORRELATION WITHIN CKD GROUP

Pearson correlation

	SERUM OPG
eGFR	-0.748
Creatinine	0.551
Total cholesterol	0.566
TGL	0.661
LDL	0.471

Correlation of serum OPG with eGFR, creatinine, total cholesterol and TGL was determined by Pearson Correlation Analysis in control group. All patients with CKD, showed a positive correlation between serum OPG with Creatinine, total cholesterol and TGL ($p < 0.01$) as shown in tables 15, 16, 17 & 19. From the table no 18, it is found that, correlation analysis by Pearson method, demonstrates an inverse relation between serum OPG and eGFR ($r = - .748$, $P<0.01$)

CHART NO 9 : CORRELATION OF OPG WITH eGFR IN CASES

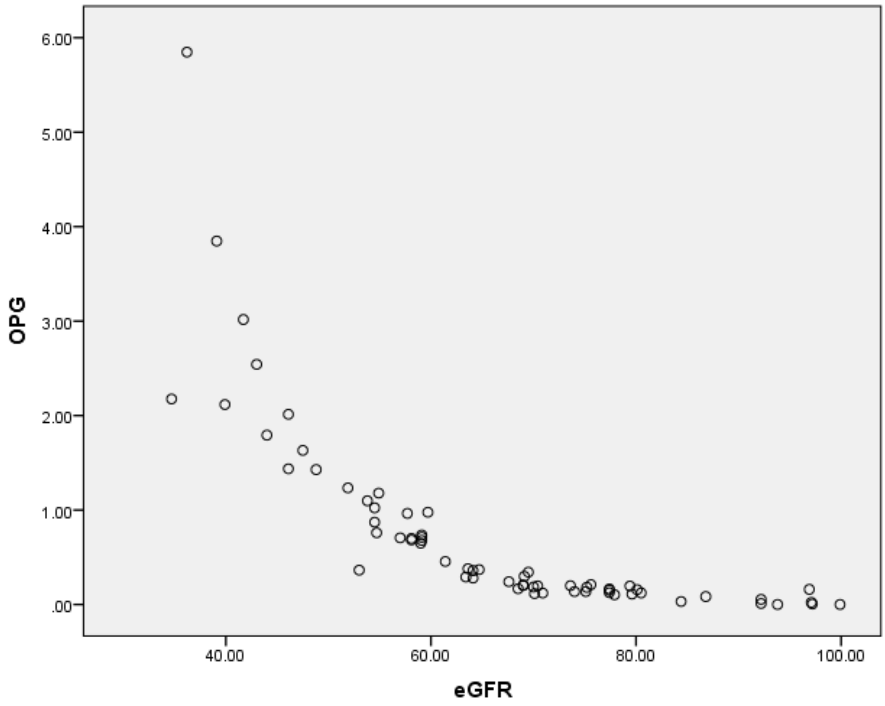


CHART NO 10 : CORRELATION OF OPG WITH eGFR IN CONTROLS

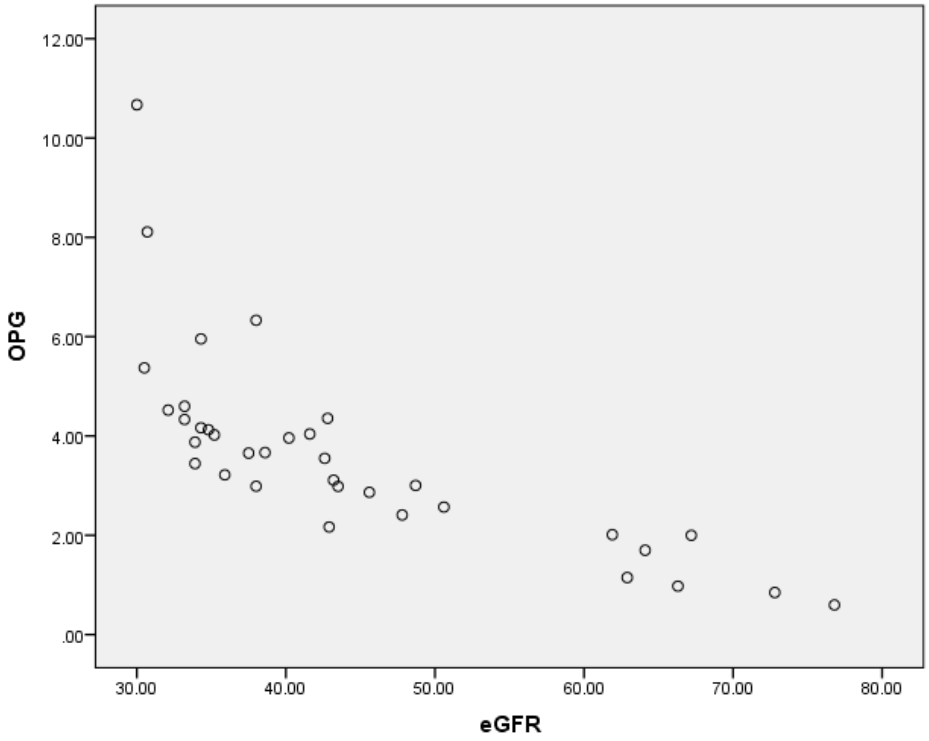


TABLE NO 20 : LINEAR REGRESSION ANALYSIS

	CKD PATIENTS	HYPERTENSIVE PATIENTS
95% Confidence Intervals		
Slope	-0.07976 to 0.008331	-0.05799 to -0.03715
Y-intercept	3.007 to 9.039	3.167 to 4.582
