ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE DEPARTMENT OF MEDICAL BIOCHEMISTRY

EVALUATION OF SERUM CYSTATIN C AND SERUM CREATININE LEVELS FOR THE DETECTION OF EARLY RENAL IMPAIRMENT AMONG PATIENTS WITH TYPE-2 DIABETES MELLITUS, ATTENDING TIKUR ANBESSA SPECIALIZED HOSPITAL

BY

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Declaration sheet

This is to certify that master's thesis entitled "Evaluation of Serum Cystatin C and Serum Creatinine Levels for The Detection of Early Renal Impairment Among Patients with Type 2 Diabetic Mellitus "in Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia", hospital based comparative cross sectional study was prepared by Tadesse Asmamaw Dejenie and Submitted to the department of Biochemistry in requirements for partial fulfillment of degree of Master's science in Medical Biochemistry. Thesis is my own original work and it has not been presented in other universities, colleges or other institutions for similar purpose. I declare that the thesis meets the acceptance standards of the university with respect to originality and quality.

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ACRONYMS AND ABBREVATIONS

ADA	American Diabetic Association
BMI	Body Mass Index
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Cr.	Creatinine
Cyst C	Cystatin C
DCCT	Diabetes Control and Complications Trial
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DRERC	Department of Ethics and Research Committee
DTPA	Diethyl-enetriamine Penta Acetic Acid
EASD	End-Stage Renal Disease
EDIC	Epidemiology of Diabetes Interventions and Complications
EDTA	Ethylene Di Amine Tetra Acetic Acid
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immunosorbent Assay
EPHI	Ethiopian Public Health Institute
FPG	Fasting Plasma Glucose
HHS	Hyperosmolar Hyperglycemic State
IDF,	International Diabetes Federation
NCDs	Non-Communicable Diseases
OGTT	Oral Glucose Tolerance Test
SPSS	Statistical Package for Social Sciences
TASH	Tikur Anbessa Specialized Hospital
T2DM	Type2 Diabetes Mellitus
UAE	Urine Albumin Excretion
WHO	World Health Organization

Abstract

Introduction: -Kidney disease is a significant problem in the diabetic population. The proportion of patients with end-stage renal disease caused by type 2 diabetes has progressively increased during the last few decades, and diabetic nephropathy is now the most common cause of end-stage renal disease in the world. Measurement of Glomerular Filtration Rate is an important parameter for assessing kidney function and serum creatinine level is the most commonly used biochemical parameter to estimate it in routine practice. Serum creatinine for estimation of GFR for the detection of renal impairment is simple but 50% of GFR can be lost before significant elevation of serum creatinine. Cystatin C is found to be a new promising marker for early detection of renal diseases.

Objective of the Study: Aim of this study was to determine the value of serum cystatin C and serum creatinine levels as well as cystatin C and creatinine based estimation of GFR for early detection of diabetic nephropathy in patients with type2 diabetes mellitus.

Methodology: A hospital based comparative cross-sectional study was conducted with a sample size of 120. To recruit the study subjects, purposive sampling technique was implemented. The parameters measured were serum creatinine and cystatin C levels.

Result and discussion: The result of this study showed serum creatinine and cystatin C levels were significantly increased in type 2 diabetic individuals compared to healthy controls. The mean ± SD value of serum creatinine was found to be ((0.87 ± 0.44) in cases and (0.63 ± 0.27) in control groups. serum cystatin C also found to be significantly (p=0.0001) higher in cases (0.92 ± 0.38) compared to controls (0.52 ± 0.20).The means ±SD of eGFR in three equations (Creatinine Equation, Cystatin C Equation and Creatinine–Cystatin C Equation) were 105.7±27.5 ml/min/m^2, 90.4 ± 28.2 ml/min/m^2 and 100 ± 29.5 ml/min/m^2 respectively.

Conclusion: Serum cystatin C was found to be a better marker of renal function and correlates better to direct measures of GFR more precisely than creatinine, because unlike serum creatinine its serum concentration is not that much influenced by age, sex and muscle mass.

