"ESTIMATION AND COMPARISON OF eGFR USING SERUM CYSTATIN C AND SERUM CREATININE FOR DETECTION OF EARLY NEPHROPATHY IN TYPE 2 DIABETES MELLITUS "

By

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In partial fulfillment of the requirements for the degree of

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IN

GENERAL MEDICINE

Under the Guidance of

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Date : **Dr. ABHISHEK KUMAR VERMA**

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ABSTRACT

ESTIMATION AND COMPARISON OF eGFR USING SERUM CYSTATIN C AND SERUM CREATININE FOR DETECTION OF EARLY NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

INTRODUCTION:

Type 2 diabetes is a common and chronic metabolic condition characterised by insulin resistance, deficiency or both and is usually irreversible. It affects more than 120 million people worldwide and is estimated to affect 370 million people by the year 2030. Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent microalbuminuria in concomitance with insulin- or non–insulin dependent diabetes. Diabetic nephropathy is currently one of the leading causes of morbidity and mortality in the diabetic population, accounting for the greatest proportion of end stage renal disease worldwide. The management of patients with diabetes and End stage renal disease contributes significantly to health care costs. This study was undertaken to evaluate the efficacy of this new molecule in assessing renal dysfunction at a phase when timely interventions can be instituted and the progression of nephropathy can be delayed.

OBJECTIVES:

1. To estimate serum cystatin C based eGFR and serum creatinine based eGFR in type 2 diabetes mellitus patients.
2. To detect early nephropathy using the above findings in type 2 diabetes mellitus patients.
3. To compare diagnosis of early nephropathy in Type 2 DM by using serum cystatin C based eGFR and serum creatinine based eGFR.

MATERIALS AND METHODS:

This is a observational prospective study. 42 patients with type 2 diabetes mellitus meeting the inclusion and exclusion criteria's admitted to R.L.Jalappa Hospital and Research center attached to Sri Devaraj Urs Medical college, Tamaka, Kolar during March 2016 to March 2017 were included in the study. The data was collected based on detailed history and clinical examination done as per the proforma along with investigations FBS,PPBS,HbA1c, serum creatinine and serum cystatin-C.

RESULTS:

Total of forty two patients satisfying inclusion criteria were included in study. The mean duration of diabetes in the study population was 8.3 years and median age of patients was 61.50 years. Males were predominant than females and majority of study group constituted by more than 60 years of age. The mean serum creatinine was 0.70 mg/dl ranging from 0.721 ± 0.256 and eGFR based on serum creatinine was in the range of 122 ± 17.96 ml/min/1.73 m^2 . The mean serum cystatin-c was 1.0 mg/dl ranging from 0.75 ± 1.20 and Mean eGFR based on serum cystatin-c was 80 ml/min/1.73m^2 with range of 81.14 ± 16.53 ml/min/1.73m^2. 37 patients had eGFR >90 ml/min/1.73m^2 based on serum creatinine. Out of whom, 26 patients had normoalbuminuria, 9 had microalbuminuria and 2 had macroalbuminuria. 5 patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine. Out of which, 3 had microalbuminuria and 2 had macroalbuminuria. 16 patients had eGFR >90 ml/min/1.73m^2 based on serum cystatin-c and all of them had normoalbuminuria. 26 patients eGFR between 60-89 ml/min/1.73 m^2, out of which, 10 patients who had normoalbuminuria, 12 patients had micro albuminuria and 4 patients had

macroalbuminuria. we found most of the patients who had normal serum creatinine based eGFR and normoalbuminuria had diabetic nephropathy (CKD stage 2) by using serum cystatin C. This study shows serum cystatin-c is highly sensitivity compared to serum creatinine to detect early diabetic nephropathy.

CONCLUSION:

Our results shows serum cystatin-C is more sensitive as compared to serum creatinine as serum cystatin-c which could detect diabetic nephropathy (CKD stage 2) in patients who had normoalbuminuria and normal eGFR based on serum creatinine. Serum Cystatin C appears to hold promise in predicting early renal dysfunction and more so as an indicator of overt nephropathy.

ABBREVATIONS

ACE	Angiotensin Converting Enzyme
ACE-I	Angiotensin Converting Enzyme Inhibitor
ACR	Albumin creatinine ratio
AF	Atrial Fibrillation
AGE	Advanced Glycation End products
ARB	Angiotensin Receptor Blocker
AT-II	Angiotensin II
CG formula	Cock croft Gault fofrmula
CKD	Chronic Kidney Disease
COPD	Chronic Obstructive Pulmonary Disease
COX-2	Cyclooxygenase-2
CSF	CerebroSpinal Fluid
CVD	Cerebrovascular Disease
CYS C	Cystatin c
DCCT	Diabetes control and complications trial
DKD	Diabetic Kidney Disease
DM	Diabetes Mellitus
DTPA	Diethylene Triamine Pentaacetic Acid
ECM	Extracellular matrix
EDTA	Ethylene Diamine Tetra Acetic acid
eGFR	estimated Glomerular Filtration Rate
ESRD	End stage renal disease
FDA	Food and Drug Administration
GFR	Glomerular Filtration Rate
HbA1c	Glycosylated Hemoglobin
HMGCoA	3-hydroxy-3-methylglutaryl-coenzyme A
HTN	Hypertension
IgG	Immunoglobulin G
IHD	Ischemic Heart disease
Kf	Filtration fraction

mRNA	messenger RiboNucleic Acid
MDRD	Modification of Diet in Renal Disease outcome prevention evalution
MICRO-HOPE	Micro albuminuric heart
NaCl	Sodium Chloride
NKF –KDOQI	National Kidney Foundation kidney disease dialysis outcomes quality initiatives.
NICE	National institute for health and care excellence
NIDDM	Non insulin dependent diabetes mellitus
NPDR	Non Proliferative Diabetic Retinopathy
NSAID	Non Steroidal Antiinflammatory Drug
PDR	Proliferative Diabetic Retinopathy
PENIA	Particle Enhanced Nephelometric ImmunoAssay
PPAR	Peroxisome proliferation activated receptor
PVD	Peripheral Vascular Disease
RAGE	Receptors for advanced glycation end product
ROS	Reactive oxygen species
RRT	Renal Replacement Therapy
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumour necrosis factor -α
UKPDS	United Kingdom Prospective Diabetes Study
UTI	Urinary tract infection

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INTRODUCTION

Type 2 diabetes is a common and chronic metabolic condition characterised by insulin resistance, deficiency or both and is usually irreversible. Type 2 Diabetes was previously referred to as non insulin dependent or adult onset diabetes and accounts for 90-95% of the total diabetic cases. Its complications results in reduced life expectancy and reduced quality of life. These include macrovascular disease, leading to an increased prevalence of coronary artery disease, peripheral vascular disease, and stroke, and microvascular damage, causing diabetic retinopathy, neuropathy and nephropathy. [(1)]

It affects more than 120 million people worldwide and is estimated to affect 370 million people by the year 2030. The greatest increases in prevalence are anticipated to occur in the Middle East, sub- Saharan Africa and India. It is the most common metabolic disorder in India. It is assuming epidemic proportions with the Asian-Indian phenotype being more susceptible for the development of the disease. [(2)]

Among Indians, the onset of type 2 diabetes occurs at a younger age making them more prone to develop all the complications of diabetes due to longer duration of the disease. [(3)]Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent microalbuminuria in concomitance with insulin- or non–insulin dependent diabetes. [(4)]

Diabetic nephropathy is currently one of the leading causes of morbidity and mortality in the diabetic population, accounting for the greatest proportion of end stage renal disease worldwide. This microvascular complication is due to a progressive change in the structure and function of the kidney owing to multiple diabetes associated factors. Rate of renal function decline increases as the disease progresses and thereby morbidity and mortality too.

The management of patients with diabetes and End stage renal disease contributes significantly to health care costs. Renal failure in patients with diabetes became a major issue when numbers of diabetic patients requiring renal replacement therapy are increasing day by day. [5]

In the past few decades, there have been notable advances in our knowledge regarding the early stages of diabetic nephropathy, like the advent of interventions that can significantly slow or even reverse the progression of disease. Substantial under-diagnosis of diabetic CKD leads to loss of opportunities for prevention and inadequate treatment of patients with early diabetic nephropathy.

Previously used parameters to assess renal function include blood urea, serum creatinine and urine albumin estimation. These are known to be influenced by several physiological and pathological factors that render them insensitive or unreliable for early detection of renal dysfunction. Hence research has been going on for finding more dependable markers for estimating kidney function. Recently conducted studies have identified Cystatin C as a new and reliable marker which can be detected in early in nephropathy and helps in prompt diagnosis of early kidney failure.

This study was undertaken to evaluate the efficacy of this new molecule in assessing renal dysfunction at a phase when timely interventions can be instituted and the progression of nephropathy can be delayed.

OBJECTIVES:

1. To estimate serum cystatin C based eGFR and serum creatinine based eGFR in type 2 diabetes mellitus patients.

2. To detect early nephropathy using the above findings in type 2 diabetes mellitus patients

3. To compare diagnosis of early nephropathy in Type 2 DM by using serum cystatin C based eGFR and serum creatinine based eGFR.

REVIEW OF LITERATURE:

EPIDEMIOLOGY OF TYPE 2 DIABETES MELLITUS:

The prevalence of diabetes is rapidly increasing all over the world at an alarming rate. According to the International Federation of Diabetes, 120 million adults around the world are suffering from diabetes, and it is estimated that the numbers will reach around 642 million by 2040.[6]

The first World Health Organization (WHO) global report on diabetes demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults.[7] Diabetes has become one of the leading causes of premature illness and deaths in most countries, mainly through the increased risk of cardiovascular disease which is responsible for over 50% of deaths in persons with diabetes.[8] Although diabetes is sometimes considered the major concern for developed nations, the loss of life from premature death among persons with diabetes is greatest in developing countries. Nearly 80% of the total adult diabetics are in low- or middle-income countries.[8] India leads the World and stands at the second position after China, with 69 million persons affected by diabetes poses a daunting challenge to the sustainable development of the nation as almost every tenth adult (9.3%) in India is estimated to be affected by diabetes.[6] The WHO estimated every 26 per 100,000 persons die due to diabetes in India though it declined marginally and for males increased between 2000 and 2012. [9]

A study in India indicates that more than 50% of people with diabetes have poor glycemic control, uncontrolled hypertension, and dyslipidemia and a large percentage have diabetic vascular complications. [10] Another study on Indian data shows that the common risk factors such as greater duration of diabetes, hypertension,

poor metabolic control, smoking, obesity, and dyslipidemia are more prone to develop diabetic complications.[11]

Some of the review studies on DM showed a rising trend in the prevalence of diabetes across different parts of India.[12] The first national study on the prevalence of type 2 diabetes based on clinical data (blood glucose level >170 mg/dl) in India was done by the Indian Council Medical Research estimated diabetes prevalence of 2.1% in urban and 1.5% in the rural area in 1972–1975.[21] A national rural diabetes survey estimated 2.8% of diabetes (based on the WHO 1985 criteria [13]) in 1989–1991.[14] Subsequent studies used the WHO 1999[15]criterion estimated a high prevalence of diabetes ranging in rural area from 10% in Goa [16] to 19.8% in Karnataka [17] and in an urban area from 9.3% in Mumbai (18) to 19.5% in Ernakulam.[19] However, due to lack of clinical data at large scale, available studies provided estimates of DM for the rural, or urban area of selected states or districts and many studies used the different criterion to define DM.

The Asian Indian phenotype is associated with increased insulin resistance, greater abdominal adiposity despite lower body mass index, lower adiponectin levels and higher high sensitive C-reactive protein which makes Indians more prone to diabetes. Among Indians, the onset of type 2 diabetes occurs at a younger age making them more vulnerable to all its complications owing to longer duration of disease. [20]

Being a multi-organ disease, diabetes causes macrovascular and microvascular complications that in turn contribute to the morbidity and mortality associated with it. Nearly 30% of chronic kidney disease in our country is due to diabetic nephropathy and it is thus the single most common cause of chronic renal failure in India. [21]

DIABETIC NEPHROPATHY

DEFINITION:

Diabetic nephropathy is a syndrome characterized by persistent albuminuria (>300 mg/24 hr) that is confirmed on at least 2 occasions 3-6 months apart, a progressive decline in GFR, raised arterial BP, and enhanced cardiovascular morbidity and mortality. [22] A clinical diagnosis of diabetic nephropathy can be made if, in addition to persistent albuminuria there is co-existent retinopathy in the absence of clinical or laboratory evidence of other kidney or renal tract disease.

PATHOPHYSIOLOGY:

Diabetes leads to progressive structural alterations of the kidneys including extracellular matrix (ECM) accumulation in the mesangium, glomerular basement membrane, and tubulointerstitial tissue. The pathophysiology of diabetic nephropathy is complex and multifactorial.

Three major histological changes occur in the glomeruli of patients with diabetes.

1. Mesangial expansion, which is probably a direct consequence of hyperglycemia owing to increased matrix production or glycosylation of matrix proteins.
2. Thickening of the glomerular basement membrane.
3. Intra-glomerular hypertension induced sclerosis. This is caused by hyaline narrowing of arteries due to ischemia. [23]

- Augmentation of extracellular matrix is hallmark change in diabetic glomerulopathy.

- The earliest morphologic abnormality in diabetic nephropathy is the thickening of the GBM and expansion of the mesangium due to accumulation of extracellular matrix.

The glomerular mesangium expands initially by cell proliferation and then by cell hypertrophy. Increased mesangial stretch and pressure can stimulate this expansion, as can high glucose levels.

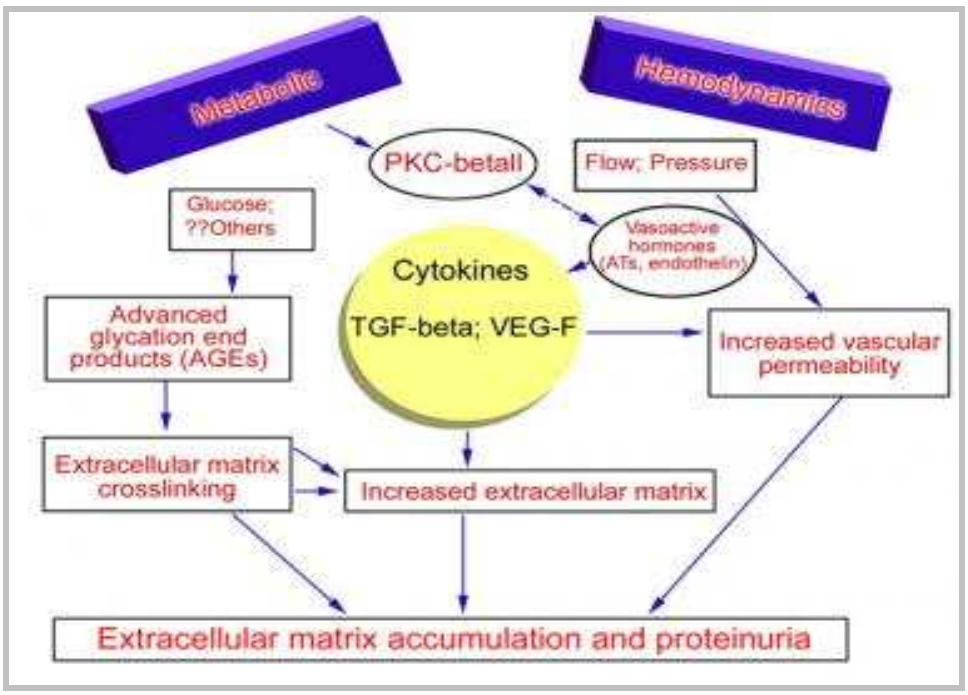


FIGURE: 1- PATHOPHYSIOLOGY OF DIABETIC NEPHROPATHY

Poor glycemic control was previously considered the sole driving factor that drives diabetic nephropathy.

However, some studies demonstrated variability in the development of renal complications despite comparable hyperglycemic control. For example, the Diabetes Control and Complications Trial (DCCT) showed that nearly 30% of type I diabetics

and 25%–40% of type II diabetics develop nephropathy despite intensive glycemic control. [24]

Variations between ethnic groups also point to the significant role of genetic background. Relatives of African Americans on renal replacement therapy secondary to diabetic nephropathy are at five fold risk of developing ESRD.[25] Additionally, the incidence of ESRD per capita in Native Americans, African Americans and Hispanics, is significantly higher than the white population. [26] The incidence of proteinuria among Pima Indians has also been increasing over the past 36 years. However, the incidence of progression to ESRD declined after 1990, possibly due to improved control of risk factors. [27]

Hyperglycemia leads to the formation of sugar-derived substances called advanced glycation end products (AGEs).

AGEs form at a constant but slow rate in the normal body, starting in early embryonic development, and accumulate with time. However, their formation is markedly accelerated in diabetes because of the increased availability of glucose. AGEs are a heterogeneous group of molecules formed from the non-enzymatic interaction of sugars like glucose with amino acid groups of proteins, lipoproteins, and nucleic acids. The initial product of this reaction is called a Schiff base, which spontaneously rearranges itself into an Amadori product, as is the case of the well-known hemoglobin A1c (A1C).

These initial reactions are reversible depending on the concentration of the reactants.

A lowered glucose concentration will unhook the sugars from the amino groups to which they are attached; conversely, high glucose concentrations will have the opposite effects, if persistent. A series of subsequent reactions, including

successions of dehydrations, oxidation-reduction reactions, and other arrangements lead to the formation of AGEs.

Several compounds, e.g., ε*N*-carboxymethyl-lysine, pentosidine, or methylglyoxal derivatives, serve as examples of well-characterized and widely studied AGEs.

Circulating AGEs have been implicated in the pathophysiology of diabetic nephropathy through mechanisms that are either receptor-dependent or receptor-independent. AGEs modify basement membrane proteins, cross-link ECM components, and increase expression of type IV collagen. These changes lead to structural alterations of the surface charge, membrane permeability, proteolytic digestion, and membrane stability. These changes disrupt intercellular interaction, and hence cause impairment of tissue function and maintenance. [(23)]

As for the receptor-dependent mechanisms, AGEs interact with a wide array of receptors on various cell types such as macrophages, monocytes, endothelial cells, podocytes, tubular epithelial cells, and smooth muscle cells.