X-intercept	111.0 to +infinity	77.17 to 87.28
R squared	0.07618	0.5691
F	2.721	83.22
DFn, DFd	1, 33	1, 63
p value	0.1085	<0.0001
Deviation from zero?	Not Significant	Significant

CHART NO 11 : LINEAR REGRESSION ANAYSIS

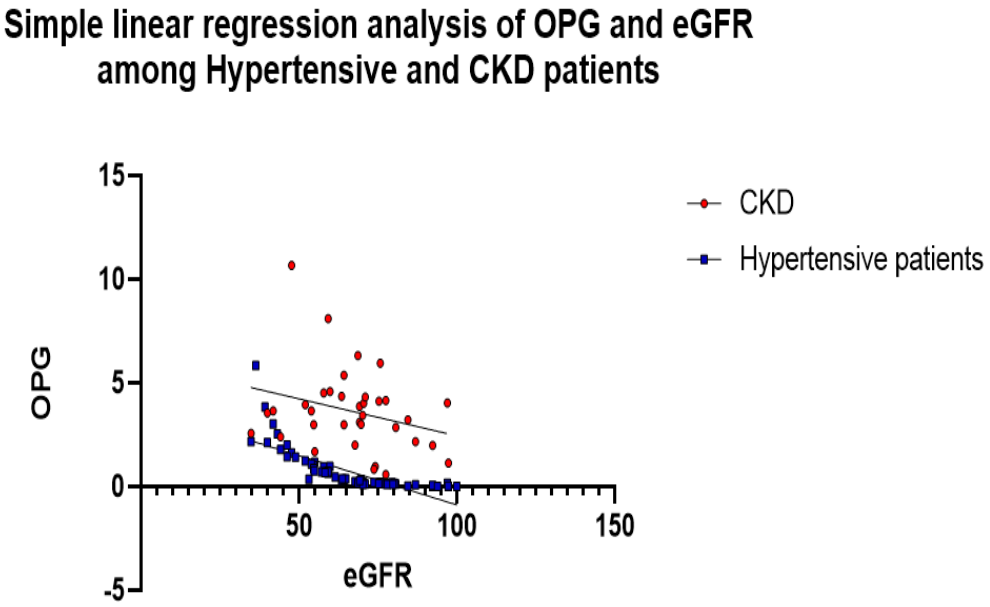
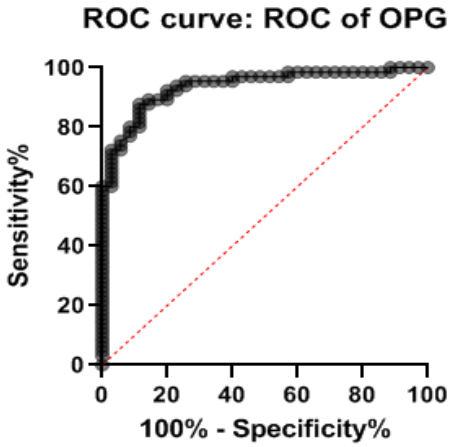


CHART NO 12: RECEIVER OPERATING CHARACTERISTIC CURVE



Area under the ROC curve	
Area	0.9378
Std. Error	0.02337
95% confidence interval	0.8920 to 0.9836
p value	<0.0001

Data	
Controls (OPG CKD)	35
Patients (OPG HT)	65

DISCUSSION

DISCUSSION

Chronic Kidney Disease is becoming an emerging public health problem in recent times due to high prevalence of non-communicable diseases,[50] most commonly diabetes and hypertension. It often remains asymptomatic until late stage and intervention becomes unsuccessful in reducing the renal damage. Henceforth, it's hightime in search for an early marker that might aid in diagnosis and prognosis of CKD among hypertensive patients in earlier stages, as far as this study is concerned.