Keywords*:* *Diabetic nephropathy; Type 2 diabetes; Serum cystatin C and creatinine*

1. Introduction

1.1. Background

Diabetes mellitus is a metabolic disorder resulting from insufficient insulin secretion or inefficient insulin action and covers a wide range of heterogeneous diseases (Ashwin-Kumar *et al.*, 2015). It can be caused by a variety of hormonal and cellular defects, which results in elevated blood glucose levels. A normal fasting glucose level is less than 110 mg/ld. (6.1 mmol) based on World Health Organization (Ryden *et al.*, 2013). According to the American Diabetic Association, normal fasting blood glucose is less than 100 mg/ld. (5.7 mmol). This level of fasting glucose is maintained in the body by an intricate balance of hormones, which work to maintain glucose levels at a steady state. Normally, insulin is released in response to rising blood glucose levels. These powerful hormones activates cellular storage of glucose, amino acids, and triglycerides in target cells, including the liver, muscle, and fat, with the end result of normoglycemia. To keep glucose levels from falling too low, other hormones, such as glucagon, corticosteroids, growth hormone, and epinephrine, increase insulin resistance to maintain adequate circulating glucose (American Diabetes Association, 2018). In the presence of diabetes, there is a diminished or complete absence of insulin response or cellular resistance to insulin. These defects coupled with a deficiency of other glucose lowering hormones, result in higher fasting and post meal glucose levels.

Diabetes can be classified into three major types, each having a different cause and management strategy. These are Type 1 diabetes (due to autoimmune B-cell destruction, leading to absolute insulin deficiency), Type 2 diabetes (due to a progressive loss of B-cell insulin secretion frequently on the background of insulin resistance) and Gestational diabetes mellitus (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation) but the most common forms of diabetes are Type 1 diabetes and type 2 diabetes which are heterogeneous diseases in which clinical presentation and disease progression may vary considerably (American Diabetes Association, 2018). The traditional paradigms of type 2 diabetes occurring only in adults and type 1 diabetes only in children are no longer accurate, as both diseases occur in both age-groups. Children with type 1 diabetes typically present with the hallmark symptoms of

polyuria/polydipsia, and approximately one-third present with diabetic ketoacidosis (DKA) (Dabelea *et al.*, 2014).

To diagnose diabetes in general, either fasting plasma glucose, random glucose, or a post 75g glucose challenge glucose level can be used. Currently, there is international consensus that a fasting blood glucose level of ≥ 126 mg/dL (7 mmol), or a random or post meal glucose tolerance level of ≥ 200 mg/dL (11.1 mmol) in the presence of symptoms of hyperglycemia confers a diagnosis of diabetes (Table 1).

Table 1: Diagnostic Criteria of DM, Tikur Anbessa hospital, Addis Ababa, Ethiopia, 2019

Assay	Description	Criteria for Diabetes
Hb A_{1C}	Performed in laboratory by method NGSP-certified and standardized to DCCT assay	≥ 6.5 %
Fasting plasma glucose	At least 8 hour fast	≥ 126 mg/dL
Random plasma glucose	In persons with symptoms of hyperglycemia or hyperglycemic crisis: Blood glucose measured at any time of day	≥ 200 mg/dL
Two-hour plasma glucose	Following a glucose load of 75 g anhydrous glucose dissolved in water	≥ 200 mg/dL

∗ADA diabetes diagnosis guidelines, 2018*

Patients with diabetes are at substantial risk for tissue injury in organs supplied by an end arterial system due to micro angiopathy. These micro vascular complications include nephropathy, retinopathy and neuropathy (Saliha Aksun *et al.*, 2018). Kidney disease is a significant problem in the diabetic population. The proportion of patients with end-stage renal disease (ESRD) caused by Type2 diabetes has progressively increased during the last few decades, and diabetic nephropathy (DN) is now the most common cause of ESRD in the world (Saliha-Aksun *et al.*, 2018).

1.2. Statement of the problem

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Moran *et al.*, 2010). According to the international diabetics federation (IFD) statistics, The global prevalence of diabetes among adults was 8.8% in 2017 and it will be projected to 9.9% in 2045 (IDF, 2017).The major driver of the epidemic is type-2 diabetes accounts for about 90% to 95% of all diagnosed cases of diabetes in adults (Krishan *et al*, 2015). The number of people with this type of DM is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. It is now a global health problem. Especially, populations of developing countries, face the greatest risk (Shaw *et al.*, 2010).The incidence and prevalence of type-2 Diabetes mellitus is increasing globally, particularly, Sub-Saharan Africa which accounts for 90% of all diabetes cases (Naomi, 2008), which in turn is related to the increase in obesity. In Ethiopia, there is no published report on the incidence, prevalence, and life expectancy of T2DM patients, but believed to be high because of lack of early screening, and shortage of facility center.