Examples of these receptors are the macrophage scavenger receptor type I and II, AGE-R1, AGE-R2, AGE-R3, receptor for AGE (RAGE), and CD36. RAGE activation by AGEs leads to activation of several signal transduction pathways that lead to the generation of reactive oxygen species (ROS) and activation of transcription factors, such as NF-kappaB. Consequently, NF-kappaB leads to the release of cytokines and growthfactors, including transforming growth factor-β1 (TGF-β1), interleukin-1β and interleukin-6, insulin-like growth factor-1, tumor necrosis factor (TNF-α), and platelet-derived growth factor. These proinflammatory growth factors play a key role in the development of diabetic complications. [(29)] Metabolic reactions result in the formation of active byproducts and free radicals known as ROS and

reactive nitrogen species. Inadequate removal of these active molecules leads to detrimental effects on the cellular level, a process known as oxidative stress. Examples of these radicals are superoxide, peroxyl, hydroxyl, and hydroperoxyl molecules. Superoxide (O2 −) is a common radical implicated in diabetic complications.

It is produced by mitochondrial electron transport chain, oxidative phosphorylation, NAD(P)H oxidase, cytochrome P-450, nitric oxide synthase, and other enzymatic processes. [29] Normally, superoxide radicals are eliminated by mitochondrial and cytosolic antioxidant defence mechanisms. Impaired clearance, as in diabetes, leads to the oxidation of membrane lipids, DNA, proteins, and carbohydrates. These alterations lead to impaired structure and function of several cellular components. There is evidence that diabetic complications arise from interplay between pathways of AGEs and oxidative stress. A study showed that diabetic glomerular lesions might undergo autoxidation by ROS and could be converted to reactive carbonyl compounds, a subgroup of AGEs. [30]

The characteristic structural changes of diabetic nephropathy, thickened glomerular basement membrane and mesangial expansion, areaccompanied by accumulation of AGEs, leading to glomerulosclerosis and interstitial fibrosis.[31]

PATHOLOGICAL CLASSIFICATION OF DIABETIC NEPHROPATHY [32]	
CLASS	PATHOLOGICAL LESION
I	Glomerular basement membrane thickening
II	Mesangial expansion
III	Nodular sclerosis with glomerulosclerosis in < 50% ofglomeruli (Kimmelstein-Wilson lesion)
IV	Advanced diabetic glomerulosclerosis: more than 50%

TABLE 1. PATHOLOGICAL CLASSIFICATION OF DIABETIC NEPHROPATHY

Risk factors for development of diabetic nephropathy [33]

UKPDS cohort of newly diagnosed individuals with type 2 diabetes shows, development of microalbuminuria was associated with:

1. Indian-Asian ethnicity.
2. Elevated systolic blood pressure.
3. Elevated plasma triglycerides.
4. Waist circumference.
5. Previous retinopathy.
6. Previous CV disease.
7. Smoking history.
8. Male gender.

And Development of macroalbuminuria was associated with the following:

1. Waist circumference.
2. Elevated systolic blood pressure.
3. Elevated LDL cholesterol and plasma triglycerides.

Development of renal impairment was associated with the following:

1. Baseline plasma creatinine level.
2. Elevated systolic blood pressure.
3. Age at diagnosis.
4. Indian-Asian ethnicity.
5. Smoking history.
6. Previous retinopathy (Retnakaran et al, 2006).

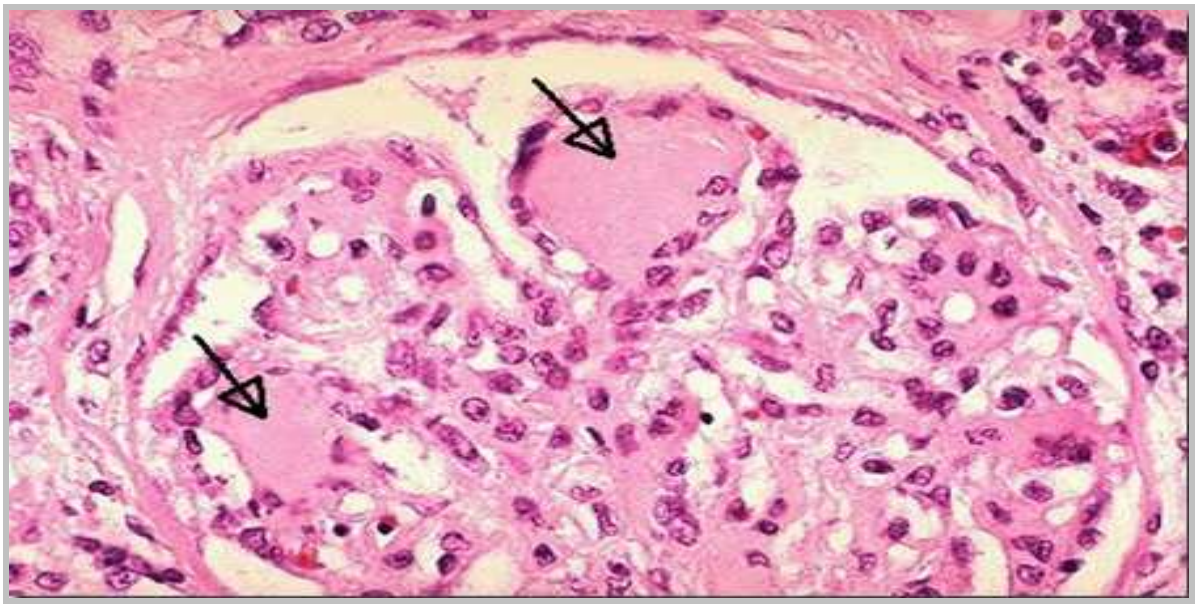


FIGURE-2 The Kimmelstein Wilson lesion (arrows) - pathognomonic of diabetic nephropathy.

NATURAL HISTORY OF DIABETIC KIDNEY DISEASE :

The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels of albumin (≥ 30 mg/day or 20 μg/min) in the urine, referred to as microalbuminuria, and patients with microalbuminuria are referred to as having incipient nephropathy.[34] Most studies dealing with the natural course of diabetic nephropathy have demonstrated a relentless, often linear but highly variable rate of decline in GFR ranging from 2 to 20 ml/min/yr, mean 12 ml/min/yr.

There occurs a reduction in the number of restrictive pores leading to loss of ultra filtration capacity (Kf) and impairment of glomerular barrier size-selectivity leading to progressive albuminuria in diabetic nephropathy. Furthermore, the extent to which ultra filtration capacity is impaired appears to be related to the magnitude of the defect in the barrier size-selectivity. The reduction in renal plasma flow is proportional to the reduction in GFR (filtration fraction unchanged), and the impact on GFR is partially offset by the diminished systemic colloid osmotic pressure.[35]

In the United Kingdom Prospective Diabetes Study (UKPDS), it was concluded that from diagnosis of diabetes, progression to microalbuminuria occurred at 2.0% per year, from microalbuminuria to macroalbuminuria at 2.8% per year, and from macroalbuminuria to elevated plasma creatinine (>or=175 μmol/L) or renal replacement therapy at 2.3% per year.

Without specific interventions, around 80% of subjects with type 1 diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of ~10–20% per year to the stage of overt nephropathy or clinical albuminuria (≥300 mg/24 h or ≥200 μg/min) over a period of 10–15 years, with hypertension also developing along the way. Once overt nephropathy occurs, without specific interventions, the glomerular filtration rate (GFR) gradually falls over a

period of several years at a rate that is highly variable from individual to individual (2–20 ml · min^{-1} · $year^{-1}$).

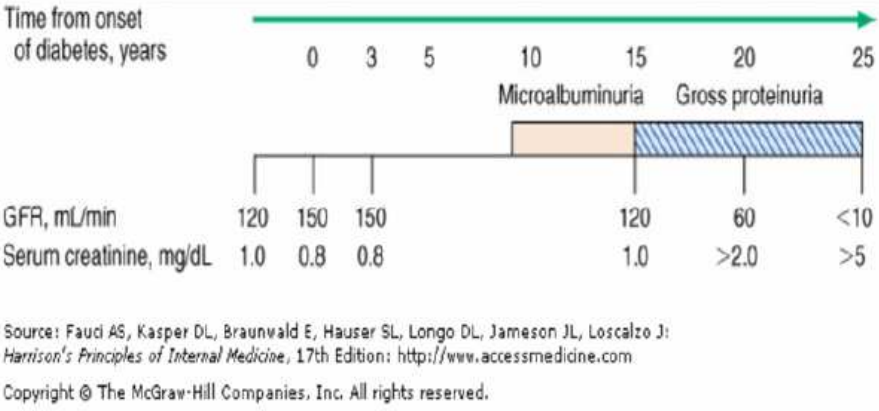
Ten years following diagnosis of diabetes, the prevalence of microalbuminuria was found to be 24.9%, of macroalbuminuria was 5.3%, and of elevated plasma creatinine or RRT (renal replacement therapy) was 0.8%. Patients with elevated plasma creatinine or RRT had an annual death rate of 19.2%. ESRD develops in 50% of type 1 diabetic individuals with overt nephropathy within 10 years and in >75% by 20 years.

There was a trend for increasing risk of cardiovascular death with increasing nephropathy with an annual rate of 0.7% for subjects in the stage of no nephropathy, 2.0% for those with microalbuminuria, 3.5% for those with macroalbuminuria, and 12.1% with elevated plasma creatinine or RRT. Individuals with macroalbuminuria were more likely to die in any year than to develop renal failure. [36]

A higher proportion of individuals with type 2 diabetes are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their diabetes, because diabetes is actually present for many years before the diagnosis is made and also because the presence of albuminuria may be less specific for the presence of diabetic nephropathy, as shown by biopsy studies. Without specific interventions, 20–40% of type 2 diabetic patients with microalbuminuria progress to overt nephropathy, but by 20 years after onset of overt nephropathy, only ~20% will have progressed to ESRD. Once the GFR begins to fall, the rates of fall in GFR are again highly variable from one individual to another, but overall, they may not be substantially different between patients with type 1 and patients with type 2 diabetes. However, the greater risk of dying from associated coronary artery disease in the older population with type 2 diabetes may prevent many with earlier stages of nephropathy from progressing to

ESRD. As therapies and interventions for coronary artery disease continue to improve, however, more patients with type 2 diabetes may be expected to survive long enough to develop renal failure.

In addition to its being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either type 1 or type 2 diabetes. Thus, the finding of microalbuminuria is an indication for screening for possible vascular disease and aggressive intervention to reduce all cardiovascular risk factors (e.g., lowering of LDL cholesterol, antihypertensive therapy, cessation of smoking, institution of exercise, etc.). In addition, there is some preliminary evidence to suggest that lowering of cholesterol may also reduce the level of proteinuria. [37]



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Time course of development of diabetic nephropathy. The relationship of time from onset of diabetes, the glomerular filtration rate (GFR), and the serum creatinine are shown. *(Adapted from RA DeFranzo, in Therapy for Diabetes Mellitus and Related Disorders, 3d ed. American Diabetes Association, Alexandria, VA, 1998.)*

FIGURE-3 PROGRESSION OF DIABETIC NEPHROPATHY

DIABETIC NEPHROPATHY IS REVERSIBLE

A study showed mesangial expansion which occurred after 7 months of diabetes was reversed within 2 months after normoglycemia which was induced by islet transplantation in streptozotocin diabetic rats[38]. After 10 years of normoglycemia, patients with diabetes had marked reversal of diabetic glomerulopathy lesions with remarkable glomerular architectural remodeling seen by light microscopy, including the complete disappearance of Kimmelstiel-Wilson nodular lesions.[38]

CURRENT TARGETS FOR INTERVENTION IN DKD:

For the prevention and treatment of diabetic nephropathy, main stay is the intensive control of the known risk factors which include-

1. Glycemic control
2. Management of hypertension
3. Hmg-coa reductase inhibitors
4. Smoking cessation
5. Dietary advice
6. Avoiding nephrotoxic agents
7. Life style advice

1. **Glycaemic control**:

Hyperglycemia is a major determinant of the progression of diabetic nephropathy in patients with either type 1 or type 2 diabetes mellitus (DM). Many study have shown intensive control can partially reverse glomerular hypertrophy and hyperfiltration, delay the development of microalbuminuria, and stabilize or even reverse microalbuminuria.[39]

In the Diabetes Control and Complications Trial (DCCT), it was observed that reduction in microvascular complications was of a smaller magnitude in patients with type 2 DM receiving intensive insulin therapy than in patients with type 1 DM. [40] Large prospective randomized control trials have demonstrated the efficacy of improved glycaemic control in preventing progression of diabetic kidney disease. The Kumamoto study revealed that with intensive glycaemic control (HbA1c < 7.1) subjects were less likely to develop new onset nephropathy and pre existing kidney dysfunction was less likely to progress.

In the UK Prospective Diabetes Study (UKPDS), 3867 patients with newly diagnosed type 2 diabetes were randomized to oral or insulin therapy versus dietary control and followed for 11 years. The difference in HbA1c was 7.0% versus 7.9%. After 9 years, there was a significant risk reduction in the intensive group, with a relative risk of 0.76 for the development of microalbuminuria.[41,42]

TABLE 2. COMMON ORAL ANTI DIABETES AGENTS USED IN TYPE 2 DM AND THEIR DOSE ADJUSTMENT IN THE CKD

DRUG CLASS	EXAMPLES	DOSE ADJUSTMENT IN NEPHROPATHY BASED ON eGFR
BIGUANIDES	METFORMIN	Dose should be reviewed (use half maximum dose with caution) if eGFR <45 ml/min/1.73m^2 . avoid if eGFR <30 ml/min/1.73m^2
SULPHONYLUREAS	GLICLAZIDE GLIMIPERIDE GLIPIZIDE	Use with care in mild or moderate impairement. Glipizide should be avoided if both renal and hepatic impairement present.
DIPEPTIDYL PEPTIDASE-4 INHIBITORS	ALOGLIPTIN LINAGLIPTIN SAXAGLIPTIN SITAGLIPTIN VILDAGLIPTIN	**Alogliptin:** use 12.5 mg if eGFR <50ml ml/min/1.73m^2 . **Linagliptin:** no dose change needed **Saxagliptin:** reduce dose to 50 mg once daily if eGFR 30-50ml ml/min/1.73m^2 . **Saxagliptin:** reduce dose to 2.5 mg in moderate to severe impairement. **Vildagliptin:** reduce dose to 50 mgonce daily if eGFR <50ml ml/min/1.73m^2
THIAZOLIDINEDIONES	PIOGLITAZONE	Contraindicated in heart failure; caution in

		cardiovascular disease
GLUCAGON LIKE PEPTIDE-1 RECEPTOR AGONISTS	EXENATIDE EXENATIDE (modified release) LIRAGLUTIDE LIXISENATIDE DULAGLUTIDE	**Standard release exenatide:** use with caution if eGFR 30-50ml ml/min/1.73m^2 avoid if eGFR <30 ml ml/min/1.73m^2. **Modified release exenatide:** avoid if eGFR <50 ml ml/min/1.73m^2. **Liraglutide:** avoid if eGFR <30 ml ml/min/1.73m^2. **Lixasenatide:** use with caution if eGFR 30-50ml ml/min/1.73m^2 avoid if eGFR <30 ml ml/min/1.73m^2. **Dulaglutide:** avoid if eGFR <30 ml ml/min/1.73m^2.
SODIUM GLUCOSE CO-TRANSPORTER-2 INHIBITORS	DAPAGLIFLOZIN CANAGLIFLOZIN EMPAGLIFLOZIN	**Dapagliglifloz in:** avoid if eGFR <60 ml ml/min/1.73m^2. **Canagliflozin:** avoid if eGFR <45 ml ml/min/1.73m^2. reduce dose to 100 mg OD if eGFR <30 ml ml/min/1.73m^2. **Empagliflozin:** avoid if eGFR <40 ml ml/min/1.73m^2. reduce dose to 10 mg OD if eGFR <60 ml ml/min/1.73m^2.

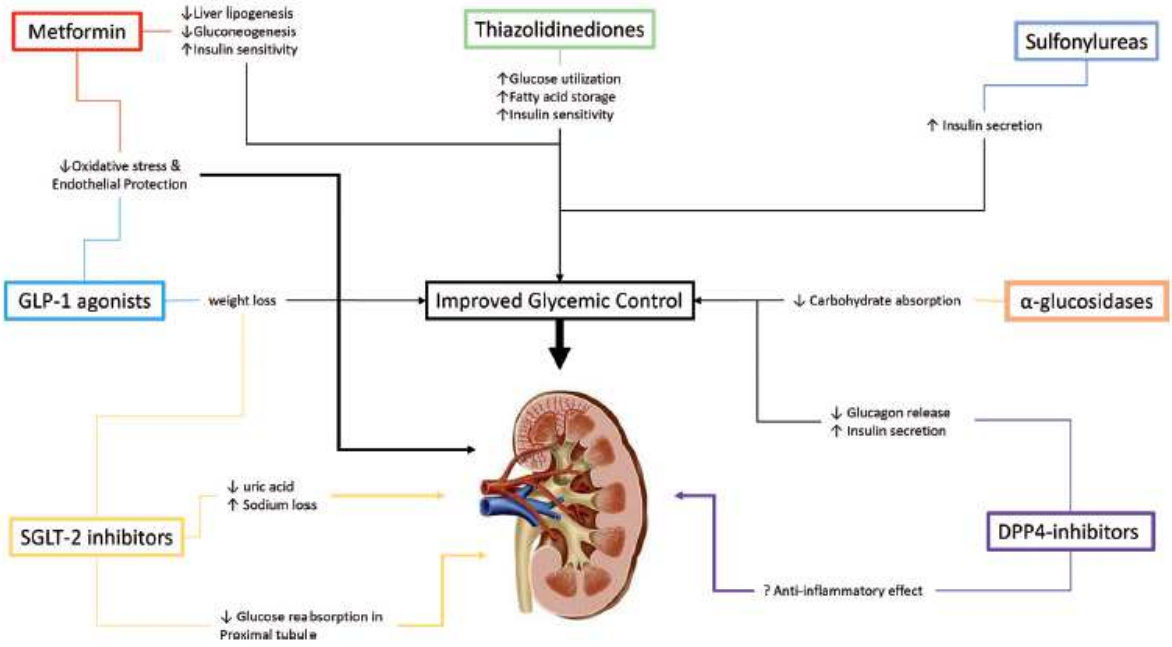


FIGURE 4: The different antihyperglycemic agents exhibit their renal protective properties through hyperglycemia-dependent and independent mechanisms.