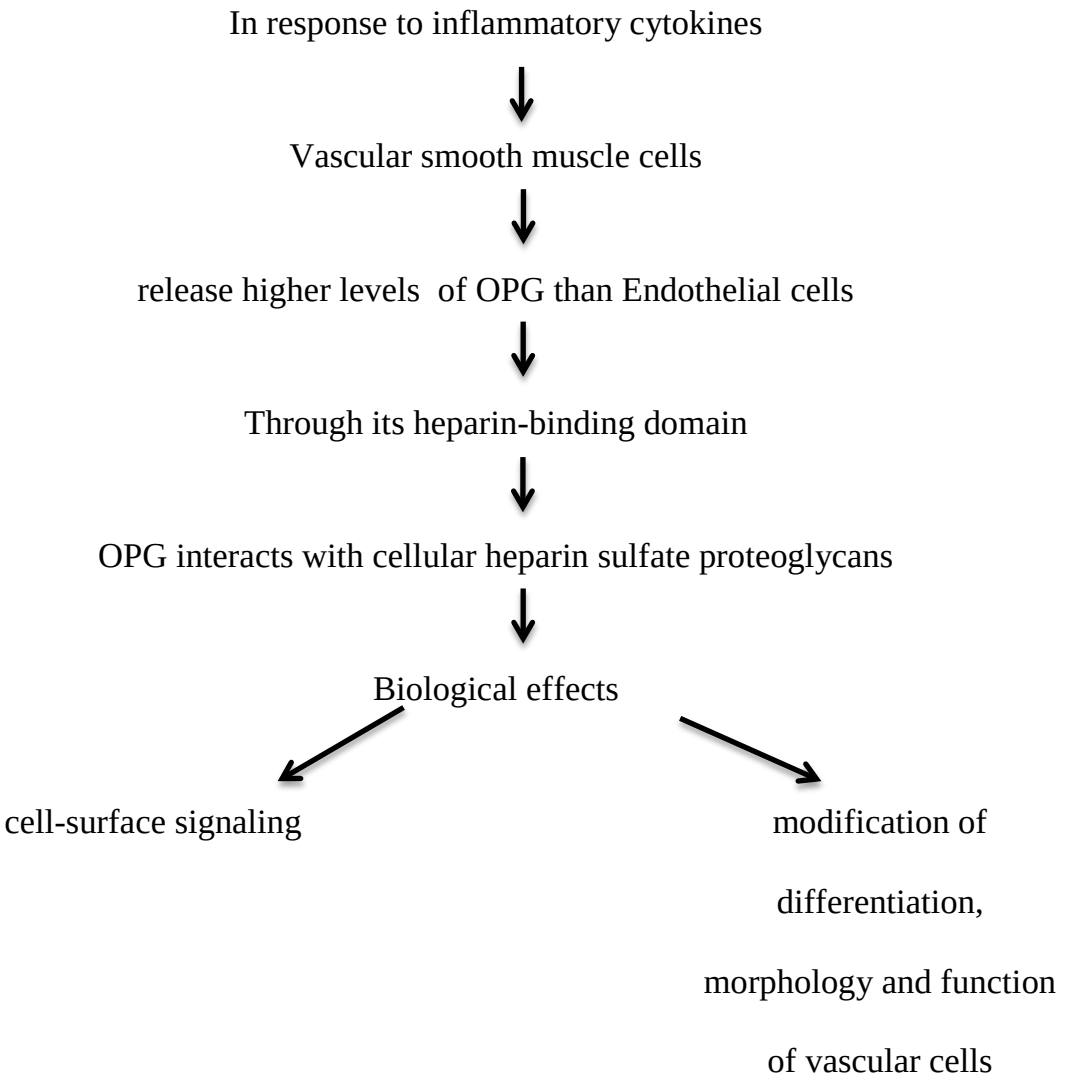
Hypertension is considered both a cause and effect of CKD and contributes to the progression of disease. As eGFR declines, the incidence and severity of hypertension increase.[51] This is in concordant with our study, showing significant association of declining eGFR with progression of hypertension.

Additionally, hypertension and CKD are both independent risk factors for cardiovascular disease.[50] When both exist together the risks of CVD morbidity and mortality are substantially increased.

OPG is mainly released from cells of skeletal and vascular system. OPG levels increases with the advancing stages of CKD.[32]

Olesen et al., in his study showed high OPG concentration in circulation was mainly due to injury to vascular system, as a result of inflammatory events.[52]

OPG/RANKL system effects on vascular system[53][54, 55]



Our study shows a significant association of circulating OPG levels with the progression of renal decline in CKD which is in concordance with the study by Li yang et al, in which there is an evidence showing the increased risk of chronic kidney disease with elevations in blood pressure. The risk of developing CKD was 1.28 times higher in patients with prehypertension than it was in individuals with normal blood pressure.[56]

The co-existence of hypertension and dyslipidemia prevalence is in the range of 15 to 31%. The co-existence of these two risk factors adversely affects the vascular endothelium, which accelerates atherosclerosis, leading to CVD. [57] Various metabolites, involved in fatty acid metabolism, alanine, aspartate and glutamate metabolism, glycerophospholipid metabolism are implicated in insulin resistance, macrophage activation, vascular remodeling and oxidized LDL formation. These are directly related to lipid profile abnormalities in hypertension.[58]

Dyslipidemia , a risk factor for adverse renal outcome in CKD especially in stages 3–5. In patients with CKD, the abnormal lipoprotein metabolism results in dyslipidemia, including hypertriglyceridemia, increased triglyceride-rich lipoprotein remnants, reduced HDL-cholesterol and increased lipoprotein.[59] The pathophysiological basis underlying dyslipidemia and CKD is related to the aggravation of atherosclerosis in the renal microcirculation, deposition of lipoprotein in glomerular structures and stimulation of cytokines and growth factors involved in inflammation and fibrogenesis.