Approximately 40% patients with type-2 diabetes eventually develop End Stage Renal Disease (ESRD) (Shima *et al.*, 2011).

Uncontrolled, chronically elevated glucose, often termed "glucose toxicity," can lead to a multiplicity of vascular complications that start long before the diagnosis of diabetes is made. This contributes to the high mortality and prevalence of complications like diabetic-nephropathy (Reimar *et al.*, 2011). Identifying and treating hyperglycemia in its earliest stages is critical to prevent complications. Unfortunately, as many as 50% of people with diabetes worldwide remain undiagnosed and untreated (Thomassian and Beverly, 2009).

Diabetic nephropathy is the leading cause of chronic renal disease in patients with diabetes mellitus (Kafrawy *et al.*, 2014). About 20-40% of type 2 diabetic patients with micro-albuminuria develop overt nephropathy, and most patients show progression to end-stage renal disease. GFR is considered the most accurate measurement of kidney disease and is reduced before the onset of clinical symptoms; it is measured or predicted using different methods (Michael *et al.*, 2006). To estimate the GFR, an endogenous substance in the blood that is cleared by the kidney is used; Serum creatinine is the most widely used substance to estimate GFR and detection of renal disease. But Creatinine concentration is influenced by sex, age, diet and muscle mass and it is only increased once GFR reduction of about 50% is

present. This leads to falsely high or low values, limiting its usefulness as an ideal marker for early diagnosis of diabetic nephropathy (Richard *et al.*, 2011). Hence, other small molecule substances, such as cystatin C, which is not influenced by age, sex, or muscle mass and dietary intake, is being explored to early detection of renal disease (Andreoli *et al.*, 2010).This new marker might reflect the early diminished GFR for early detection of renal impairment compared with traditional markers; and it is a new promising marker for early detection of renal diabetes nephropathy. So far, there is no research done on cystatin C in patients with diabetes mellitus for early detection of diabetic nephropathy in Ethiopia. Hence this study is expected to clarify the use of cystatin C as plausible markers for early detection of diabetic nephropathy.

1.3. Literature Review

1.3.1. Epidemiology of Diabetes Mellitus

The explosive increase of diabetes mellitus, a chronic metabolic and endocrine disease worldwide is a major public health concern both in developing and developed
Countries (Borah *et al.*, 2016).WHO estimates that globally, diabetes is the third highest risk factor for premature mortality which accounts for five million (14.5%) of global all-cause mortality among people aged 20–79 years, which is higher than the combined number of deaths from the three major infectious diseases (1.5 million deaths from HIV/AIDS, 1.5 million from tuberculosis, and 0.6 million from malaria in 2013), (IDF, 2015).

According to the IDF, 415 million people worldwide, or 8.8% of adults aged 20–79 years, were estimated to have diabetes in 2015. Of these, about 75% lived in low- and middle-income countries. If these trends continue, by 2040, 642 million people (or one in ten adults) will have diabetes. The largest increases will take place in the regions where economies are moving from low-income to middle-income levels (IDF, 2015).

In 2015, the IDF also estimated that, in the Africa region, 14.2 million adults aged 20–79 years had diabetes, representing a prevalence of 3.2%. The majority (59%) of people with diabetes live in cities, even though the population is predominantly (61%) rural. This region has also the highest proportion of previously undiagnosed diabetes; over two-thirds (67%) of people with diabetes being unaware they have the disease (IDF, 2015).
WHO showed that type 2 diabetes is a global problem and that populations of developing countries, minority groups and disadvantaged communities in industrialized countries face the greatest risk (Ayesha *et al.*, 2003).

Sub-Saharan Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases. In 2010, 12.1 million people were estimated to be living with diabetes in Africa, and this is projected to increase to 23.9 million by 2030. In Sub-Saharan Africa this trend is emerging in a region grappling with high rates of communicable diseases including the highest global prevalence of HIV, Tuberculosis and Malaria (Hall *et al.*, 2011).

Currently, Ethiopia has been challenged by the growing magnitude of non-communicable diseases such as diabetes. Ethiopia is among the top four countries with the highest adult diabetic populations in sub-Saharan Africa.

1.3.2. Complication of Diabetes Mellitus

Diabetes mellitus leads to acute and chronic complications. The acute complications include diabetic ketoacidosis (DKA), hyperosmolar hyperglycemic state (HHS), and hypoglycemia during treatment whereas the chronic complications include retinopathy, nephropathy, and neuropathy (Worku *et al.*, 2010). Patients with all forms of diabetes of sufficient duration are vulnerable to these complications, which cause serious morbidity and mortality. Our study was focused on one of these common complications which is diabetic nephropathy caused by type-2 DM.