2. Hypertension control

Antihypertensive therapy slows the development of diabetic glomerulopathy. This is particularly significant when lowering of systemic blood pressure is accompanied with concomitant decrease of glomerular capillary pressure. Blood pressure control is needed to retard the progression of diabetic nephropathy and other complications.[43]

In the UKPDS, they observed 12% risk reduction in diabetic complications with each 10 mm Hg drop in systolic pressure, the lowest risk being associated with a systolic pressure below 120 mm Hg. [44]

In diabetic nephropathy, activation of the local renin-angiotensin system occurs in the proximal tubular epithelial cells, mesangial cells, and podocytes. Angiotensin II (ATII) itself contributes to the progression of diabetic nephropathy.

ATII is stimulated in diabetes despite the high-volume state typically seen with the disease, and the intrarenal level of ATII is typically high, even in the face of lower systemic concentrations. ATII preferentially constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressures. In addition to its hemodynamic effects, ATII also stimulates renal growth and fibrosis through ATII type 1 receptors, which secondarily upregulate TGF-β and other growth factors.[45]

Specific use of agents that block the renin-angiotensin system appears to be particularly beneficial in the prevention or slowing of progression of diabetic nephropathy. Angiotensin blockade with Angiotensin converting enzyme inhibitors (ACE-I) like ramipril or Angiotensin Receptor Blockers (ARB) like valsartan and irbesartan should be started early in diabetic kidney disease and may have its greatest benefit in prevention or reversal of early kidney disease.[46,47]

I. Angiotensin-converting enzyme (ACE) inhibitors:

ACE inhibition has been shown to delay the progression of development of diabetic nephropathy. A large trial of ACE inhibitor shown, only 7% of patients with microalbuminuria experienced progression to overt nephropathy; however, in the placebo-treated group, 21% of patients experienced progression to overt nephropathy. The beneficial effect of ACE inhibition is long lasting on preventing progression from microalbuminuria to overt diabetic nephropathy and it is associated with the preservation of a normal glomerular filtration rate (GFR). [48]

A study done on normotensive type 2 diabetic patients with microalbuminuric who received enalapril or placebo for 5 years, 12% of those in the actively treated group experienced diabetic nephropathy, with a rate of decline in kidney function of 13%, and 42% of those in the placebo group experienced nephropathy.

Meta-analysis has shown that ACE inhibitors are superior to beta-blockers, calcium channel blockers and diuretics, in reducing urinary albumin excretion in normotensive and hypertensive type 1 and type 2 DM patients.[49] This superiority is pronounced in the normotensive state, whereas it is diminished progressively with progressive blood pressure reduction. The antiproteinuric effect of ACE inhibition varies considerably in patients with diabetic nephropathy. Individual differences in the renin-angiotensin system (RAS) may influence this variation.

In addition to its beneficial cardiovascular effects, also has significant beneficial effect on the progression of diabetic retinopathy and on the development of proliferative retinopathy.

ACE inhibitors reduce the risk of progression of overt type 1 diabetic nephropathy to end-stage renal disease (ESRD) Although it improves glomerular permeability in patients with type 1 DM it does not do so in patients with type 2 DM.

II. ANGIOTENSIN RECEPTOR BLOCKERS:

Two studies (the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) Study and the Irbesartan Diabetic Nephropathy Trial (IDNT) demonstrated that angiotensin II receptor blockers (ARBs) are more superior to conventional therapy and amlodipine in slowing the progression of overt nephropathy.[50]

These trials were performed with ARBs and not ACE inhibitors.

Microalbuminuria-Heart Outcomes Prevention Evaluation (MICRO-HOPE) Trial which shown difficult to choice between an ARB and an ACE inhibitors.

This trial shown ramipril reduced the risk for myocardial infarction, stroke, or cardiovascular death by 26% after 2 years. Perhaps the more interesting question arise

is whether the combination of an ACE inhibitor and an ARB is more effective than either drug alone. [51] One meta-analysis showed that ACEI + ARB reduced 24-hour proteinuria to a greater extent than ACEI alone. However, this benefit was associated with small effects on GFR, serum creatinine, potassium, and blood pressure. [48]

A study done by Imai et al results of which shows that combined treatment with ACE inhibitors and ARBs significantly decreased blood pressure, proteinuria, and rate of change of reciprocal serum creatinine; however, higher cardiovascular death was reported among the olmesartan-treated patients compared with placebo. Major adverse cardiovascular events and all–cause data were similar between the 2 groups. Hyperkalemia was more frequent in the olmesartan–treated group than in the placebo group. These findings shows similar finding as shown in previous studies that combined therapy for patients with diabetic nephropathy may improve short-term biomarkers but is not associated with improvement in long-term hard endpoints. [52]

III. Direct renin inhibitors

A study done by Persson et al they observed the combination of aliskiren and irbesartan to be more antiproteinuric in type 2 diabetes mellitus than was monotherapy with either drug. [53] This study assessed the effect of aliskiren which is a direct renin inhibitor, on proteinuria in patients with type 2 DM (n = 26) and compared the effect with that of placebo, irbesartan (an ARB), and the combination of aliskiren and irbesartan.

Results showed combination therapy with aliskiren and irbesartan reduced albuminuria by 71%, more than did either monotherapy (aliskiren monotherapy 48%; irbesartan monotherapy 58%). Use of direct renin inhibitors with ARBs or ACEIs is no longer recommended.

vitamin D supplementation may be useful in reducing proteinuria in patients with diabetic nephropathy as observed in the research which suggest that vitamin D may have a role in renin inhibition.

Patients with diabetic nephropathy with stage 3 or more chronic kidney disease should be evaluated for their vitamin D and parathyroid hormone status as recommended by the National Kidney Foundation- Kidney Disease Dialysis Outcomes Quality Initiative (NKF-KDOQI). [54] Patients should be given vitamin D supplementation based on status vitamin D levels. As per One randomized controlled trial vitamin D supplementation may reduce proteinuria in patients with diabetic nephropathy.[55,56]

IV. Endothelin Antagonist Therapy

It has been demonstrated antifibrotic, anti-inflammatory, and antiproteinuric effects in experimental studies.

A randomized controlled trial done on the effect of the endothelin-A antagonist avosentan on urinary albumin excretion rate in 286 patients with diabetic nephropathy, macroalbuminuria, and a blood pressure of < 180/110 mm Hg found that all dosages of avosentan, administered along with the standard treatment with an ACE inhibitor or an ARB, reduced the mean relative urinary albumin excretion rate (-16.3% to -29.9%, relative to baseline). [57]

3. HMG-CoA reductase inhibitors:

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, or statins, are also being studied for delaying nephropathy progression. Statins may have unique benefits independent of lipid lowering capacity. In animal models of diabetic

nephropathy, statin therapy was found to block intracellular signaling and decreases the mRNA expression of TGF-β which has been implicated in the pathophysiology of the disease.[58]

The development of microalbuminuria is mostly associated with an elevation in triglycerides, LDL cholesterol and total cholesterol. The risk of CV disease is significantly high in these individuals and, therefore, aggressive management of dyslipidaemia is often indicated in these patients.

Study shown that statin therapy reduces the rate of macrovascular complications and GFR decline in people with diabetes, independent of their baseline cholesterol levels (Collins et al, 2003).

As per NICE recommendation statin therapy for the primary prevention of CV disease in all people with type 1 diabetes. Atorvastatin 20 mg once daily in type 2 diabetes should be given if the 10-year risk of developing CV disease is 10% or greater, when calculated using the QRISK2 assessment tool. In patient with CKD, high doses of statin therapy may be indicated if a greater than 40% reduction in non-HDL cholesterol is not achieved. The use of bile acid sequestrants, fibrates, nicotinic acid or omega-3-fatty acid compounds either alone or in combination with a statin is no longer recommended for the primary or secondary prevention of CV disease (NICE, 2014b).

4. Smoking

The association between development diabetic nephropathy and the smoking has been long known (Telmer et al, 1984). Researchers found that smoking is an independent predictor of progression of nephropathy despite blood pressure control and use of ACE inhibitors (Chuahirun et al, 2003). Rossing et al (2004) did one study

in that they found that heavy smokers (>20 cigarettes daily) had a significantly greater decline in GFR compared to those smoking <20 cigarettes daily and non-smokers, but no significant difference was found between all smokers and non-smokers in the rate of progression.

Cessation of smoking should be adviced to all patients with both type 1 and type 2 diabetes, especially when other risk factors for CV disease are present (for example, microalbuminuria).

5. Dietary recommendation:

Diabetics with renal disease are salt-sensitive and minimizing salt intake can help in reaching blood pressure goals, with secondary benefits of decreased stroke risk, regression of left ventricular hypertrophy, and reduction in proteinuria. A low-sodium diet of 2.3 g or lower (5.8 g of NaCl) is advocated in patients with diabetes with any degree of proteinuria. [59,60] It has also been suggested that in incipient nephropathy protein restriction to 0.6-0.8 g/kg/d may prove useful in slowing GFR decline.

A meta-analysis observed the effects of dietary protein restriction (0.5-0.85 g/kg/d) in diabetic patients suggested a beneficial effect on the GFR, creatinine clearance, and albuminuria.

The American Diabetic Association suggests diets of various energy intake (caloric values) depending on the patient. With advancing renal disease, protein restriction of as much as 0.8-1 g/kg/d may retard the progression of nephropathy.

When nephropathy is advanced, the diet should reflect the need for phosphorus and potassium restriction, with the use of phosphate binders.

6. Avoidance of nephrotoxins:

It is important to avoid nephrotoxic agents, if possible, in patients with diabetic nephropathy. Nonsteroidal anti-inflammatory drugs (NSAIDs) can cause a significant drop in GFR in patients with diabetic nephropathy, particularly when used with angiotensin-blocking agents aspirin at higher dosages and other NSAIDs (including COX-2 inhibitors should be avoided if possible considering their potential for renal toxicity.

Radio-contrast media are also particularly nephrotoxic for diabetics. Even with a normal serum creatinine level, patients with diabetes and proteinuria should be volume loaded 12 hours before and after exposure to contrast, if not contraindicated. Diuretics should be temporarily discontinued, and hyperglycemia should be controlled. [61]

7. LIFE STYLE ADVICE

Tight glycemic control retard the development of diabetic nephropathy. In Diabetes Control and Complications Trial (DCCT), Type 1 dibetics were were randomized to intensive or conventional insulin treatment groups and followed for an average of 6.5 years. Included patients had 7.2% versus 9.2% of hemoglobin A1c (HbA1c) levels. They observed there was risk in the development of microalbuminuria and a 54% reduction in the development of macroalbuminuria in the intensive treatment group. [62] In the UK Prospective Diabetes Study (UKPDS), patients with newly detected type 2 diabetes were randomized to oral or insulin therapy versus dietary control and followed for 11 years. They noticed difference in HbA1c levels in both groups which was 7.0% versus 7.9% in oral and insulin receiving patients respectively. [63] There was a significant risk reduction for diabetic

nephropathy in the intensive group, with a relative risk of 0.76 for the development of microalbuminuria. The complete correction of hyperglycemia with pancreatic transplantation in type 1 diabetics has led to a dramatic resolution in glomerular and tubular expansion and fibrosis over time. [60]There was a significant reduction in basement membrane thickening and mesangial expansion on repeat biopsies over time in patient with drop in HbA1c from an average of 8.7% to 5.5% in eight transplanted patients. Renal fibrosis may be reversible as in this study they observed glomerular sclerosis appeared to resolve, showing that, although it took 10 years after transplantation to see these significant changes.

NICE (national institute for health and care excellence) recommendations in diabetic nephropathy: [65]

- Ask all diabetic patients with or without detected nephropathy to bring in a first-pass morning urine.
- Specimen once a year. Send this for laboratory estimation of ACR in the absence of proteinuria or UTI.
- Measure creatinine level and estimate GFR annually at the time of performing ACR.
- Repeat the ACR if an abnormal result is obtained for next two clinic visits but within a maximum of 3–4 months. If further specimen (of two more) is also abnormal (>3.0 mg/mmol). Then result to be Confirming with microalbuminuria.
- Consider referral to nephrologist if ACR is increased and any of the following apply:

1. Significant or progressive retinopathy,
2. Blood pressure is particularly high or resistant to treatment,
3. The person previously had a documented normal ACR and develops heavy proteinuria (ACR >100 mg/mmol),
4. Significant haematuria is present,
5. GFR has worsened rapidly,
6. The person is systemically ill.

- Start ACE inhibitor and titrate to maximum dose (with monitoring of renal function) if ACR is raised. Special attention is needed with women of child-bearing age.

I. Use ARB if ACR is raised and ACE inhibitors are poorly tolerated.
II. Discuss the significance of a raised ACR with the individual.
III. Avoiding nephrotoxins, such as non-steroidal analgesics, is essential.
IV. Maintain blood pressure <130/80 mmHg if microalbuminuria confirmed (NICE, 2009).

FUTURE TRENDS IN PREVENTION OF NEPHROPATHY:

1. Reduction of Advanced Glycation Endproducts with valsartan, ramipril and metformin.
2. Protein kinase C inhibition.
3. Anti TNF-β agents- Peroxisome Proliferator Activated Receptor (PPAR) agonists such as Gemfibozil With our increasing knowledge of the pathophysiology of diabetic kidney disease, a number of new avenues have opened that promise to delay or even reverse the progression of chronic renal

impairment in diabetic subjects. The emphasis is on early institution of therapy necessitating earlier detection of renal function decline. For decades, serum creatinine, creatinine based estimations of glomerular filtration and urine albumin estimation have been used as markers of kidney function.

The term chronic renal failure applies to the process of continuing relatively irreversible reduction in nephron number and typically corresponds to CKD stages 3-5. Therefore Target population for early interventions are stage 2 and above with a corresponding GFR > 60 ml/min.[61]

TABLE 3- NATIONAL KIDNEY FOUNDATION STAGES OF CHRONIC KIDNEY DISEASE

TABLE.3 **NATIONAL KIDNEY FOUNDATION STAGES OF CHRONIC KIDNEY DISEASE**	
STAGE	**GFR (ml/min/ 1.73 m^2)**
0	>90 with risk factors for CKD
1	≥90 with demonstrated kidney damage
2	60-89
3	30-59
4	15-29
5	<15

Many primary care physicians rely on serum creatinine estimations for screening of renal impairment owing to its convenience and low cost. Creatinine is the break down product of creatine and phosphocreatine, both of which are found almost exclusively in muscle. Thus, creatinine production varies with age, sex and presence of any factor that muscle mass is influenced by, including diet. Creatinine is freely filtered by the glomerulus but also actively secreted by the tubules in very small amounts. Evidence suggests that the secretion of creatinine varies substantially both in the same individuals over time and between different individuals. Particularly troublesome is the fact that the proportion of total renal creatinine excretion due to tubular secretion increases with decreasing renal function, which could have a dampening effect on serial measurements in individuals, because glomerular filtration rate could fall more rapidly than indicated by either serum creatinine.

This implies that concentrations of creatinine in serum can remain within normal range even in the presence of significant renal impairment. Use of eGFR equations were thought to provide more accurate approximation of renal function. [67] The most widely used serum creatinine-based formulas in adults for estimated GFR (eGFR) are the Cockcroft-Gault (CG) and Modification of Diet in Renal Disease Study (MDRD). Recently, a new Chronic Kidney Disease Epidemiology Collaboration equation has been developed. Review of the literature revealed that CG and MDRD formulae correctly assigned overall only 64% and 62%, respectively, of the subjects to their actual K/DOQI-CKD classification's GFR groups as determined by measured GFR (mGFR). [68] On the other hand, a number of normal plasma constituents can interfere with creatinine measurement. Glucose, fructose, pyruvate, acetoacetate, uric acid, ascorbic acid, and plasma proteins can all cause the Jaffe colorimetric assay to yield falsely high creatinine values. [69] The sensitivity of serum

creatinine in the detection of CKD is consequently poor and it will fail to identify half of the patients with crucial stage prior to onset of stage 3 CKD (GFR of 30–60mL/min/1.73m2). [70] The MDRD equation was originally validated in CKD patients but its use is unclear for healthy individuals, or in patients with eGFR ≥90 mL/min/1.73m2 . There are also many situations in which these equations have not been validated; such as in patients with diabetes, extreme body sizes and in certain races. At higher levels of kidney function, the bias and precision of the MDRD estimate is poorer, tending to underestimate true GFR in most studies.

TABLE 4: QUANTIFICATION OF ALBUMINURIA

QUANTIFICATION OF ALBUMINURIA			
CATEGORY	**SPOT COLLECTION (µg/g creatinine)**	**24 hrs collection (mg/day)**	**Timed collection (µg/min)**
NORMAL	**<30**	**<30**	**<20**
MICROALBUMINURIA	**30-299**	**30-299**	**20-199**
MACROALBUMINURIA	**>300**	**>300**	**>200**

Urine protein excretion is now been used widely for screening of diabetic patients with early nephropathy. Screening for microalbuminuria can be performed by three methods:

1. Measurement of the albumin-to-creatinine ratio in a random spot collection
2. 24-h collection with creatinine, allowing the simultaneous measurement of creatinine clearance and
3. Timed (e.g., 4-h or overnight) collection.

There is a 40% day to day variability in albumin excretion rate in a given individual because of which two of three specimens collected within a 3- to 6-month period should be abnormal before considering a patient to have crossed one of the diagnostic thresholds for incipient or overt nephropathy.