In our study, marked dyslipidemia is observed among CKD, which is in concordant with the observations of Khurana et al.,[60] profound dysregulation of lipid metabolism among CKD patients has a potential impact on cardiovascular disease and energy metabolism.

Lee et al., in their study showed elevated OPG levels and its association with vascular calcification in hemodialysis patients , that in turn associated with increased risk of CV mortality in dialysis patients. High serum OPG to be an independent predictor of CV events in CKD, especially on dialysis patients [61] Hence, elevated OPG in CKD group, in our study can have a role in predicting cardiovascular events and mortality, that warrants a long term follow up.

In our study, the increase in serum osteoprotegerin levels with decreasing kidney function, was found to be correlated negatively with eGFR and positively with serum creatinine. This is well evident from the scatter plot between eGFR and OPG in Hypertensive patients.

Linear regression analysis perfomed in our study as shown in chart 11, among hypertensive patients showed a significant inverse relationship between eGFR and OPG with p value < 0.0001, which suggests that OPG can be a predictor of renal dysfunction among hypertensive patients.

Serum OPG levels were increased with impairment of renal function. In our study, we performed ROC curve analysis between hypertensive patients and patients with CKD. In ROC, area under the curve is 0.9378 with 95% CI, with optimal cut-off value of OPG 2.143 ng/ml. It has 93 % sensitivity and 80% specificity. Hence, OPG can be used as an early marker for decline in renal function in hypertensive patients.

CONCLUSION

CONCLUSION

Our study suggest that OPG plays a significant role in CKD progression among hypertensive patients. So, the estimation of serum OPG level may aid in early diagnosis of kidney dysfunction and also in predicting the progression of disease.

LIMITATIONS

LIMITATIONS

- Small sample size of the study.
- Large cohort studies are warranted to confirm the clinical significance of OPG in prediction of kidney dysfunction in hypertensive patients.
- Other chronic kidney disease markers like Cystatin C would have been measured.

FUTURE PROSPECTS OF THE STUDY

FUTURE IMPLICATIONS FOR GLOBAL HEALTH

In contrast to the natural decline and successful eradication of many devastating infectious diseases, there is rapid growth in the prevalence of metabolic and vascular disease in developing countries. Non communicable diseases are becoming increasingly prevalent in these countries, for which lifestyle changes such as physical inactivity, dietary habits and weight gain may have an impact. Therefore, it follows that there will be a proportionate increase in vascular and renal disease. Futuristic plans must be focused for improved screening for early detection, prevention and treatment and must start considering options for improved availability of renal replacement therapies.

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ANNEXURES

GOVERNMENT MOHAN KUMARAMANGALAM

MEDICAL COLLEGE & HOSPITAL
SALEM, TAMILNADU
College: Phone No.0427-2383313 Fax No:0427-2383193
E-Mail ID: deangmkmcslm@gmail.com
Hospital: Phone No: 0427 - 2210674, 2210757 Fax : 0427 - 2210876
E-Mail ID: msgmkmchsalem@gmail.com

Communication of Decision of the Institutional Ethics Committee(IEC)

Ref. No. GMKMC&H/4341/IEC/02/2018-74 **Date: 15.11.2018**

Protocol title	"SERUM OSTEOPROTEGERIN-CAN IT PREDICT CHRONIC KIDNEY DISEASE AMONG HYPERTENSIVES?"
Guide/Principal Investigator	Dr.R.RANGARAJAN, MD (BIOCHEMISTRY) Head of the, Dept. of Biochemistry, GMKMCH, Salem-01
Primary Investigator	Dr.C.JANANI, First year Post Graduate MD (BIOCHEMISTRY), GMKMC, Salem-30
Name & Address of Institution	Govt. Mohan Kumaramangalam Medical College & Hospital, Salem, Tamil Nadu.
Type of Review	☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y)	09.10.2018
Date of previous review, if revised application:	Nil
Decision of the IEC	☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:	Nil
Recommended for a period of :	From December 2018 to November 2019

Please note *

- Inform IEC immediately in case of any Adverse events and Serious adverse events.
- Inform IEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above. Annual report to be submitted to IEC.
- Members of IEC have right to monitor the trial with prior intimation.

Signature of Member Secretary

VICE PRINCIPAL
Govt. Mohan Kumaramangalam
Medical College,
SALEM - 636 030.

By E-Mail / RPAD

From

Dr.R. BALAJINATHAN, MD.,
Dean,
Govt. Mohan Kumaramangalam
Medical College, Salem – 30.