1.3.2.1. Diabetic-nephropathy

Diabetic nephropathy is the most important long-term complication of diabetes mellitus. It is the most common cause of end-stage renal disease requiring dialysis and characterized by albuminuria, subsequent proteinuria, declining glomerular filtration rate and elevated blood pressure (Piwowar *et al.*, 1999). It is one of the major chronic micro-vascular complications in diabetes mellitus and a leading cause of end-stage renal disease (ESRD), accounting for nearly half of all incident cases of ESRD in most of the countries worldwide (Zhou *et al.*, 2016), and it is predominantly due to type 2 diabetes mellitus. An increasing number of type 2 diabetic patients live long enough for nephropathy and ESRD to develop, since the treatment of diabetes, hypertension and coronary heart disease has improved (Mussap *et al.*, 2002). Many studies have reported that diabetic nephropathy develops in 25–35% of patients with T2DM (Rao *et al.*, 2014).

Diabetic-nephropathy (DN) increases, the overall 10-year mortality among type-2 diabetic patients at least 6 folds compared to healthy age matched non-diabetic individuals (Usama *et al.*, 2017). The earliest stage of DN is characterized by renal hyper-function and hypertrophy. Few years later, persistent increase in urine albumin excretion (UAE) develops. This stage is called the stage of incipient nephropathy characterized by UAE > 30 mg/day, >20 mg/min, or urine albumin to creatinine ratio (ACR) > 30 mg/g of creatinine. The persistent increase in UAE is initially associated with increased glomerular filtration

rate (GFR). However, GFR shows consistent decline that becomes pronounced with the continuous increase of UAE above 300 mg/day, 200 lg/min, or when urine ACR exceeds 300 mg/g (Dounousi *et al.*, 2015)

In 2014, the American Diabetes Association (ADA) and the National Kidney Foundation reached an agreement in which DN was referred to as the chronic kidney disease caused by DM, with a persistent estimated glomerular filtration rate (eGFR) of < 60 ml per min per 1.73 m^2 or a urinary albumin/creatinine ratio (ACR) of > 30 mg/g for more than 3 months (Tuttle *et al.*, 2014). The Joint Committee on Diabetic Nephropathy recommended the combination of the 24-houralbuminuria excretion rate (AER) and glomerular filtration rate (GFR) as the new classification of DN (Haneda *et al.*, 2015). Microscopically, DN is characterized by diffuse or nodular glomerulosclerosis, afferent and efferent hyaline arteriolosclerosis, and tubule interstitial fibrosis and atrophy (Alsaad *et al.*, 2007).

The progression of DN is accompanied by a decrease in GFR among patients with T2DM.GFR and albuminuria have long been considered twin manifestations of DN(De Boer and Steffes, 2007). However, recent researches have shown that normoalbuminuric patients with T2DM may have significant renal damage(Macisaac *et al.*, 2004). The Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that 24% of the examined DM patients with baseline eGFR level > 60 ml per min per 1.73 m^2 progressed to DN within one year; however, they did not experience microalbuminuria or macroalbuminuria (Molitch *et al.*, 2010). Therefore, GFR is superior to AER or ACR in the early diagnosis of DN. Recently, serum cystatin C has been considered as biomarker for the diagnosis of kidney damage. Several studies have shown that serum cystatin C is found to be a better marker of decreased GFR than serum creatinine (Zhou *et al.*, 2016).

Glomerular filtration rate (GFR) is the volume of plasma that can be completely cleared of a particular substance by the kidneys in a unit of time (Laterza *et al.*, 2002). The "gold standard" for determining GFR is to measure the clearance of exogenous substances such as inulin, iohexol, 51Cr-EDTA, 99mTc-labeled diethylenetriamine penta acetic acid (DTPA), or 125I-labeled iothalamate (Laterza *et al.*, 2002). However, these techniques, are time-

consuming, labor-intensive, expensive, and require administration of substances that make them incompatible with routine monitoring. Thus, the measurement of endogenous blood substances to estimate GFR is a common practice. Properties of an ideal endogenous blood substance to estimate GFR should include release into the blood stream at a constant rate, freely filtered by the glomerulus, no reabsorption or secretion by the renal tubules, and exclusive elimination via the kidneys (Stevens *et al.*, 2006).