Albuminuria, though a sensitive marker for renal function decline may be elevated over baseline values in presence of exercise within 24 h, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, pyuria, and hematuria making it of limited value in these frequently encountered settings.[71] Importantly, a considerable proportion of patients with type 2 diabetes develop impaired renal function without having significant albuminuria.[72] Also, renal risk of diabetic patients increases progressively with increasing albuminuria, even within the range of normoalbuminuric values. This result has led to the recent consideration of abandoning the concept of microalbuminuria altogether.[73]

eGFR is the most important measure of the renal efficiency for clearing various substances from the blood. A decrease in eGFR precedes end-stage renal failure in all forms of progressive kidney disease and knowledge of eGFR is therefore critical in the prevention and management CKD. Accurate determination of eGFR requires the use of invasive protocols based on injected exogenous substances such as Inulin, 125-Iothalamate, Iohexol, 51Cr-EDTA or 99mTc- DTPA. Such procedures are labour intensive, costly, and not entirely free of potential for harm.

CYSTACIN C AS NEW MARKER FOR DIABETIC NEPHROPATHY

These practically encountered disadvantages of currently available parameters of renal function have led to a search for more dependable markers for kidney function. Several low molecular weight proteins have been evaluated as endogenous markers of GFR with Cystatin C commanding the most attention. The history of Cystatin C dates back to 1961 when Jorgen Clausen described the occurrence in human cerebrospinal fluid (CSF) of a "cerebrospinal fluid-specific" protein, which he named γ-CSF [(74)] Cystatin C has since been found in urine, human plasma, ascitic and pleural fluid. Known also as γ-trace, the complete amino acid sequence of human cystatin C was determined in 1981 by Grubb and Lofberg.

Cystatin C is a 122-amino acid, 13-kDa protein that is a member of the family of cysteine proteinase inhibitors whose function is thought to be modulation of the intracellular catabolism of proteins.[(75)] It is encoded by the 'housekeeping type" CST3 gene, and produced by all nucleated cells at a constant rate.[(76)] It is freely filtered by the glomerulus and is largely reabsorbed and catabolised in the proximal tubules. Early studies have suggested no apparent tubular secretion of cystatin C.[(77)] Although its clearance cannot be measured because of its catabolism, its plasma or serum concentration is a good measure of GFR, with possible advantages over more established markers such as serum creatinine.[(78)]

For serum cystatin C determination the particle-enhanced nephelometric immunoassay (PENIA) was introduced by Dade Behring (now Siemens) in 1997 and is the only one approved presently by the FDA.[(79)] The advantages of using the PENIA include faster turnaround time and lack of interference by other substances that affect serum creatinine measurements. The main limitation of cystatin C immunoassay determination lies in the standardisation of platforms which will require

an international reference standard preparation to allow for valid comparisons. The potential utility of serum cystatin C in the laboratory lies in its capability to detect early renal failure, i.e. at stage 2 CKD (i.e. GFR level of 60 to 90 ml/min/1.73 m2).[80] The reciprocal of cystatin C levels has been found to correlate well with measured GFR and many equations based on cystatin C in specific populations have been developed.

A Belgium- based study by Willems D, Wolff F, Mekhali F and co-workers that compared renal markers to Cr-EDTA clearance in 67 diabetic patients with normal creatinine showed that Cystatin C was a more sensitive parameter than creatinine for the detection of an incipient nephropathy in diabetes.[81]

Similarly, a meta-analysis of available data from various studies to compare the accuracy of Cystatin C and Creatinine in relation to a reference standard of GFR was performed by Dharnidharka VR, Kwon C, Stevens G, et al. The study involved 4496 subjects and established that the overall coefficient of correlation was significantly greater for 1/cystatin c (mean r = 0.816) in comparison to 1/creatinine.[82]

TABLE 5. CYSTATIN C BASED eGFR EQUATIONS FOR ADULTS

TABLE 5. CYSTATIN C BASED eGFR EQUATIONS FOR ADULTS	
Grubb A et al [83]	eGFRgrubb= 84.69 x cystatin $c^{-1.680}$ (x 0.948 if female)
Le Bricon et al [84]	eGFR= 78/cystatin C (mg/l) + 4
Hoek et al [85]	eGFR = 80.35/cystatin C (mg/l) -4.32
Larsson et al [86]	eGFR = 77.239 x cystatin C (mg/dl) $^{-1.2623}$
Macisaac et al [87]	eGFR = 86.7/cystatin C (mg/dl)- 4.2
Rule et al [88]	eGFR = 76.6 x cystatin C (mg/dl) $^{-1.16}$

J. Surendar, S. Anuradha, Berty Ashley, et al studied 209 subjects at the Madras Diabetes Research Foundation in the year 2008. The subjects were divided into 5 groups depending on glucose tolerance, presence or absence of nephropathy and retinopathy. The study concluded that Cystatin C levels increase and Cys C-GFR levels decrease with increasing severity of glucose intolerance.(89)

Several similar studies in the past decade have established the diagnostic accuracy of serum Cystatin C over serum creatinine for stage 1 and 2 chronic kidney disease.(90)

However, there are few studies that have been performed in the Indian subcontinent that support currently available data. Our study intends to assess the performance of Cystatin C in detecting early phase of kidney dysfunction in Diabetics in the Indian clinical setting.

MATERIAL AND METHODS

- **SOURCE OF DATA:**

The study included 42 diabetic patients who presented to RLJ Hospital Kolar attached to SUAHER during the study period extending from March 2016 – March 2017.

INCLUSION CRITERIA :

Type 2 diabetes mellitus patients with serum creatinine based eGFR > 60 ml/min/1.73m^2 (as determined by the Cockroft and Gault formula) were included in the study.

EXCLUSION CRITERIA:

Patients with-

- Thyroid dysfunction.
- Steroids or immunosuppressants usage including asthmatics.
- Malignancy.
- Ischemic heart disease or Congestive Cardiac failure.
- Urinary tract infections.
- Myocardial infarction
- Stroke

METHODS:

Informed consent was taken from all the participants.

Detailed history was obtained from the study participants. Meticulous examination was done.

The following laboratory tests were done:

1. Serum creatinine
2. Blood urea
3. Serum Cystatin C
4. Fasting Blood Sugars
5. Post Prandial Blood sugars
6. Glycated haemoglobin (HbA1C)

ESTMATION OF SERUM CYSTATIN C LEVEL :

Cystatin C was estimated by immunonephelometric method using Siemens' BNTM II Prospec nephelometer. Nephelometry works on the following principle: Polysterene particles coated with specific antibodies to Cystatin C aggregate when mixed with samples containing

human Cys C. These aggregates scatter a beam of light passed through the sample.

The intensity of scattered light is proportional to the amount of Cystatin C in the sample. Reference range for Cystatin C was 0.83-1.1 mg/L.

STUDY DESIGN:

An observational Prospective study was performed in which 42 patients were included . eGFR using serum Cystatin C was estimated in all the patients and was compared to eGFR estimated using serum creatinine by Cockroft and Gault formula[91]

Sample size:

Has been estimated based on the outcome of Cystatin C levels in the early renal impaired diabetic subjects from the study by J surender et al. Considering SD of 0.12, at 5% alpha error and at 95% Confidence level sample size of 38 was obtained and had been included in the study.

With 10% Non-response rate 38 + 3.8 ≈ 42 subjects had been included in the study

Formula used:

Sample size = $Z_{1-\alpha/2}{}^2 p(1-p)/d^2$

Here

- Z = Standard normal variate

[at 5% type 1 error ($p<0.05$), it is 1.96 and at 1% type 1 error(p,0.01),it is 2.58].

As in majority of studies, p values are considered significant below 0.05, hence 1.96 is used in formula.

- p = Expected proportion in population based on previous studies or pilot studies.

Here p = 21.9 or 0.219 and q (1-p) = 78.1 or 0.781

- d = Absolute error or precision which has to be decided by researcher.

d = 10% or 0.1

Z = at 1% alpha error = 2.58

SD = 0.12

d= 5% error

RESULTS

Statistical methods:

Descriptive analysis: Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables.

Inferential statistics:

Quantitative outcome:

The association between categorical explanatory variables and quantitative outcome was assessed by comparing the mean values. The mean differences along with their 95% CI were presented by Independent sample t-test.

Categorical outcome:

The association between explanatory variables and categorical outcomes was assessed by cross tabulation and comparison of percentages. Odds ratio along with 95% CI is presented. Chi square test was used to test statistical significance.

Linear regression:

The association between quantitative explanatory and outcome variables was assessed by calculating person correlation coefficient and the data was represented in a scatter diagram.Uni-variate linear regression analysis was also performed. The association between quantitative explanatory variables and ordinal variables was assessed by spearman's rank correlation. The regression coefficients and their 95% CI were presented.

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.[92]

Table 5: Descriptive analysis of Age in study population (N=42)

Parameter	Mean±STD	Median	Min	Max	95% C.I.for EXP(B)	
					Lower	Upper
Age (in years)	61.33 ± 12.30	61.50	43.00	90.00	57.50	65.17

In the study, median age of study population was 61.50 years and age range was 61.33 ± 12.30 years.

Table 6: Descriptive analysis of Age group in study population (N=42)

Age group	Frequency	Percentage
<60 years	20	47.62%
>60 years	22	52.38%

We observed in our study majority of the patients were more than 60 years of age which constitute 52.38% and patients less than 60 years constitute 47.62%.

Table 7: Descriptive analysis of Gender in study group (N=42)

Gender	Frequency	Percentage
Male	27	64.29%
Female	15	35.71%

In the present study majority of included patients were males who constitutes 64.29% followed by females who constitute 35.71%.

Fig 5: Pie chart representing Gender distribution in study group (N=42)

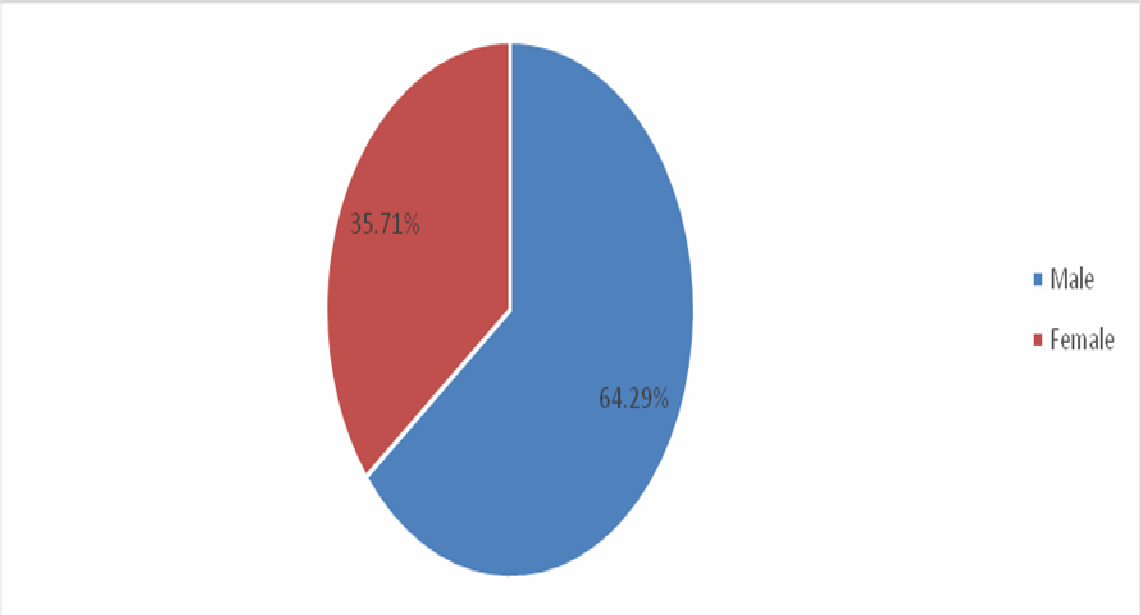


Table 8: Descriptive analysis of Duration of diabetic mellitus in yrs. in study population (N=42)

Duration of diabetic mellitus in yrs.	Frequency	Percentages
<5 years	14	33.33%
5 to 10 years	18	42.85%
>10 years	10	23.82%

In the study, we observed majority of patients had duration of diabetes between 5 to 10 years who constitute 42.85 % (18) followed by less than 5 years duration who constituted 33.33% (14) and patients with diabetes duration more than 10 years who constituted 23.82% (10).

Fig 6: Pie chart showing duration of diabetic mellitus in years in study population (N=42)

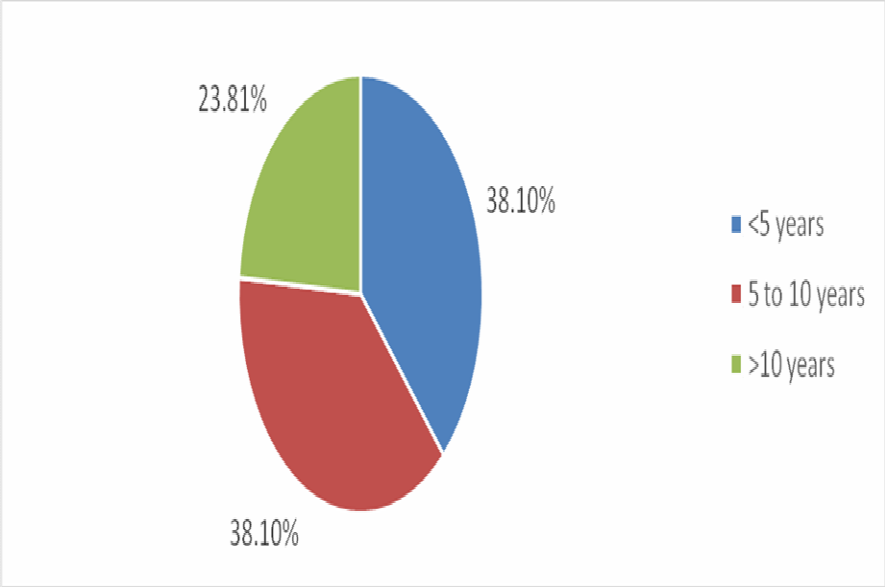


Table 9: Descriptive analysis of HbA1C (%) category in study population (N=42)

HbA1C(%) category	Frequency	Percentage
<7%	12	28.57%
≥7%	30	71.43%

In the present study we observed HbA1C value <7% in 28.57 % (12) and ≥ 7% in 71.43% (30) patients.

Fig 7: Pie chart of HbA1C (%) categories in study population (N=42)

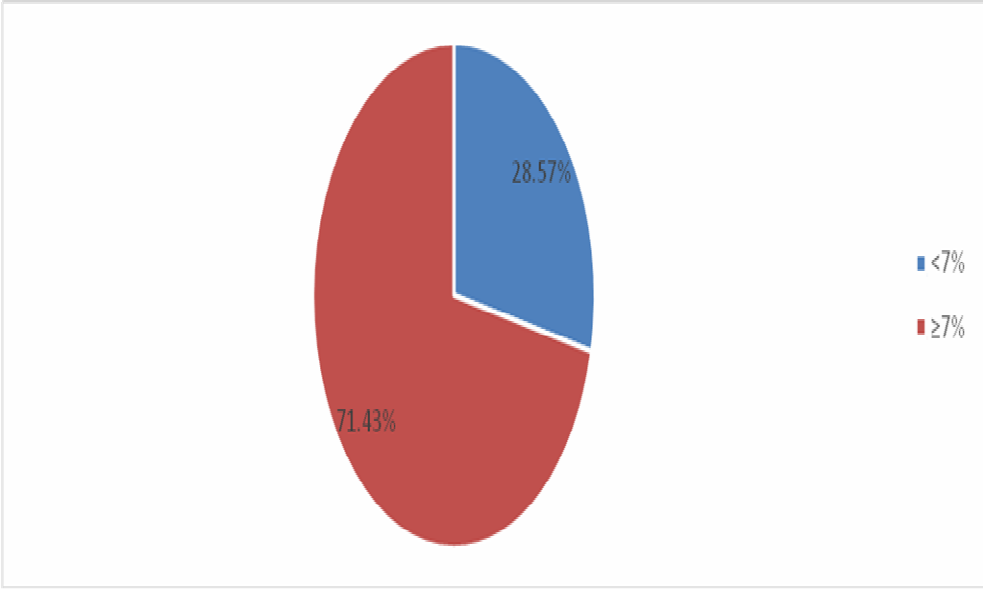


Table 10: Association of Age groups with Gender of study population (N= 42)

Age group	Gender		Chi square	P-value
	Male	Female		
<60 years	14 (70%)	6 (30%)	.543	0.461
>60 years	13 (59.09%)	9 (40.91%)		

In the study, among the males, patients with less than 60 years constitute 47.61% and patients with age more than 60 years constitute 52.39%. Males were in the majority in the both less than 60 years and more than 60 years groups.