To

The Controller of Examination,
The Tamil Nadu Dr.M.G.R. Medical University,
No.69, Anna Salai,
Guindy, Chennai – 600 023.

Ref.No.1697/MEI(PG)/2018, Dated :16.12.2020

Sir,

Sub : Medical Education – Govt. Mohan Kumaramangalam Medical College, Salem – 3rd year Post Graduate Students in Biochemistry of this College – Requesting in change of Dissertation Guide – Requisition submitted – Forwarded - Regarding.

Ref : Application submitted by the individuals dated 01.12.2020.

****

I am forwarding herewith the following Post Graduate Students studied in Md (Biochemistry) of this College, requesting to change the name of the guide in their dissertation, for your kind perusal and necessary action.

1) Dr.V. Hariraj
2) Dr.M. Ganesan
3) Dr.C. Janani

This is for your kind information and necessary further action.

DEAN
16/12/2020

Encl: Original Requesting letters – 3

Copy to the above individuals

..... through the HOD of Biochemistry of this College.

Copy to the HOD of Biochemistry of this College.

PROFORMA

"SERUM OSTEOPROTEGERIN- Can it predict Chronic Kidney Disease among hypertensives?"

Name: Age/sex: Op/Ip no:

Presenting complaints :

Past History : DM HT Thyroid Epilepsy TB

Any systemic illness causing renal damage

Congenital/intrinsic renal damage

Previous History of Surgery: Yes / No

Treatment History :

Antihypertensive medications – Yes /no

Drug details

Duration

Personal History:

Menstrual History:

General Examination:

Pallor / Icterus / Pedal Edema

BP: Wt: Ht:

System Examination:

P/A: CVS : RS: CNS:

Lab investigations :

Hb %

Blood sugar(mg/dl)

Blood urea (mg/dl)

Sr. creatinine (mg/dl)

Sr. calcium (mg/dl)

Sr. total protein (g/dl)

Sr. albumin (g/dl)

Sr. uric acid (mg/dl)

Total cholesterol(mg/dl)

TGL (mg/dl)

HDL (mg/dl)

cLDL (mg/dl)

Sr. Osteoprotegerin levels(ng/ml)

eGFR

Stage of CKD

PATIENT CONSENT FORM

STUDY TITLE: ***"SERUM OSTEOPROTEGERIN - Can it predict Chronic Kidney Disease among hypertensives?"***

DEPARTMENT OF BIOCHEMISTRY, GMKMCH, SALEM

PARTICIPANT NAME: AGE: SEX: IP.NO

I confirm that I have understood the purpose of the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.

I have been explained about the possible complications that may occur during and after medical procedure. I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason.

I understand that investigator, regulatory authorities and the ethical committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study. I hereby consent to participate in this study.

Time :	Patient name;
Date :	Signature / Thumb Impression
Place :	Name and signature of the Investigator

ஆராய்ச்சி ஒப்புதல் படிவம்

பெயர்	:	தேதி	:
வயது	:	உள்நோயாளி எண்	:
பாலினம்	:	ஆய்வு சேர்க்கை எண்	:

இந்த ஆய்வின் நோக்கம் மற்றும் விவரங்கள் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது. இவ்ஆயிவில் இருந்து நான் எந்த நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எனக்கு எந்த பாதிப்பும் இல்லை என்பதையும் தெளிவாக புரிந்து கொண்டேன்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆய்வின் போதோ என்னுடைய பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டார்கள் என்பதையும் அறிந்து கொண்டேன்.

இந்த ஆய்வில் எவ்வித நிர்பந்தமும் இன்றி எனது சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகின்றேன்.

நான் சுயநினைவுடனும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் சேர்த்துக் கொள்ள சம்மதிக்கின்றேன்.