GFR is accepted as the best overall measure of kidney function (Shlipak *et al.*, 2013). Normal values, which are related to age, sex, and body size, are approximately 130 ml per minute per 1.73 m2 in young men and 120 ml per minute per 1.73 m^2 in young women. Mean values decline as persons age (Shlipak *et al.*, 2013).

An ideal endogenous marker of GFR should have a production rate that is constant, should be cleared from the circulation only by glomerular filtration, should be freely filtered at the glomerulus and should neither be secreted nor reabsorbed intact by the proximal or distal renal tubule (Newman and David, 2002).

Creatinine which is a chemical waste molecule that is generated from muscle metabolism, meets only one of these criteria: it is freely filtered at the glomerulus but not very sensitive since its levels significantly increase when more than 50% of the GFR is reduced (Mussap *et al.*, 2002). its concentration may be also significantly influenced by several extra-renal factors (age, sex, muscle mass, changes in tubular secretion, dietary intake etc.) especially in elderly female patients with reduced muscle mass, measurement of serum creatinine may grossly underestimate the reduction in the GFR (Shlipak *et al.*, 2013).

Creatine can be obtained from the diet or can be synthesized in the liver and kidney from arginine, glycine, and methionine then transported to most creatine utilizing cells like muscle and brain cells through the blood stream by a specific protein transporter (Brosnan *et al.*, 2011)

Creatinine formation is a two-step process, transamidination of arginine and glycine (transfer of amidine group from arginine to Glycine) to form guanidoacetate (Glycocyamine) at kidney followed by methylation of Glycocyamine at liver to form active creatine (Brosnan *et al.*, 2011).

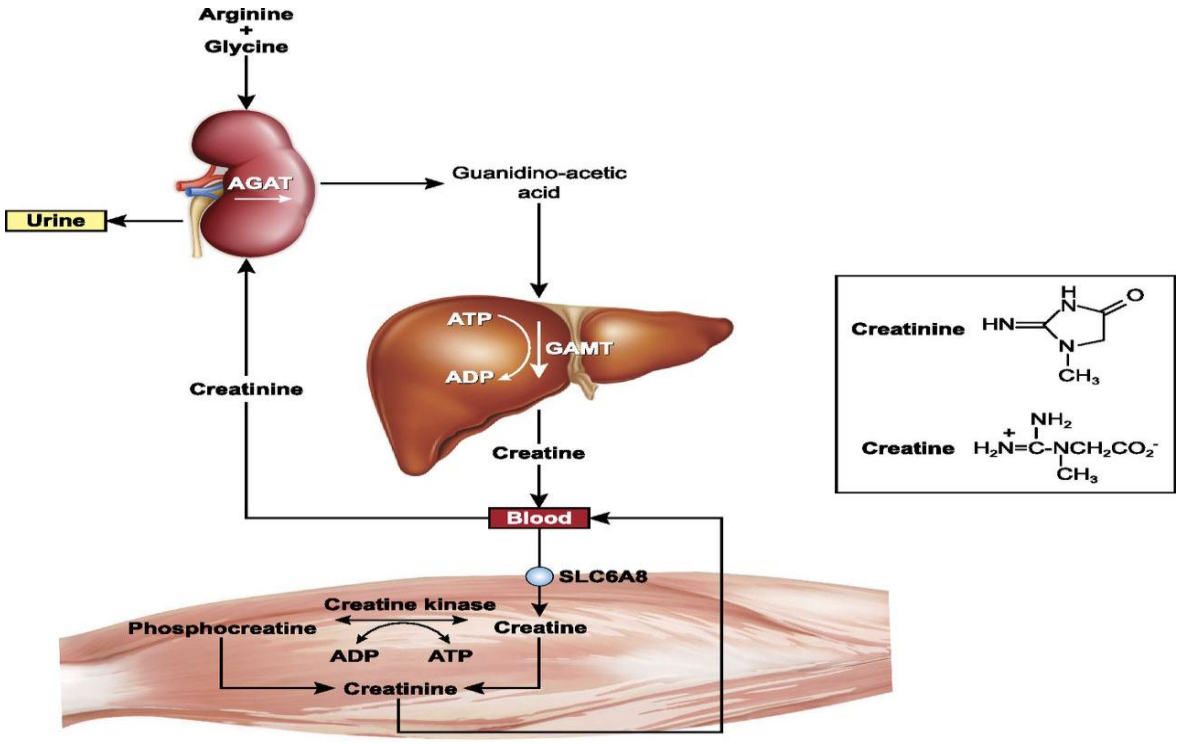

Figure 1: Schematic representation of creatinine synthesis pathway, Tikur Anbessa specialized teaching hospital, Addis Ababa, Ethiopia, 2019; (adopted from Xiaoyan* et al., *2016)