Fig 8:Association of Age group with Gender of study population

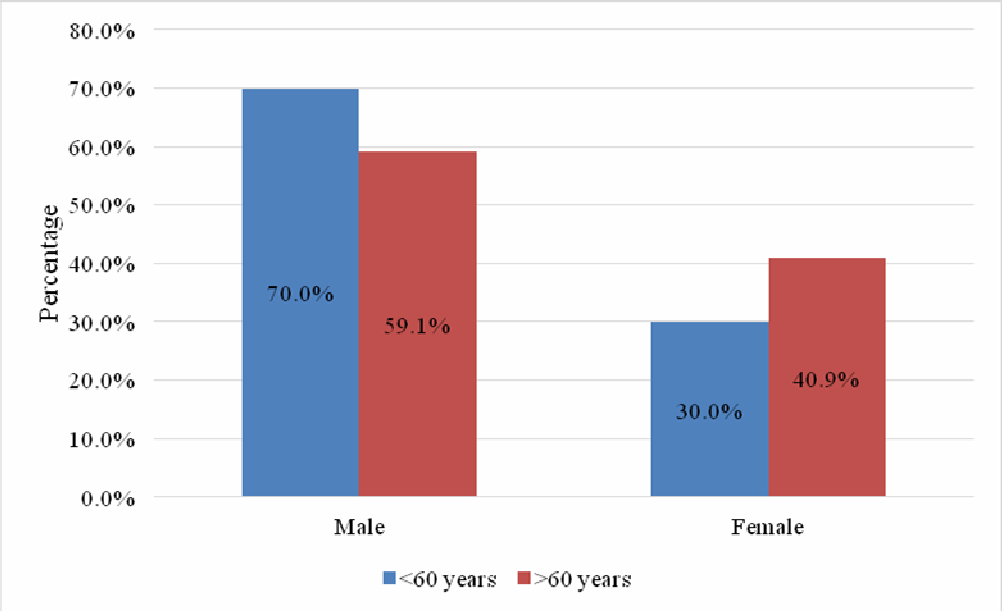


Table 12: Descriptive analysis for Serum creatinine(mg/dl), Serum creatinine based eGFR (ml/min/1.73 m2), Serum cystatin C (mg/l), Serum cystatin C based eGFR(ml/min/1.73 m2), Urine albumin (mg/dl), HbA1C (%), Fasting blood sugar(mg/dl), Post prandial blood sugar (mg/dl), in study population (N=42)

Parameter	Mean ±STD	Median	Min	Max	95% C.I.for EXP(B)	
					Lower	Upper
Serum creatinine(mg/dl)	0.721 ± 0.256	0.70	0.20	1.20	0.64	0.80
Serum creatinine based eGFR (ml/min/1.73 m2)	122 ± 17.96	125.50	76.00	146.00	116.40	127.60
Serum cystatin C (mg/l)	0.968 ± 0.132	1.00	0.75	1.20	0.93	1.01
Serum cystatin C based eGFR (ml/min/1.73 m2)	81.14 ± 16.53	80.00	60.00	116.00	75.99	86.30
Urine albumin (mg/dl)	103.2 ± 125.6	24.00	6.50	11.20	64.09	142.42
HbA1C (%)	7.940 ± 1.059	7.95	6.50	11.20	7.61	8.27
Fasting blood sugar(mg/dl)	169.7 ± 36.77	165.50	120.00	266.00	158.28	181.20
Post prandial blood sugar(mg/dl)	264.0 ± 53.15	253.00	200.00	400.00	247.46	280.59

In the study population serum creatinine was in the range of 0.721 ± 0.256 ml/dl and eGFR based on it was in the range of 122 ± 17.96 ml/min/1.73 m^2.

Serum cystatin-C was in the range of 0.968 ± 0.132 mg/l eGFR based on it was in the range of 81.14 ± 16.53 ml/min/1.73 m^2.Other parameters in the study such as urine albumin were in the range of 103.2 ± 125.6 mg/dl, HbA1C 7.940 ± 1.059 %, FBS 169.7 ± 36.77 mg/dl and PPBS 264.0 ± 53.15 mg/dl.

Table 12: Descriptive analysis of HbA1C (%) category in study population (N=42)

HbA1C(%) category	Frequency	Percentage
<7%	12	28.57%
≥7%	30	71.43%

In the present study we observed HbA1C value <7% in 28.57 % (12) and ≥7% in 71.43% (30) patients.

Table 13: Comparison of mean Serum creatinine (mg/dl) across study groups (N=42)

HbA1C(%)	Serum creatinine(mg/dl) Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
<7%	0.77 ± 0.27	0.06	-0.11	0.24	.476
≥7%	0.7 ± 0.25				

In the study patients with HbA1C <7% had serum creatinine in the range of 0.77 ± 0.27 mg/dl and HbA1C ≥7% had serum creatinine in the range of 0.7 ± 0.25 mg/dl.

Table 14: Comparison of mean Serum creatinine based eGFR (ml/min/1.73 m2) across study groups (N=42)

HbA1C(%)	Serum creatinine based eGFR (ml/min/1.73 m2) Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
<7%	134.17 ± 8.5	17.03	5.72	28.34	***.004***
≥7%	117.13 ± 18.52				

Patients with HbA1C <7% had eGFR based on serum creatinine in the range of 134.17 ± 8.5 ml/min/1.73 m2 and patients with HbA1C ≥7% had eGFR based on serum creatinine in the range of 117.13 ± 18.52. This results show patients with Hb1AC ≥7% had slightly low eGFR based on serum creatinine.

Fig 9:Comparison of mean Serum creatinine based eGFR (ml/min/1.73 m2) across study groups

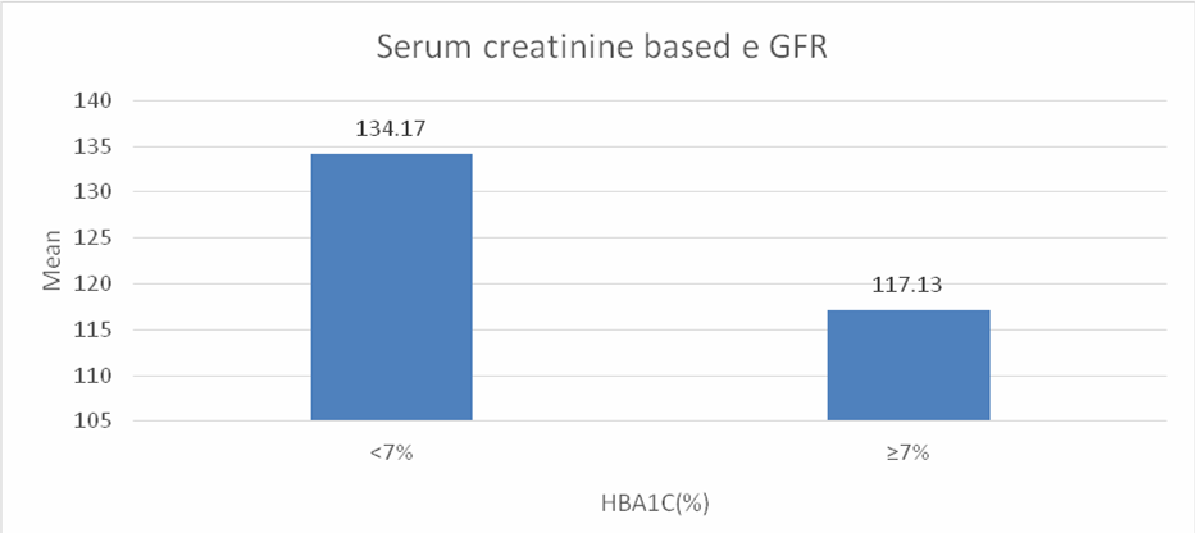


Table 15: Comparison of mean Serum cystatin C (mg/l) across study groups (N=42)

HbA1C(%)	Serum cystatin C (mg/l) Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
<7%	0.91 ± 0.13	-0.09	-0.18	0.00	***.048***
≥7%	0.99 ± 0.13				

In the study patients with HbA1C <7% had serum cystatin-c in the range of 0.91 ± 0.13 mg/l and HbA1C ≥7% had serum cystatin-c in the range of 0.99 ± 0.13 mg/l.

Table 16: Comparison of mean Serum cystatin c based e GFR (ml/min/1.73 m2) across study groups (N=42)

HBA1C (%)	Serum cystatin c based e GFR(ml/min/1.73m^2) Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
<7%	91.42 ± 15.41	14.38	3.78	24.99	***.009***
≥7%	77.03 ± 15.34				

Patients with HbA1C <7% had eGFR in the range of 91.42 ± 15.41 ml/min/1.73 m2 based on serum cystatin-C and patients with HbA1C ≥7% had eGFR in the range of 77.03 ± 15.34. based on serum cystatin-C. This results show patients with Hb1AC ≥7% had significant low eGFR based on serum cystatin-C compared to eGFR based on serum creatinine.

Fig 10:Comparison of mean Serum cystatin c based e GFR (ml/min/1.73 m2) across study groups

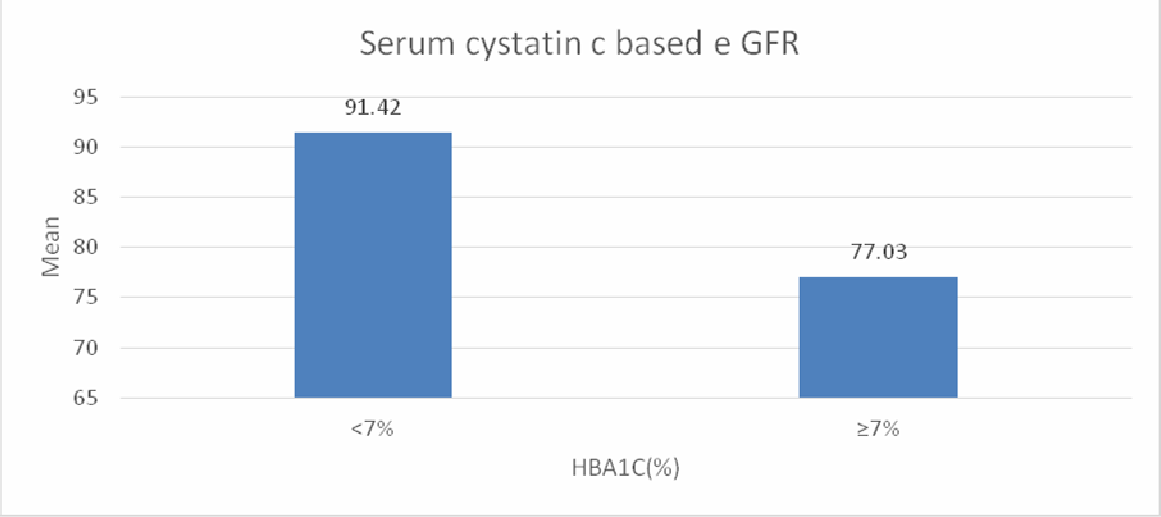


Table 17: Correlation between HbA1C and Serum creatinine based Egfr and Serum cystatin C based eGFR in the study group (42)

Parameter	r value	P value
Serum creatinine based eGFR (ml/min/1.73 m2)	-0.586	<0.001
Serum cystatin C based eGFR(ml/min/1.73 m2)	-0.510	<0.001

Fig 11:Correlation between HbA1C and Serum creatinine based eGFR

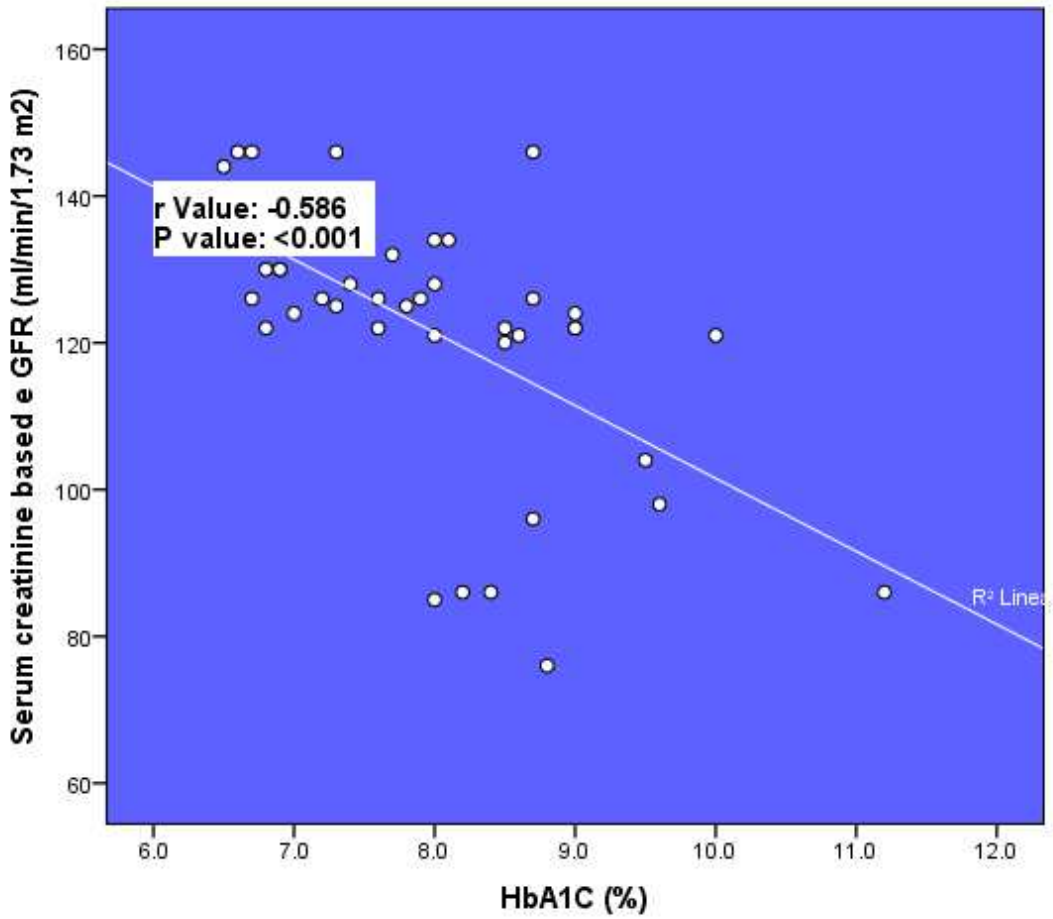


Fig 12:Correlation between HBA1C and Serum cystatin c based e GFR

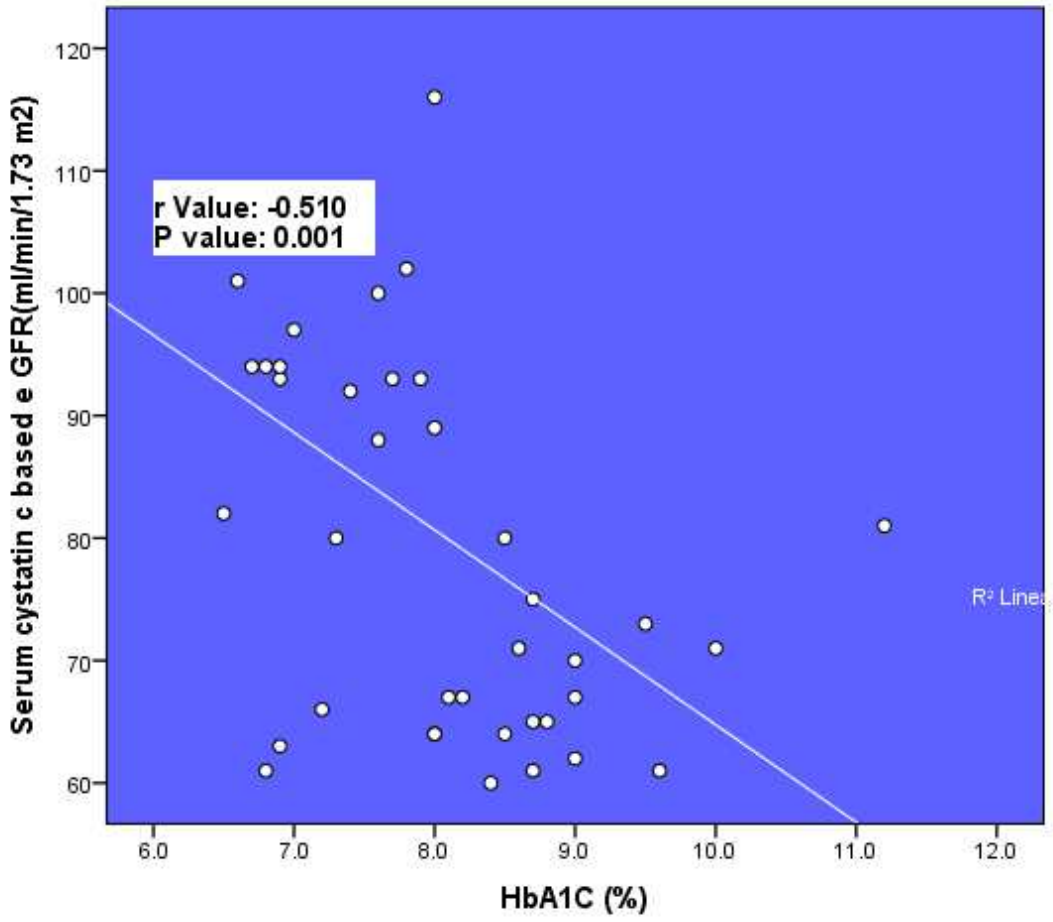


Table 18: Correlation between FBS and Serum creatinine based eGFR, Serum cystatin-C based e GFR in the study group (42)

Parameter	r value	P value
Serum creatinine based eGFR (ml/min/1.73 m2)	-0.511	0.001
Serum cystatin C based eGFR(ml/min/1.73 m2)	-0.470	0.002