ஆராய்ச்சியாளர் ஒப்பம் **பங்கேற்பாளர் ஒப்பம் (அ)**

இடது பெருவிரல் ரேகை

MASTER CHART - CASES

S. No	age	sex	BMI	HT (YRS)	HT (mm Hg)	glucose	urea	creat	t.chol	TGL	HDL	LDL	T.pro	alb	cal	UA	eGFR	OPG
1	53	M	23.50	New	140/100	110	14	0.9	179	115	77	79	5.7	3.5	10.8	2.7	97.2	0.005
2	64	F	30.31	New	130/90	78	15	0.9	151	104	65	65.2	5.8	3.1	10.5	4.4	67.6	0.241
3	40	F	24.14	New	140/90	92	31	0.8	166	147	53	83.6	4.9	2.9	6.8	3.3	92.2	0.011
4	65	F	35.54	New	150/100	90	32	0.8	213	172	65	113.6	6.8	3.3	7	5.8	77.4	0.165
5	59	F	35.20	New	130/80	121	26	1.1	189	220	48	97	5.9	2.6	6.8	3.9	54.9	1.179
6	62	M	25.20	New	130/90	103.6	31	1.07	165	243	31.7	84.7	7.08	3.95	8.8	4.6	74	0.137
7	43	M	21.09	New	140/90	118	38	1.2	186	234	53	86.2	7.7	4.2	6.6	3.6	73.6	0.199
8	51	M	34.22	New	140/100	86	29	1	206	204	56	109.2	7.3	3.4	6.5	3.8	86.8	0.084
9	37	F	33.32	6 months	140/80	99	22	1.31	245	354	48	126.2	5.9	2.8	9.1	3.6	51.9	1.234
10	60	F	33.33	6 months	160/100	85	31	1.6	245	311	39	143.8	6	2.9	10.2	4.5	34.7	2.176
11	60	F	24.67	6 months	130/90	116	26	1.1	215	181	53	125.8	7.6	3.7	7.6	4.1	54.5	1.023
12	39	F	24.14	7 months	140/90	98	30	0.9	259	289	55	146.2	7.3	4.1	8.3	4.2	80.5	0.122
13	61	F	30.33	8 months	140/80	129	24	0.9	179	230	48	85	6.8	3.5	6.8	3.9	69	0.206
14	33	F	37.53	9 months	160/90	106	22	0.8	213	172	65	113.6	6.8	3.3	7	5.6	96.9	0.16
15	63	F	27.33	1	120/80	121	27	1.4	242	237	43	151.6	5.8	2.7	8.9	6.7	39.9	2.117
16	60	F	19.66	1	150/90	197	31	0.9	191	308	48	81.4	7.7	3.9	8.8	5.1	69.5	0.342

S. No	age	sex	BMI	HT (YRS)	HT (mm Hg)	glucose	urea	creat	t.chol	TGL	HDL	LDL	T.pro	alb	cal	UA	eGFR	OPG
17	62	F	27.76	1	170/90	121	24	1.1	215	181	53	125.8	7.6	3.7	7.6	5.5	53.8	1.098
18	49	M	26.72	2	170/100	112	31	1.3	194	229	61	87.2	6.8	3.8	7.8	4.4	64.1	0.279
19	59	F	27.88	2	140/90	79	37	1.32	195	179	47.9	111.3	6.9	4.3	9.5	5.7	44	1.793
20	64	M	30.47	2	150/100	102	21	1.3	197	215	26	128	6.4	3.1	10	5.1	57.7	0.964
21	61	F	23.63	2	140/100	119	14	0.9	198	200	42	116	5.7	3.3	10.8	4.2	69	0.201
22	55	M	23.05	2	130/80	95	26	1	185	163	63	89.4	6.1	3.3	10.5	3.1	84.4	0.032
23	40	F	26.70	3	160/100	119	22	1	207	265	52	102	6.4	3.7	7.5	3	70.4	0.197
24	35	M	26.39	3	140/90	98	34	1.31	245	354	48	126.2	5.9	2.8	9.1	6.5	70	0.185
25	65	M	31.63	3	120/80	107	26	1.09	216	151	55.7	130.1	7.6	4.1	7.1	5.5	70.9	0.121
26	64	M	29.21	3	130/80	158	27	1.7	176	100	57	99	7.3	3.4	7.5	3	41.7	3.017
27	59	M	24.85	3	180/110	119	31	1.3	220	424	37	98.2	7.1	3.3	9	4.5	59.7	0.976
28	55	M	26.23	3	110/80	97	26	1.1	213	258	49	112.4	6.3	2.9	7	5.8	75.2	0.181
29	55	F	18.19	3	120/80	83	24	1	207	256	70.5	85.3	7.6	3.9	9.1	6.5	63.4	0.291
30	62	F	28.35	4	200/120	121	21	0.9	191	411	41	67.8	7.1	3.8	10.2	4	68.5	0.167
31	37	F	32.46	4	150/90	73	19	1.1	151	213	36	72.4	5	3.3	7.8	5.5	64.1	0.359
32	51	F	30.84	4	150/90	123	26	1.3	208	213	46.8	118.6	7.9	4	7.8	6.4	47.5	1.631
33	65	F	24.45	4	130/90	60	15	0.8	225	120	45	156	5.3	3.1	10.2	2.2	77.4	0.151