Human cystatin C which is a member of the cystatin superfamily (inhibitors of cysteine proteinases), and a non-glycosylated basic protein with a low molecular mass (13 kD) has been intensively studied in the last few years and suggested as a serum marker of changes in GFR and used as early marker of renal impairment (Mussap *et al.*, 2002). It is freely filtered by the glomerulus, and produced by all nucleated cells is currently under investigation as promising and a replacement for serum creatinine in estimating of the GFR and early detection of renal failure (Dharnidharka *et al.*, 2002 ; Marco *et al.*, 2007). The low molecular mass of cystatin C, in combination with its stable production rate, indicates that the plasma concentration of cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), this makes cystatin C an excellent promising marker of GFR (Sjostrom *et al.*, 2005). In adults, serum Cyst-C concentration is closely correlated with GFR (Donadio *et al.*, 2001).

The study which was conducted by Kunal Gupta *et al*, showed that the Cystatin C values were raised even in the patients in whom clinical albuminuria had not yet started, and hence

it acts an earlier marker than microalbuminuria to determine nephropathy(Kunal Gupta *et al.*, 2016). Results of this study showed that serum Cystatin C may be considered as an early marker, but microalbuminuria and serum creatinine are commonly used marker for nephropathy, for declining renal function, in diabetic subjects.

Takir *et al.* studied 78 T2DM patients and found that the values of Cystatin C were significantly increased in the normoalbuminuric group and this showed that serum cystatin C may be an early marker of diabetic nephropathy. The serum cystatin C level of microalbuminuric patients was found to negatively correlate with eGFR (Takir *et al.*, 2016). Other study which was conducted by Bruce *et al* also found that the serial measures of serum cystatin C accurately detect trends in renal function in patients with normal or elevated GFR and provide means for studying early renal function decline in diabetes(Pucci *et al.*, 2007). The degree of correlation between a reference GFR procedure and reciprocal cystatin C concentration was found to be superior to the reciprocal creatinine concentration in several studies. A more definitive assessment requires an evaluation of the diagnostic sensitivity and specificity of cystatin C for the detection of a decreased GFR(Newman and David, 2002). This has been performed using a variety of cystatin C, creatinine and GFR methods. In the majority of studies which formally compared the areas underreceiver operator characteristic curves, cystatin C performed significantly better than creatinine (Newman and David, 2002).

1.3.2.2. Structure of cystatin C

It has a crystal structure characterized by short alpha helix and a long alpha helix running across a large antiparallel 5 stranded beta sheet and has 2 disulfide bonds (fig1).

Cystatin C contains 120 amino acids but is synthesized as a pre-protein (indicating an extracellular function) (Newman and David, 2002). It is the product of a 7.3-K base gene and located in short arm of chromosome 20 which contains majority of type 2 cystatin genes and pseudo genes (Newman and David, 2002). It is dimer macromolecular complex formed by two, usually covalently bound, molecule.

The human cystatin family presently comprises 11 identified proteins. Two of these, Cystatin A and B, form the family 1 Cystatin and are mainly, or exclusively, intracellular proteins, while Cystatin C, D, E, F, S, SA and SN are mainly extracellular and/or

transcellular proteins and constitute the family 2 Cystatins. The family 3 cystatins are mainly intravascular proteins, produces vasoactive peptide & involved in coagulation (Kunal Gupta *et al.*, 2016).

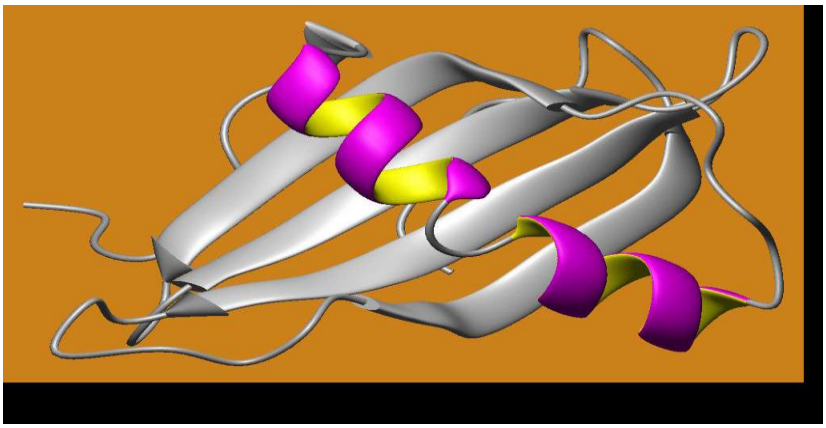

Figure 2: structure of cystatin C, Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, 2019, Adopted from: (Brijesh et al 2018),