Fig 13:Correlation between FBS and Serum creatinine based eGFR

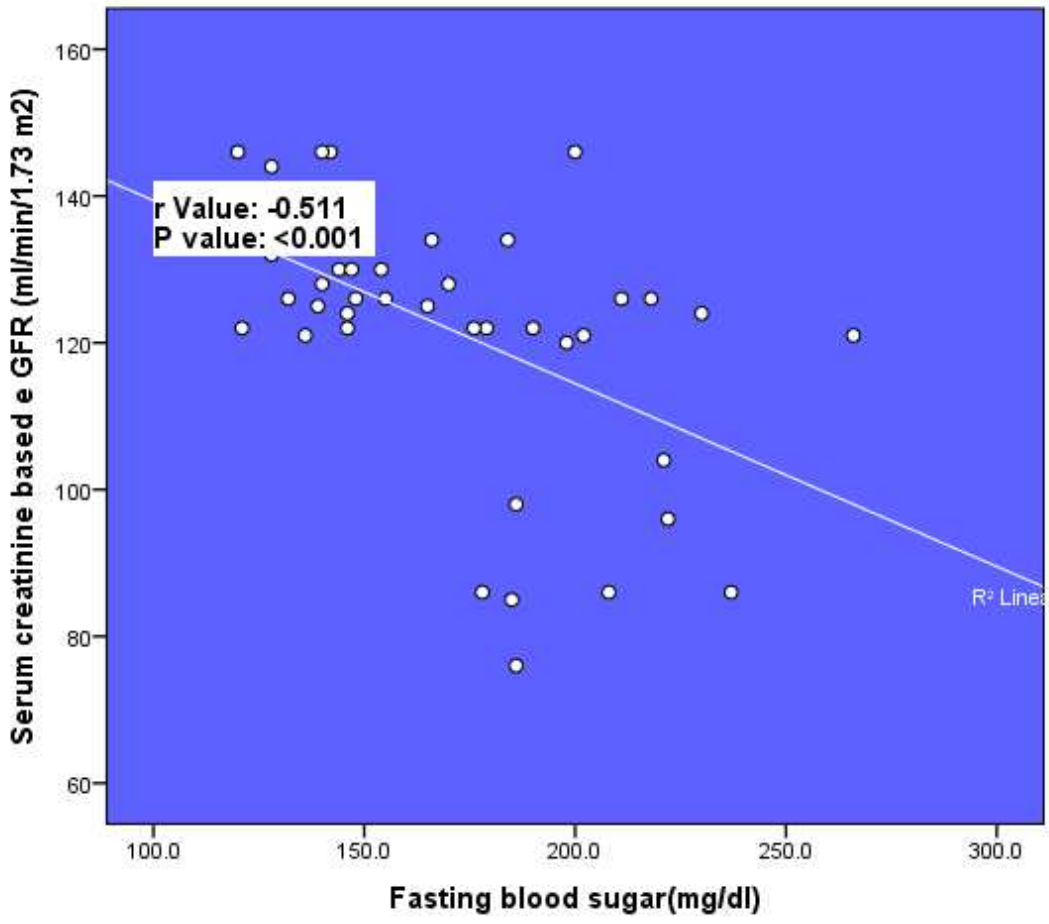


Fig 14:Correlation between FBS and Serum cystatin c based e GFR

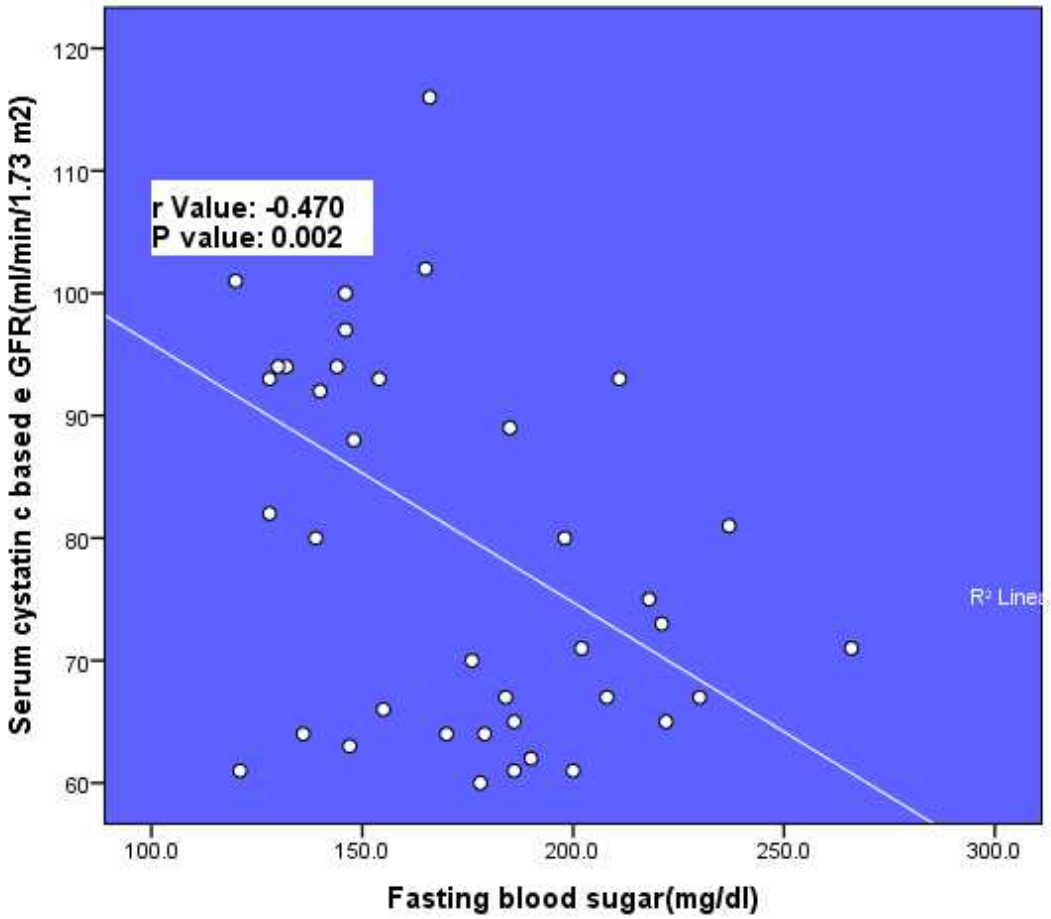


Table 19: Correlation between PPBS and Serum creatinine based eGFR, Serum cystatin-C based eGFR in the study group (42)

Parameter	r value	P value
Serum creatinine based eGFR (ml/min/1.73 m2)	-0.390	0.011
Serum cystatin C based eGFR(ml/min/1.73 m2)	-0.389	0.011

Fig 15:Correlation between PPBS and Serum creatinine based eGFR

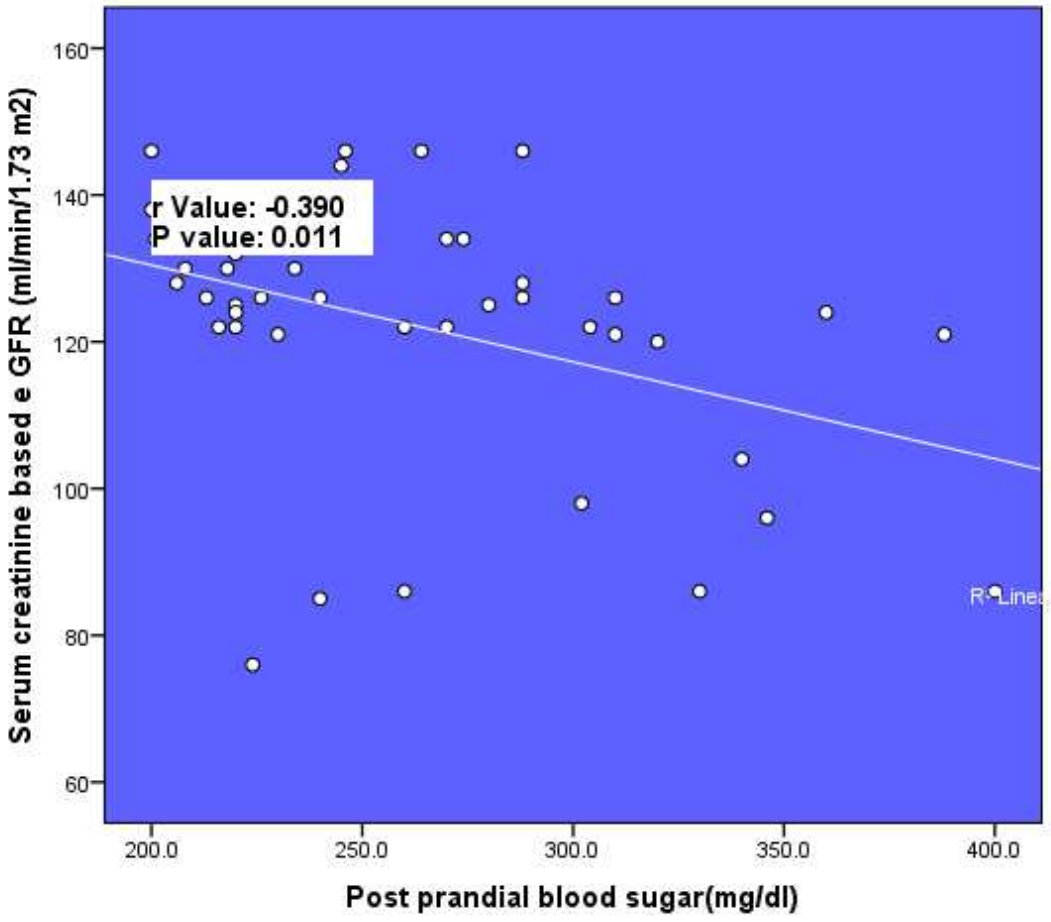


Fig 16:Correlation between PPBS and Serum cystatin-c based e GFR

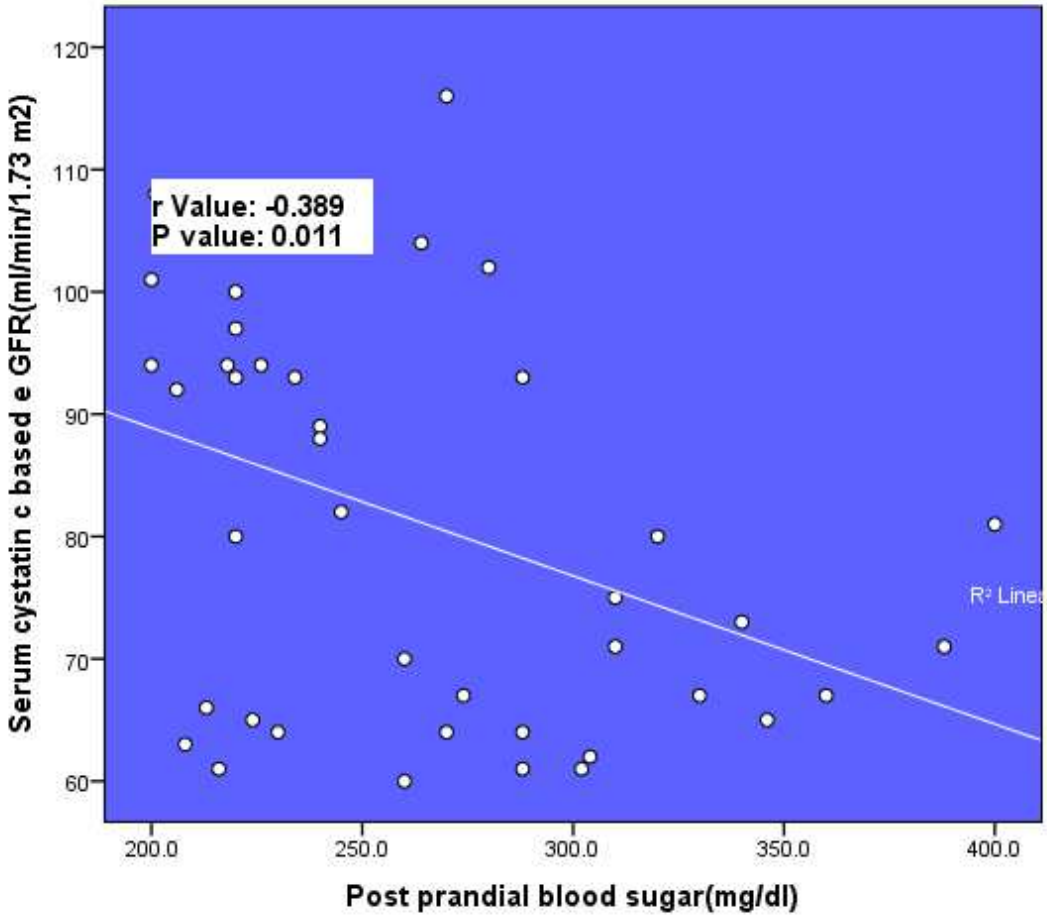


Table 20: Association of serum creatinine based eGFR category with serum cystatin-c based eGFR category of study population (N=42)

SERUM CYSTATIN-C BASED eGFR (ml/min/1.73 m2) CATEGORY	SERUM CREATININE BASED eGFR (ml/min/1.73 m2) CATEGORY		Chi square	P-value
	>90	**60 to 89**		
>90	16 (43.24%)	0 (0%)	3.493	0.062
60 to 89	21 (56.76%)	5 (100%)		

In the study we measured the eGFR based on serum creatinine and eGFR based on serum cystatin-C.

We found :

- Out of 42, 37 (88%) patients had eGFR > 90 ml/min/1.73m^2 and 5 (12%) patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine .

- Out of 42, 16 (38.80%) patients had eGFR > 90 and 26 (61.90%) patients had between 60-89 based on serum cystatin-C.

	Kappa statistics	Std. Error	P value
Measure of Agreement	0.154	0.070	0.062

Fig 17:Correlation between Serum cystatin C based eGFR and Serum creatinine based eGFR

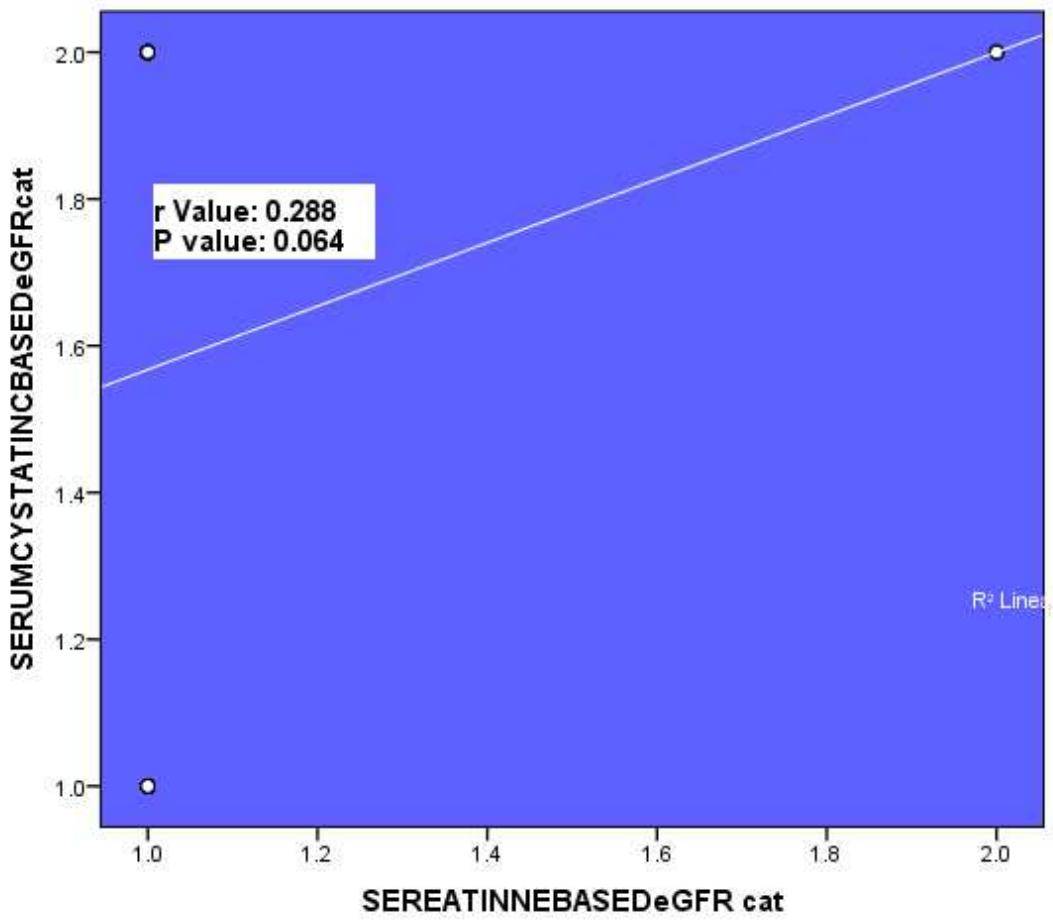


Table 21: Comparison of mean Age across study groups (N=42)

SERUM CREATININE BASED eGFR (ml/min/1.73m^2) category	Age Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
>90	58.59 ± 10.19	-23.01	-32.49	-13.52	***<0.001***
60 to 89	81.6 ± 5.94				

In present study we observed eGFR based on serum creatinine comparatively was low in elderly than younger age group.

Table 22: Comparison of mean Age across study groups (N=42)

SERUM CYSTATIN-C BASED eGFR (ml/min/1.73 m2) category	Age Mean± STD	Mean difference	95% CI		P value
			Lower	Upper	
>90	51.81 ± 8.35	-15.38	-21.70	-9.06	***<0.001***
60 to 89	67.19 ± 10.62				

In present study we observed eGFR based on serum cystatin-c comparatively was low in elderly than younger age group.

Table 23: Association of serum creatinine based eGFR category with Gender of study population (N=42)

Gender	SERUM CREATININE BASED eGFR CATEGORY		Chi square	P-value
	>90	60 to 89		
Male	25 (67.57%)	2 (40%)	1.458	0.227
Female	12 (32.43%)	3 (60%)		

We observed 25 (92.59%) male patients had eGFR >90 ml/min while 2 (7.41%) males had eGFR 60-89 ml/min based on serum creatinine out of 27 male patients. 12 (80%) female patients had eGFR >90 ml/min while 3 (20%) female had eGFR 60-89 ml/min based on serum cystatin-c out of 15 female patients.

Table 24: Association of serum cystatin-c based eGFR category with Gender of study population (N=42)

Gender	SERUM CYSTATIN -C BASED eGFR CATEGORY		Chi square	P-value
	>90	**60 to 89**		
Male	11 (68.75%)	16 (61.54%)	.224	0.636
Female	5 (31.25%)	10 (38.46%)		

We observed 11 (40.74%) male patients had eGFR >90 ml/min while 16 (59.26%) male had eGFR 60-89 ml/min based on serum cystatin-C out of 27 male patients . 5 (33.33%) female patients had eGFR >90 ml/min while 10 (66.67%) female had eGFR 60-89 ml/min based on serum cystatin-c out of 15 female patients.

Table 25: Descriptive analysis of Comorbidites in study population (N=42)

Comorbidies	Frequency	Percentage
HTN	9	20%
PVD	1	3%
Seizure	1	1%

In the study 9 (20%) patients had hypertension, 1 (3%) had peripheral vascular disease and 1 (3%) patient had seizure disorder.