S. No	age	sex	BMI	HT (YRS)	HT (mm Hg)	glucose	urea	creat	t.chol	TGL	HDL	LDL	T.pro	alb	cal	UA	eGFR	OPG
34	48	F	21.02	4	160/100	98	16	0.9	219	149	50	139.2	5.7	2.9	9.6	4.2	75.6	0.21
35	65	F	25.78	4	120/80	125	23	1	161	125	57	79	7.4	3.9	9.5	5.9	59.1	0.679
36	52	M	25.31	5	130/80	73	31	1.07	165	243	31.7	84.7	7.08	3.95	8.8	4.8	79.4	0.196
37	60	F	19.81	5	140/90	94	21	0.8	209	138	55	126.4	6.5	3.4	10.9	3.4	80.1	0.157
38	65	F	25.68	5	170/100	96	26	1	160	82	59	84.6	5.9	3.9	9.5	5.9	59.1	0.736
39	52	f	22.48	5	150/90	108	24	1	186	196	74	72.8	6.2	3.4	6.5	3.8	64.7	0.369
40	65	M	31.60	5	150/90	87	21	1.1	151	213	36	72.4	5	3.3	7.8	6.4	70.1	0.115
41	60	F	27.23	5	150/100	101	19	0.8	175	110	59	94	7.2	3.5	7.8	4.4	77.4	0.125
42	60	M	29.76	5	170/90	86	26	1.6	221	110	47	152	6.1	3.2	10.8	2.8	46.1	2.013
43	58	M	21.00	5	150/90	97.6	19	1.32	195	179	47.9	111.3	6.9	4.3	9.5	4.2	59	0.649
44	60	F	34.69	6	120/70	232	29	1.1	195	474	37	63.2	7.1	3.7	10.1	4	54.5	0.871
45	63	M	22.83	6	150/90	93	24	1.3	195	169	57	104.2	6.9	4.3	9	4.5	58.1	0.699
46	65	F	26.89	6	190/100	115	26	1	186	137	34	124.6	7.1	3.8	7.1	5.5	59.1	0.712
47	55	M	32.43	6	190/130	102	37	1.3	154	109	49	83.2	7.4	4.3	6.2	4.2	61.4	0.455
48	50	M	26.92	6	160/90	104	34	1.3	229	189	46	145.2	6.2	3.1	8.8	4.3	63.6	0.379
49	52	M	25.51	6	145/95	69	29	1.2	264	299	39	165.2	6.3	2.9	10.5	4.1	69.1	0.298
50	60	M	21.63	7	150/90	98	24	0.8	166	147	53	83.6	4.9	2.9	6.8	3.9	97.1	0.022

S. No	age	sex	BMI	HT (YRS)	HT (mm Hg)	glucose	urea	creat	t.chol	TGL	HDL	LDL	T.pro	alb	cal	UA	eGFR	OPG
51	50	M	29.01	7	150/90	94	30	1.1	189	220	48	97	5.9	2.6	6.8	5.5	77.9	0.103
52	47	M	27.91	7	160/100	89	31	1.5	269	203	42	186.4	5.9	2.8	9.5	4.2	54.7	0.759
53	65	F	30.45	7	170/100	129	29	1.3	237	178	48	153.4	6.2	3	8.5	3.9	43	2.542
54	65	F	25	7	170/90	120	22	1.03	269	319	40	165.2	6.5	3.1	10.1	4	57	0.706
55	47	M	21.35	7	150/90	112	35	1.5	247	221	49	153.8	7.1	3.8	7.6	4.2	53	0.364
56	63	F	19.14	7	150/100	96	24	0.7	205	170	34	137	7.2	3.4	6.2	4.2	92.2	0.054
57	49	F	28.76	7	130/90	73	23	0.9	268	204	62	165.2	7.3	4.1	8.2	4.6	75.1	0.136
58	63	M	22.05	8	150/90	107	19	1.3	143	161	42.4	68.4	7.3	3.9	8.9	6.7	58.1	0.682
59	58	M	26.57	8	130/90	102	26	0.9	191	308	48	81.4	7.7	3.9	8.8	6.2	93.8	0
60	49	M	24.15	8	140/90	112	17	0.9	189	263	38	98.4	6.1	3	7.9	4.1	99.9	0
61	61	F	23.49	9	180/100	121	21	0.8	221	342	58	94.6	6.8	3.5	11.3	3	79.6	0.11
62	54	F	28.22	9	150/100	101	27	1.6	245	311	39	143.8	6	2.9	10.2	4	36.2	5.848
63	63	M	24.80	10	160/90	123	32	1.5	179	386	36	65.8	7.5	4	6.8	3.3	48.8	1.428
64	55	F	26.70	10	140/90	91	26	1.3	295	219	42	209.2	6.1	3.6	6.6	3.6	46.1	1.437
65	43	F	29.70	20	150/100	107	28	1.6	268	204	62	165.2	7.3	4.1	8.2	4	39.1	3.847