1.4. Significance of study

End stage renal disease (ESRD) became one of the major killer diseases in the world particularly Sub-Saharan counties including Ethiopia, because of rapid rise of its risk factors like diabetes mellitus particularly T2DM and this affects most productive adults and results in death. Even for diabetic patient is under treatment, there is a risk of development of diabetic nephropathy because the risk is related to the length of time the person has diabetes. Therefore, it is vital to work towards prevention of diabetic renal disease or at least slowing down of the disease is important.

In Ethiopia early screening, detection, and identification of kidney disease in diabetes patients is very rare as a result its complication become common. As increase in the number of people with diabetes will also have a major impact on dialysis and transplant need and this is very difficult due to various reasons such as lack of facility and its affordability. Therefore, early screening of diabetes nephropathy is very important to minimize such problems.

This study was aimed to evaluate the GFR via serum cystatin C and serum creatinine and compare them for the early detection of diabetic nephropathy among T2DM patients because the early detection of DN is very important and can improve patient outcomes.

1.5. Hypothesis

Serum Cystatin C marker is superior to serum creatinine marker for early detection of diabetic nephropathy

2. Objective of the Study

2.1. General objective

To determine the value of serum cystatin C and serum creatinine levels as well as cystatin C and creatinine based estimation of GFR for early detection of diabetic nephropathy among T2DM patients in Tikur Anbesa specialized teaching hospital, Addis Ababa, Ethiopia.

2.2. Specific objectives

- To assess serum cystatin C in diabetes patients and compare with apparently healthy control groups
- To determine serum creatinine in diabetes patients and compare with apparently healthy control groups
- To compare serum cystatin C based estimation of GRF with serum creatinine based estimation of GFR for the assessment of early renal failure

3. Materials and Methods

3.1. Study Area

This study was conducted at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. TASH is a large referral teaching hospital, under the administration of Addis Ababa University, located in Lideta Sub City of Addis Ababa. This referral hospital has about 700 beds and is the main teaching hospital for both clinical and preclinical training of most disciplines.

3.2. Study Design and period

Institutional based Comparative Cross-Sectional Study design was conducted from April, 2019 to Jun, 2019.

3.3. Source Population

Includes all previously diagnosed T2DM adult patients who have treatment follow up at the diabetic center of Tikur Anbesa Specialized Hospital

3.4. Study Population

Patients: Adult males and females who have T2DM for different period of time from the diabetic center of TASH and those who have attended during the study period and fulfill the inclusion criteria.

Controls: Adult subjects who were willing to provide signed voluntary informed consent to participate and give blood sample for the purpose of the study and no have known medically recorded diseases like urinary tract infections, malignancies, liver disease, thyroid gland dysfunction, congestive heart failure, hypertensive, HIV and those pregnant women were taken as control group for this study.

3.5. Eligibility criteria for T2DM cases

3.5.1. Inclusion Criteria

Both sexes of type-2 diabetic patients diagnosed and confirmed to be type 2 diabetics and had treatment follow up and patients above 18 years were included.

3.5.2. Exclusion Criteria

Patients who had urinary tract infections, malignancies, liver disease, thyroid gland dysfunction, congestive heart failure, hypertensive, HIV, women who were pregnant and patients with any other diseases that can cause nephropathy were excluded from this study

3.6. Sampling Technique

To recruit study subjects, purposive none probability sampling technique was used and T2DM patients who came to diabetic center during the study period and those who meet the inclusion criteria were selected for this study.