Fig 18: Pie chart of Comorbidies in study population (N=42)

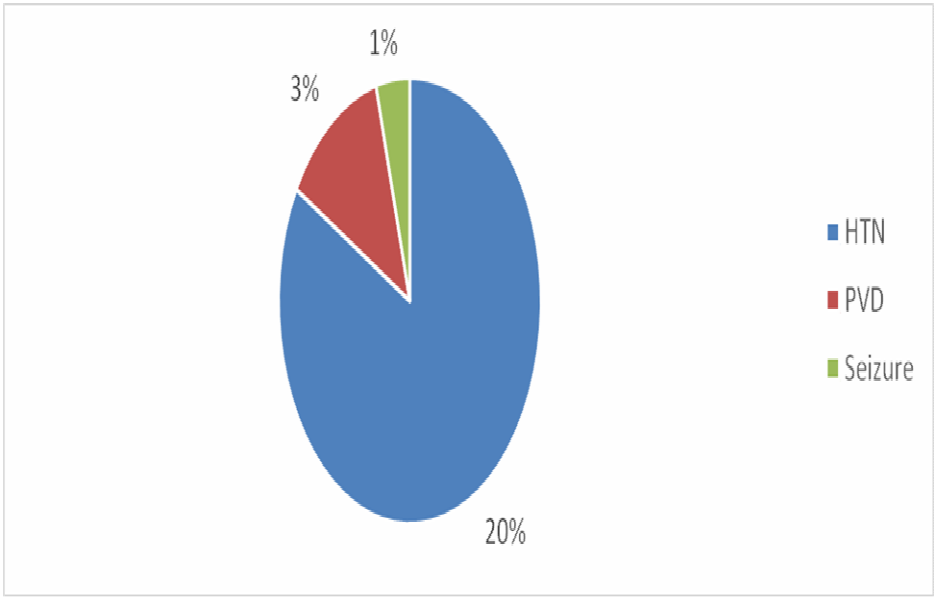


Table 26: Descriptive analysis of Retinopathy in study population (N=42)

Retinopathy	Frequency	Percentage
NPDR	7	17%
PDR	1	2%
HYPERTENSIVE	5	12%

In the study group 7 (17%) patients had non proliferative diabetic retinopathy (NPDR), 1 (2%) patient had proliferative retinopathy (PDR) and 5 (12%) had hypertensive retinopathy.

Fig 19: Pie chart of retinopathy in study population (N=42)

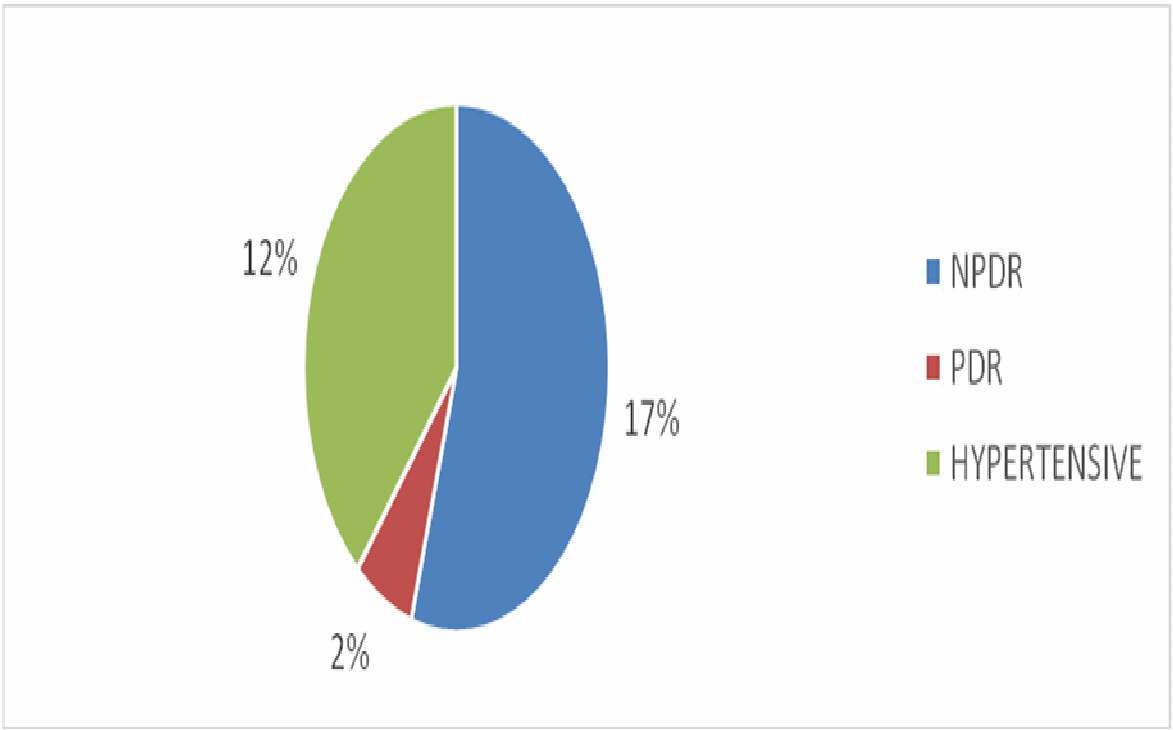


Table 26: Descriptive analysis of Retinopathy in study population (N=42)

Retinopathy	Frequency	Percentage
NPDR	7	17%
PDR	1	2%
HYPERTENSIVE	5	12%

In the study group 7 (17%) patients had non proliferative diabetic retinopathy (NPDR),

1 (2%) patient had proliferative retinopathy (PDR) and 5 (12%) had hypertensive retinopathy.

Fig 19: Pie chart of retinopathy in study population (N=42)

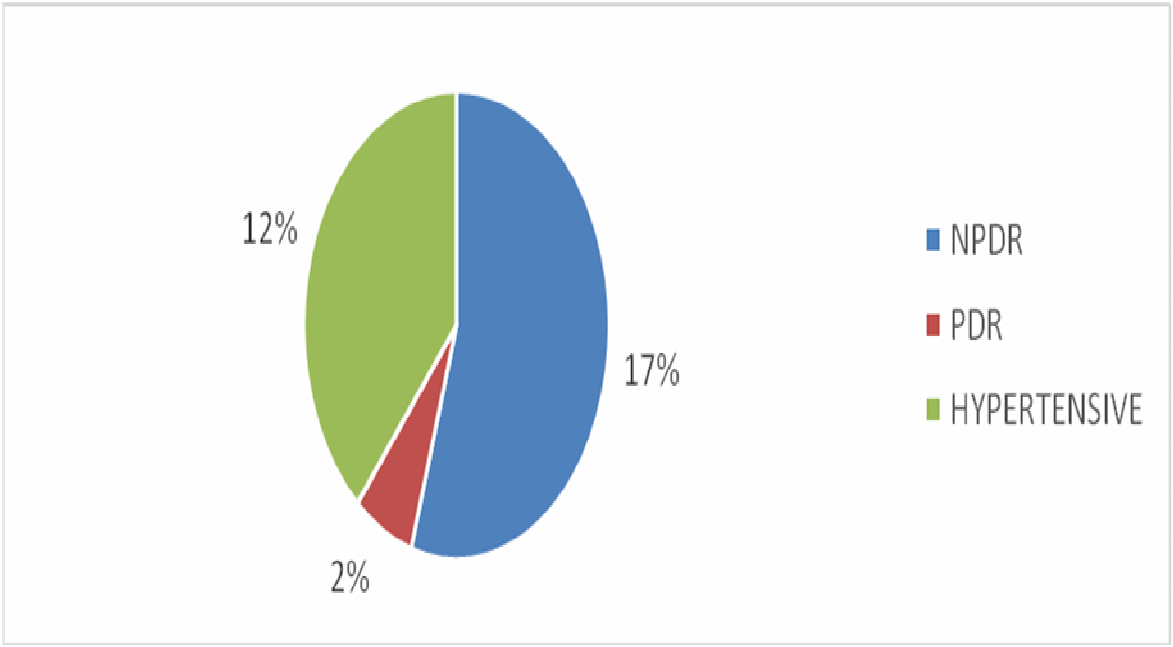


Table 27: Descriptive analysis of CKD stages based on serum cystatin c-in study population (N=42)

CKD stages based on serum cystatin C	Frequency	Percentage
CKD Stage1	16	38%
CKD Stage2	26	62%

In the present study a significant number of patients 26 (62%) patients had CKD stage-2 while 16 (38%) patients had CKD stage 1.

Fig 20: Pie chart of CKD stages based on serum cystatin C in study population (N=42)

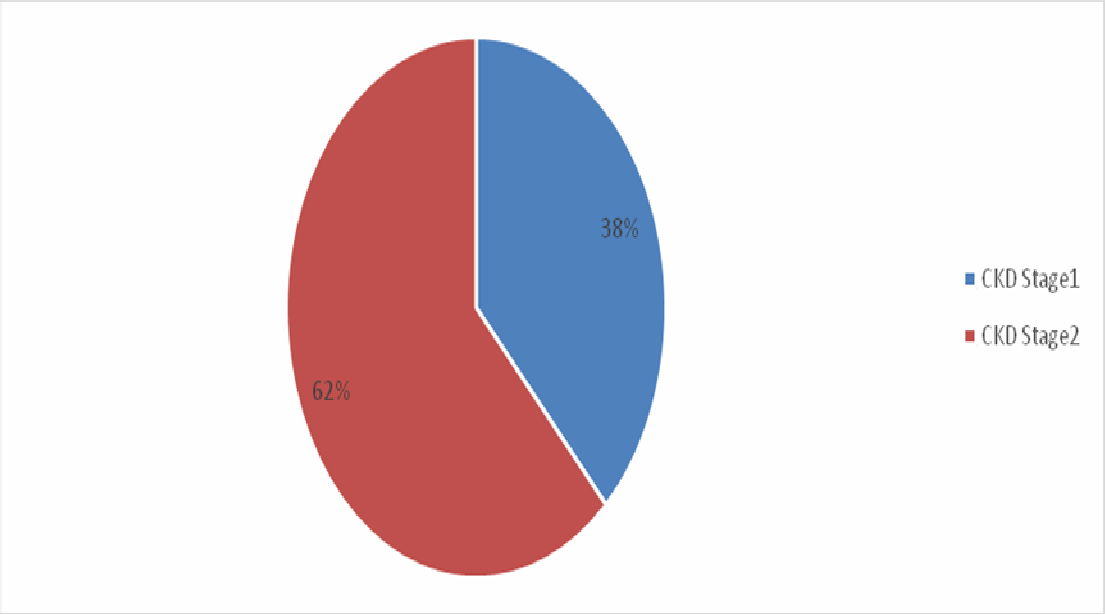


Table 28: Descriptive analysis of CKD stages based on serum creatinine in study population (N=42)

CKD stages based on serum creatinine	Frequency	Percentage
Normal	35	83.33%
CKD Stage1	4	9.6%
CKD Stage2	3	7.14%

In the present study a significant number of patients 35 (83.33%) patients had normal eGFR, 4 patient (9.6%) had CKD stage-1 and 3 (7.14%) patients had CKD stage2 which is significantly lower than serum cystatin-C.

Fig 21: Pie chart of CKD stages based on serum creatinine in study population (N=42)

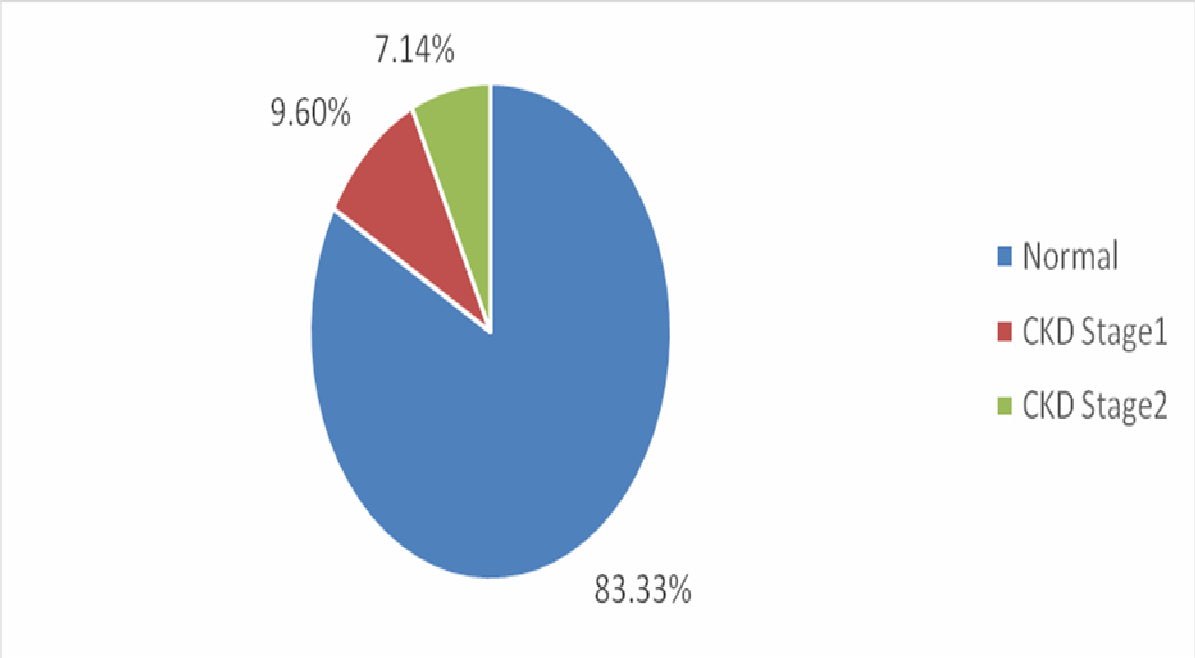


Table 29: Descriptive analysis of Albuminuriain study population (N=42)

Albuminuria	Frequency	Percentage
Narmo albuminuria	28	67%
Micro albuminuria	12	29%
Marco albuminuria	4	9%

Out of 42 patients 28 (67%) had normal albuminuria, 12 (29%) had micro albuminuria and 4 (9%) had Marco albuminuria.

Fig 22: Pie chart of Albuminuria in study population (N=42)

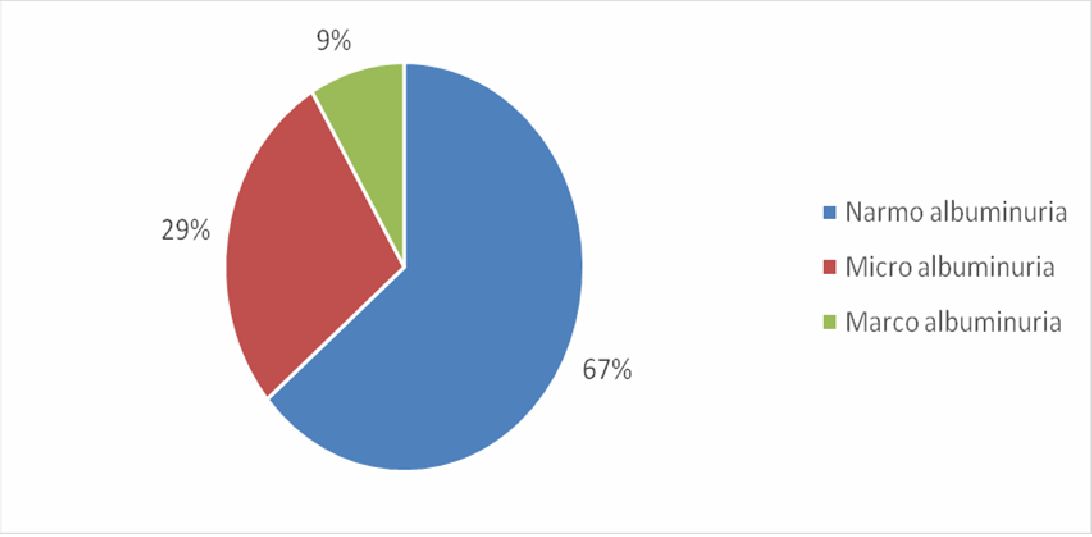


Table 30: Association of urine albumin with sereum creatinine based eGFR category of study population (N= 42)

Serum CREATININE BASED eGFR	urine albumin		
	Normal	micro albuminuria	macro albuminuria
>90	26 (70.27%)	9 (24.32%)	2 (5.41%)
60 to 89	0 (0%)	3 (60%)	2 (40%)

Table 31: Association of urine albumin categories with serum creatinine based eGFR cat of study population (N= 42)

SERUM CREATININE BASED eGFR	urine albumin	
	Normal albuminuria	Micro+macro albuminuria
>90	26 (70.27%)	11 (29.73%)
60 to 89	0 (0%)	5 (100%)

In the study, we observed :

- ✓ 26 patients had normoalbuminuria. Out of which 26 patients had eGFR >90 ml/min/1.73m^2 based on serum creatinine
- ✓ 16 patients had micro and macroalbuminuria-
- ✓ 11 patients had eGFR >90 ml/min/1.73m^2 and
- ✓ 5 patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine

Table 32: Association of urine albumin categories with serum cystatin-C based eGFR category of study population (N= 42)

SERUM CYSTATIN-C BASED eGFR CATEGORY	urine albumin 3 categories		
	Normal	micro albuminuria	macro albuminuria
>90	16 (100%)	0 (0%)	0 (0%)
60 to 89	10 (38.46%)	12 (46.15%)	4 (15.38%)

Table 33: Association of urine albumin categories with serum cystatin-C based eGFR category of study population (N=42).

SERUM CYSTATIN-C BASED eGFR CATEGORY	urine albumin categories	
	Normal	micro+macro albuminuria
>90	16 (100%)	0 (0%)
60 to 89	10 (38.46%)	16 (61.54%)

In the study, we observed :

- 26 patients had normoalbuminuria. Out of which –

  -16 patients had eGFR >90 ml/min and

  - 10 patients had eGFR between 60 to 89ml/min based on serum cystatin-c.

- 16 patients had micro and macroalbuminuria, all of them had eGFR based on serum cystatin C between 60-89 ml/min.

DISCUSSION

In this study we estimated and compared eGFR based on serum creatinine and based on serum cystatin-c levels to detect early diabetic nephropathy.

The mean duration of diabetes in the study was 8.3 years and median age of patients was 61.50 years and age range was 61.33 ± 12.30 years.

In the study males were predominant than females. Majority of the patients were more than 60 years of age which constitute 52.38% (22) and patients less than 60 years constitute 47.62%.

Out of 42 included diabetic patients 20 (47.61%) had hypertension, 3 (7.1%) had peripheral vascular disease (PVD) and 1 (2.38%) had seizure.