MASTER CHART - CONTROLS

S. No	Age	Sex	HT-(Yrs)	HT (mm Hg)	Glucose	Urea	Creat	t.chol	TGL	HDL	LDL (cal)	T.pro	Alb	Cal	UA	eGFR	OPG	Stage
1	48	F	7	140/90	121	48	1	189	231	49	93.8	5.8	2.9	7.9	4.4	62.9	1.149	II
2	42	F	6	180/100	93	43	1.1	206	138	45	133.4	7.2	4.2	8.8	5.5	61.9	2.012	II
3	56	M	4	155/100	87	59	1.2	193	158	39	122.4	6.9	3.6	9.1	4.3	67.2	1.999	II
4	52	M	6	160/100	110	44	1.1	211	163	32	146.4	7.6	3.8	7.5	4.1	76.8	0.597	II
5	49	M	8	140/90	101	46	1.3	267	172	44	188.6	7.1	3.7	7.3	3.7	64.1	1.699	II
6	58	M	4	140/100	95	51	1.2	230	136	38	164.8	7.9	3.9	8.2	7.2	66.3	0.976	II
7	39	M	6	160/100	88	54	1.24	255	172	41	179.6	6.3	2.9	8.6	4.9	72.8	0.847	II
8	49	F	5	150/90	94	43	1.42	273	134	41	205.2	6.5	3.5	7.6	5.5	42.9	2.168	III - B
9	62	F	7	140/90	123	53	1.6	256	163	40	183.4	6.8	3.6	7.9	5.7	40.2	3.96	III - B
10	58	M	3	150/90	106	39	1.5	243	136	36	179.8	7.2	4.1	8.2	4.6	50.6	2.569	III - A
11	48	M	6	140/100	70	42	1.8	193	116	38	131.8	7.1	4.2	7.2	4.9	43.5	2.985	III - B
12	44	F	4	180/110	96	54	1.4	183	132	36	120.6	7.3	4.6	7.1	6.1	45.6	2.865	III - A
13	49	M	6	170/100	89	49	1.8	210	175	39	136	7.6	4.3	8.3	4.2	43.2	3.11	III - B
14	34	F	6	160/100	118	43	1.6	206	157	45	129.6	7.1	4.2	9.2	4.1	41.6	4.04	III - B
15	51	M	9	150/90	117	49	1.8	239	165	42	164	7	3.9	6.6	5.2	42.6	3.55	III - B

S. No	Age	Sex	HT-(Yrs)	HT (mm Hg)	Glucose	Urea	Creat	t.chol	TGL	HDL	LDL (cal)	T.pro	Alb	Cal	UA	eGFR	OPG	Stage
16	42	M	8	140/90	92	52	1.7	271	172	40	196.6	6.9	3.8	10.1	3.9	48.7	3.004	III - A
17	60	F	8	160/100	76	40	1.5	227	202	42	144.6	6.5	3.2	9.5	4	37.5	3.654	III - B
18	58	F	14	150/90	84	39	1.5	301	196	39	222.8	6.7	3.6	6.6	4.3	38	2.987	III - B
19	55	M	10	130/80	89	56	1.6	296	173	36	225.4	6.8	3.5	8.9	6.3	47.8	2.41	III - A
20	65	M	9	160/100	82	74	2.1	268	209	40	186.2	7.3	4.2	9	4.6	32.1	4.521	III - B
21	63	M	13	160/100	96	69	2.3	364	220	35	285	6.5	3.6	7.8	5.3	33.9	3.876	III - B
22	49	M	6	150/90	111	58	2.1	264	187	37	189.6	5.9	2.9	6.5	5	35.9	3.217	III - B
23	58	F	10	160/100	78	76	1.6	271	169	39	198.2	6.3	3.4	7.1	5.2	35.2	4.021	III - B
24	49	M	7	150/100	86	63	2.2	326	203	32	253.4	6.7	3.4	7.5	4.3	33.9	3.444	III - B
25	52	M	8	140/90	91	59	2.2	356	236	37	271.8	6.9	3.3	6.2	4.7	33.2	4.333	III - B
26	56	M	9	140/100	101	47	1.9	341	264	36	252.2	7.3	4	6.8	5.2	38.6	3.666	III - B
27	60	M	4	130/90	79	52	2.1	296	243	43	204.4	7.2	4.2	7.7	4.6	33.2	4.597	III - B
28	49	F	11	140/90	84	49	1.7	240	224	49	146.2	6.9	3.6	8.8	4.7	34.8	4.125	III - B
29	53	F	16	150/90	110	59	1.4	271	239	48	175.2	6.4	3.2	9	4.5	42.8	4.356	III - A
30	58	M	9	140/90	96	51	1.9	298	243	54	195.4	5.9	2.9	7.4	5	38	6.33	III - B
31	64	M	13	150/100	83	47	4	271	219	43	184.2	5.5	2.5	8.6	5.6	30.5	5.371	III - B

S. No	Age	Sex	HT-(Yrs)	HT (mm Hg)	Glucose	Urea	Creat	t.chol	TGL	HDL	LDL (cal)	T.pro	Alb	Cal	UA	eGFR	OPG	Stage
32	59	F	10	140/90	97	55	1.9	365	257	37	276.6	6.6	3.7	6.9	5.1	30	10.67	III - B
33	64	M	9	150/100	79	48	2	229	168	44	151.4	6.5	3.9	7.6	6.2	34.3	4.165	III - B
34	61	M	7	140/90	88	55	2.1	286	218	39	203.4	7	4	8.1	4.1	34.3	5.954	III - B
35	63	M	11	150/100	73	49	2.3	325	308	42	221.4	7.3	4.1	7.2	4.3	30.7	8.11	III- B