Glycemic control was assessed by FBS (fasting blood sugar), PPBS (postprandial blood sugar) and glycated haemoglobin (HbA1C). Out of 42 patients, 30 (71.42%) had ≥7.0 % HbA1C while 12 (18.68 %) had <7.0%. We observed there was significant difference in serum cystatin-c levels in patients with HbA1C ≥7.0 % compared to patients with Hb1AC <7.0%. There was no significant difference in serum creatinine in patients with Hb1AC <7.0% OR ≥7.0 %.

Patients with HbA1C <7% had eGFR in the range of 134.17 ± 8.5 ml/min/1.73 m^2 based on serum creatinine and patients with HbA1C ≥7% had eGFR in the range of 117.13 ± 18.52 based on serum creatinine. On the analysis we found, patients with Hb1AC ≥7% had slightly low eGFR based on serum creatinine.

Patients with HbA1C <7% had eGFR in the range of 91.42 ± 15.41 ml/min/1.73 m^2 based on serum cystatin-c and patients with HbA1C ≥7% had eGFR in the range of 77.03 ± 15.34 ml/min/1.73 m^2 based on serum cystatin-c. On the

analysis, we found patients with Hb1AC ≥7% had significant low eGFR based on serum cystatin-c compared to eGFR based on serum creatinine.

Our results are similar to a study done by Jocelyn Eid Fares et al who concluded that HbA1C may act differently in terms of developing nephropathy, depending on the variation in the levels of HbA1C and more precisely, depending on the frequency of the acute ‘‘jumps” in the HbA1C. However, this effect seems to be more pronounced among those who have higher values of HbA1C considered as poorly controlled. [93]

Another study done by Dipsha Kriplani et al found that There is a strong correlation between the increase in the levels of glycosylated hemoglobin with the corresponding rise in the levels of microalbuminuria and serum creatinine. [94]

The mean serum creatinine was 0.70 mg/dl ranging from 0.721 ± 0.256. creatinine clearance was estimated by Cockroft and Gault equation which varied between 122 ± 17.96 ml/min/1.73 m^2 with mean of 125.50 ml/min/1.73 m^2 .

The mean serum cystatin-c was 1.0 mg/dl ranging from 0.75 ± 1.20. Mean eGFR based on serum cystatin-c was 80 ml/min/1.73m^2 with range of 81.14 ± 16.53 ml/min/1.73m^2.

We measured the eGFR based on serum creatinine and serum cystatin-c. 37 (88%) patients had eGFR more 90 ml/min/1.73 m^2 and 5 (12%) had eGFR between 60-89 ml/min/1.73 m^2 based on serum creatinine; 26 (61.90%) patients had eGFR between 60-89 ml/min/1.73 m^2 and 16 (38.80%) patients had eGFR more than 90 ml/min/1.73 m^2 based on serum cystatin-c. we observed that most of the patients who had normal eGFR that is >120 ml/min/1.73 m^2 or stage 1 CKD (>90 ml/min/1.73 m^2) based on serum creatinine had eGFR 60-89 ml/min/1.73 m^2 based on serum cystatin-c. In our study 5 (11.90%) out of 42 diabetic patients found to have diabetic

nephropathy (CKD stage 2) based on serum creatinine levels while 26 (61.90%) out of 42 diabetic patients had diabetic nephropathy (CKD stage 2) based on serum cystatin-c.

This finding shows serum cystatin-c is more sensitive in detecting diabetic nephropathy compared to serum creatinine.

Serum cystatin-c based eGFR was calculated with the help of following equation- eGFR = 76.6 ×cystatin C [mg/L] −1.16.

This equation was originally used in post renal transplant recipients.[95] Using the Grubb's equation it was found that, among this population, eGFR was consistently over-estimated. Previous studies revealed that variables like age, sex and race did have a tendency to influence CysC GFRs in defined settings and in some cases the CysC equations over-estimated the GFR.[96,97] On using the equation proposed by Rule et al, taking readings above a cut off value of Cystatin C of 0.8 mg/L was found to have a better correlation with 24 hr urine creatinine clearance (r=0.83) than Cockroft and Gault estimates(r=0.41).

Study done by Yi-Sun Yang et al shown similar results and concluded serum cystatin c may be useful especially in detecting early impairement of renal function, and in condition where creatinine or urine albumin measurement is a problem. In addition, serum cystatin C also may be a marker for glomerular dysfunction in type 2 diabetic nephropathy.

Harmoinen et al and Mussap et al showed that serum cystatin C is more sensitive than serum creatinine for the estimation of GFR in type 2 diabetic patients and Tan et al showed the same in type 1 diabetic patients. [98-100]

In 52 Caucasian patients with type 2 diabetes, cystatin C was found to be a better marker of kidney disease measured by serum creatinine or Cockcroft and Gault

GFR estimation. The study clearly demonstrated that serum cystatin-C concentration progressively increased as glomerular filtration rate decreased.

Cystatin C undoubtedly proved to be of greater benefit than serum creatinine based assay in those patients whose GFR was < 60 ml/min consistent with the findings of Yoshiji Ogawa et al. who asserted that cystatin-C was a good predictor of overt nephropathy.[96] Confounding variables of Ischemic heart disease and hypothyroidism did not cause statistically significant change in the GFR.

In our study we observed 37 patients had eGFR >90 ml/min/1.73m^2 based on serum creatinine. Out of whom, 26 patients had normoalbuminuria, 9 had microalbuminuria and 2 had macroalbuminuria.

5 patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine. Out of which, 3 had microalbuminuria and 2 had macroalbuminuria.

16 patients had eGFR >90 ml/min/1.73m^2 based on serum cystatin-c and all of them had normoalbuminuria.

26 patients eGFR between 60-89 ml/min/1.73 m^2, out of which, 10 patients who had normoalbuminuria, 12 patients had micro albuminuria and 4 patients had macroalbuminuria.

The above findings reflect sensitivity of serum cystatin-c which could detect diabetic nephropathy (CKD stage 2) even when patients had normoalbuminuria and normal eGFR based on serum creatinine. It shows that cystatin C levels of serum could be reliable marker for detecting renal dysfunction in type 2 diabetic patients with normoalbuminuria.

Similar results obtained by Durga Prasad Kedam et al concluded that cystatin C was best for discriminating between microalbuminuria and normoalbuminuria in those with type-2 diabetes resulting in the conclusion that cystatin C might be a more

useful marker than creatinine for detection of early diabetic nephropathy in type 2 patients with diabetes.[102]

Other study done by Yun Kyung Jeon et al concluded that the cystatin C levels of serum and urine increased with increasing degree of albuminuria, reaching higher levels in macroalbuminuric patients. Especially, in normoalbuminuric patients, serum and urine cystatin C were identified as independent factors associated with eGFR < 60 mL/min/1.73 m2 estimated by MDRD equation. The cystatin C levels of serum and urine could be useful markers for renal dysfunction in type 2 diabetic patients with normoalbuminuria.[103]

We observed among patients with diabetic nephropathy, 7 (17%) patients had non proliferative diabetic retinopathy (NPDR), 1 (2%) patient had proliferative retinopathy (PDR) and 5 (12%) had hypertensive retinopathy.

Investigations which can detect accurate eGFR which are based on intravenous injection of exogenous substances like Inulin, 125-Iothalamate, Iohexol, 51Cr-EDTA or 99mTc- DTPA are invasive, labour intensive, costly, and not entirely free of potential for harm.

Hence estimation of eGFR based on serum cystatin C can considered as a relatively easy, fast and cost effective alternative.

CONCLUSION

This study was a prospective observational study done at a tertiary medical college hospital. Total of forty two patients satisfying inclusion criteria were studied. The mean duration of diabetes in the study was 8.3 years and median age of patients was 61.50 years.

In the study males were predominant than female population. Majority of the patients were more than 60 years of age who constitute 52.38% (22) and patients less than 60 years constitute 47.62% (20).

This study was undertaken to assess the utility of Serum Cystatin C in detecting early renal dysfunction in type 2 diabetes. Serum Cystatin c was determined by immunonephelometry. Creatinine clearance was estimated by Cockroft and Gault formula.

In the study most of the patients who had normal eGFR that is >120 ml/min/1.73 m^2 or stage 1 CKD (>90 ml/min/1.73 m^2) based on serum creatinine had eGFR between 60-89 ml/min/1.73 m^2 based on serum cystatin-c and 5 (11.90%) out of 42 diabetic patients found to have diabetic nephropathy (CKD stage 2) based on serum creatinine levels while 26 (61.90%) out of 42 diabetic patients had diabetic nephropathy (CKD stage 2) based on serum cystatin-c.

This result shows serum cystatin-C is more sensitive as compared to serum creatinine.

Patients with Hb1AC ≥7% had significant low eGFR based on serum cystatin-c compared to eGFR based on serum creatinine.

In our study we observed 37 patients had eGFR >90 ml/min/1.73m^2 based on serum creatinine. Out of whom, 26 patients had normoalbuminuria, 9 had microalbuminuria and 2 had macroalbuminuria.

5 patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine. Out of which, 3 had microalbuminuria and 2 had macroalbuminuria.

16 patients had eGFR >90 ml/min/1.73m^2 based on serum cystatin-c and all of them had normoalbuminuria.

26 patients eGFR between 60-89 ml/min/1.73 m^2, out of which, 10 patients who had normoalbuminuria, 12 patients had micro albuminuria and 4 patients had macroalbuminuria.

This finding shows sensitivity of serum cystatin-c which could detect diabetic nephropathy (CKD stage 2) in patients who had normoalbuminuria and normal eGFR based on serum creatinine.

This is one of the first studies of its kind in India. Cystatin c has opened new avenues for research in the Indian context. With a larger database representative of the Indian population, a generalization of these study results can probably be made.

SUMMARY

- Total of forty two patients satisfying inclusion criteria were included in study.
- The mean duration of diabetes in the study population was 8.3 years and median age of patients was 61.50 years.
- Males were predominant than females and majority of study group constituted by more than 60 years of age.
- Glycemic control was assessed by FBS, PPBS and glycated haemoglobin. Most of the patients (71.42%) had HbA1C ≥7% and rest had Hb1AC <7.0%.
- There was significant difference in serum cystatin-c levels in patients with HbA1C ≥7.0 % compared to patient with Hb1AC <7.0% and there was no significant difference in serum creatinine in patients with Hb1AC <7.0% OR ≥7.0 %.
- Patients with Hb1AC ≥7% had slightly low eGFR based on serum creatinine while patients with Hb1AC ≥7% had significantly low eGFR based on serum cystatin-c compared to eGFR based on serum creatinine.
- The mean serum creatinine was 0.70 mg/dl ranging from 0.721 ± 0.256 and eGFR based on serum creatinine was in the range of 122 ± 17.96 ml/min/1.73 m^2 .
- The mean serum cystatin-c was 1.0 mg/dl ranging from 0.75 ± 1.20 and Mean eGFR based on serum cystatin-c was 80 ml/min/1.73m^2 with range of 81.14 ± 16.53 ml/min/1.73m^2.
- In our study we observed 37 patients had eGFR >90 ml/min/1.73m^2 based on serum creatinine. Out of whom, 26 patients had normoalbuminuria, 9 had microalbuminuria and 2 had macroalbuminuria.

- 5 patients had eGFR between 60-89 ml/min/1.73m^2 based on serum creatinine. Out of which, 3 had microalbuminuria and 2 had macroalbuminuria.
- 16 patients had eGFR >90 ml/min/1.73m^2 based on serum cystatin-c and all of them had normoalbuminuria.
- 26 patients eGFR between 60-89 ml/min/1.73 m^2, out of which, 10 patients who had normoalbuminuria, 12 patients had micro albuminuria and 4 patients had macroalbuminuria.
- In the included patients, 7 (17%) patients had non proliferative diabetic retinopathy (NPDR), 1 (2%) patient had proliferative retinopathy (PDR) and 5 (12%) had hypertensive retinopathy.
- Most common other comorbidities were hypertension in 47.61% followed by peripheral vascular disease (PVD) in 1 patient (2.38%) and 1 (2.38%) had seizure.
- we found most of the patients who had normal serum creatinine based eGFR and normoalbuminuria had diabetic nephropathy (CKD stage 2) by using serum cystatin C.
- This study shows serum cystatin-c is highly sensitivity compared to serum creatinine to detect early diabetic nephropathy.
- Serum Cystatin C appears to hold promise in predicting early renal dysfunction and more so as an indicator of overt nephropathy.

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ANNEXURES

PROFORMA

TITLE: ESTIMATION AND COMPARISON OF eGFR USING SERUM CYSTATIN C AND SERUM CREATININE FOR DETECTION OF EARLY NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

Name : Age :

Sex: Hosp no:

DOA: DOD:

HOPI:

History suggestive of UTI:

Past history:

DM duration:

Thyroid dysfunction:

Other known chronic illness:

Hypertension /IHD/ Asthma

Treatment history:

Family history:

Personal history:

Smoking/ Ethanol:

EXAMINATION:
Vital parameters: Pulse: BP: JVP:

Other findings on general examination:

Examination of thyroid:

Systemic examination:
CNS:

Fundus exam:

CVS:

RS:

PA:

- Investigations:

  FBS PPBS

- Urinalysis:

  Alb: Sugar:

- Renal function tests:

  BUN

  S. Creatinine:

  S Creatinine based eGFR

  S. Cystatin C

  Cystatin C based eGFR

Diagnosis:

INFORMED CONSENT FORM

ESTIMATION AND COMPARISON OF eGFR USING SERUM CYSTATIN C AND SERUM CREATININE FOR DETECTION OF EARLY NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

STUDY NUMBER:

SUBJECT'S NAME: **HOSPITAL NUMBER:**

AGE:

Study on serum cystatin C in diabetic patients to detect early impairement of renal function in type 2 DM will bring to limelight the importance and potentiate the clinical application of serum cystatin C as an early marker for renal dysfunction and early treatment.

If you agree to participate in the study, we will collect information (as per proforma) from you or a person responsible for you or both. We will collect the treatment and relevant details from your hospital record. This information collected will be used for only dissertation and publication. This study has been reviewed by the institutional ethical committee. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study after accepting the below terms.

I understand that I remain free to withdraw from the study at any time and this will not change my future care. I have read or have been read to me and understood the purpose of the study. The procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information that will be collected and disclosed during the study have been explained to me. I have had the opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction. I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information for dissertation.

Subject name

(Parents / Guardians name)

DATE: **SIGNATURE /THUMB IMPRESSION**

MASTER CHART

Sl No	IP NO.	AGE (in years)	SEX (M/F)	DURATION OF DM	SERUM CREATININE (mg/dl)	S. CREATININE BASED e GFR (ml/min/1.73 m^2)	SERUM CYSTATIN C (mg/l)	SERUM CYSTATIN C BASED e GFR (ml/min/1.73 m2)	URINE ALBUMIN (mg/dl)	HbA1C (%)	FBS	PPBS
1	74928	85	M	20	1	85	0.84	89	385	8	185	240
2	27218	57	M	9	0.2	122	1.2	62	16	9	190	304
3	174268	75	M	16	0.8	104	1	73	24	9.5	221	340
4	604161	56	F	8	0.4	124	1.13	67	156	9	230	360
5	77450	49	M	2	0.7	130	0.9	93	18	6.9	154	234
6	282791	52	M	3	0.9	120	1	80	200	8.5	198	320
7	304427	48	F	3	0.3	125	0.8	102	16	7.8	165	280
8	81154	45	F	4	0.6	130	0.86	94	18	6.8	144	218
9	280198	51	M	5	0.7	126	0.9	93	8	7.9	211	288
10	49655	73	M	22	1	96	1.1	65	26	8.7	222	346
11	75220	53	M	5	0.4	134	0.8	108	16	6.6	123	201
12	287028	61	M	4	0.6	122	1.2	61	28	6.8	121	216
13	302955	54	M	6	0.9	125	1	80	22	7.3	139	220
14	287045	64	M	9	0.7	126	0.9	88	210	7.6	148	240
15	288246	70	M	7	0.4	128	1.12	64	226	8	170	288
16	280198	62	F	7	0.9	124	0.8	97	22	7	146	220
17	260765	65	F	14	0.5	130	1.1	63	28	6.9	147	208
18	243388	80	F	16	0.8	86	1	67	288	8.2	208	330
19	272332	70	M	8	0.4	126	1	75	126	8.7	218	310
20	284090	90	F	20	0.7	76	0.99	65	450	8.8	186	224
21	78399	43	M	3	1.1	134	0.75	116	10	8	166	270
22	300868	53	M	3	1.2	126	1.15	66	22	7.2	155	213
23	283545	75	F	26	0.9	86	1.1	60	146	8.4	178	260
24	166738	65	F	13	0.6	121	1	71	345	10	266	388
25	289043	78	M	6	1	86	0.92	81	76	11.2	237	400
26	283306	48	F	2	1.2	140	0.75	106	18	6.8	132	210
27	288983	48	M	4	1	144	1	82	12	6.5	128	245
28	292554	78	M	18	0.7	122	1.1	64	26	8.5	179	270
29	289349	49	M	8	0.9	146	0.85	101	16	6.6	120	200
30	289483	52	M	3	1	128	0.9	92	8	7.4	140	206
31	284037	43	F	1	1.2	146	0.8	104	16	6.7	142	264
32	393574	65	M	7	0.6	134	1.1	67	24	8.1	184	274
33	297505	48	M	2	0.6	126	0.9	94	18	6.7	132	226
34	287877	70	M	7	0.5	146	0.75	104	22	7.3	140	246
35	299971	66	F	8	0.6	98	1.12	61	280	9.6	186	302
36	344260	68	F	13	0.4	122	1	70	274	9	176	260
37	244378	48	M	3	0.6	138	0.9	94	14	6.9	130	200
38	162066	58	F	9	0.8	146	1.15	61	212	8.7	200	288
39	244378	66	F	8	0.7	121	1	71	165	8.6	202	310
40	185780	70	M	8	0.6	122	0.8	100	19	7.6	146	220
41	301068	75	M	7	0.4	121	1.11	64	324	8	136	230
42	252526	50	M	2	0.8	132	0.9	93	6.6	7.7	128	220