3.7. Sample Size Determination

The sample size for the study was assumed by using single proportion population formula.

$$n = \frac{\left(Z_{1-\alpha/2}\right)^2 P(1-P)}{d^2}$$

Where,

n=estimated sample size, z=standard normal distribution corresponding to significance level at $\alpha = 0.05$, thus z=1.96 and d= allowable margin of error (5%)

P = expected prevalence or proportion of T2DM

Due to lack of population-based study on T2DM in Ethiopia, an estimate prevalence of 50% was considered.

$$\mathbf{n= \frac{(1.96)^2 \text{ x } (0.50\ (1-0.50))}{(0.05)^2} = 384}$$

However, many international reports regarding serum cystatin C and serum creatinine measurement on T2DM were based on relatively small sample size which were various from 30 to 150 due to practical constraints but power analysis and sample size calculation were not reported in any of these studies. Therefore, by considering a number of factors such as cooperation and attrition, practical constrains like time, subject availability and finance, the sample size of 60 T2DM patients and 60 healthy controls (a total of 120) were included for this study.

3.8. Data collection methods and Procedure

A structured questionnaire was used to collect the data like, sex, age, residency, occupation, educational status, weight, height, smoking and alcohol consumption history and other medical information like time of diagnosis (duration of diabetes) and types of medication they use (for patients only). The English version questionnaire was translated to Amharic version and retranslated back to English to check its consistency. The Amharic version of the questionnaire was used to collect the data.

3.9. Study variables

3.9.1. Dependent variables

- Serum creatinine
- Serum cystatin C

3.9.2. Independent variables

- Socio demographic factors (age, sex, residence, occupation, educational status)
- Behavioral factors (smoking, alcohol use)
- Anthropometric parameters (height, weight and BMI)
- Clinical factors (duration of DM, medication use)

3.10. Operational Definition

Fasting blood glucose (FBG): - Fasting is defined as no caloric intake for at least 8 hrs.

Diabetic nephropathy: - Referred as the chronic kidney disease caused by DM, with a persistent estimated glomerular filtration rate (eGFR) of < 60 ml per min per 1.73 m2 or a urinary albumin/creatinine ratio (ACR) of > 30 mg/g for more than 3 months (Tuttle Kr *et al.*, 2014).

End stage renal failure: - is defined as eGFR< 15 mL/min/1.73 m^2 (Levin *et al.,* 2013).

3.11. Data Quality Control

In order to assure the quality of data, validity of the questionnaire was maintained by pretested questionnaire. Orientation was given to data collectors on the objective of the study, data collection process and relevance of the study prior to data collection. The completed questionnaire was cross checked daily for inconsistencies. Throughout the course of the data collection data collectors were supervised. The data were checked for completeness on site and before data entry the incomplete data were discarded.

3.12. Blood sample collection and processing

After maintenance of all aseptic precautions, 5mL of venous blood sample was drawn before breakfast (minimum of eight hours of fasting) from each participant by trained BSc nurses. Then it was transferred to a clean, dry serum separator tube. Then the sample was centrifuged and the serum was transferred to nunc tube and preserved at -20 ^{0}C until transfer to National References Laboratory for Clinical Chemistry, Ethiopian Public Health Institute (EPHI) through ice bag. Then serum cystatin C and serum creatinine were analyzed using automated chemistry analyzer in EPHI. Creatinine derived Equation (CKD-EPI 2009), Cystatin C derived Equation (CKD-EPI 2012) and both Creatinine–Cystatin C derived Equation (CKD-EPI mix) for eGFR were calculated and compared for early detection of diabetic-nephropathy.

3.13. Biochemical assays and Laboratory analysis

A. Determination of serum cystatin C

Cystatin C which a cysteine protease inhibitor with a molecular weight of 1300 Daltons has been identified as a new, promising marker for early detection of kidney failure (Adeera, 2005).

Principle of the test

Serum cystatin C was assessed by COBAS 6000 analyzer. This assay was based on the quantitative sandwich enzyme immunoassay technique. The surface of the wells of the micro titer plate was coated with polyclonal anti-human cystatin C-specific antibodies. Then diluted standards, diluted quality controls, and diluted samples were pipetted into the wells. Any human cystatin C present was captured by the immobilized antibodies, and unbound protein was washed away after the first incubation period. Thereafter, horseradish peroxidase-conjugated polyclonal anti-human cystatin C antibodies was added to the wells and incubated. A substrate solution (H202) was added to the wells. The enzymatic reaction yielded a blue product that turned yellow when the acidic stop solution was added.

B. Determination of serum creatinine

Creatinine which is a chemical waste molecule that is generated from muscle metabolism is widely available, rapidly measured and relatively inexpensive indicator of kidney function related to change in GFR. Despite this wide use, it is regarded as an insensitive marker for early changes in kidney function and affected by factors such as muscle mass, age, gender,

and race (John, 2018). It was assessed by COBAS 6000 analyzer through the principle of enzymatic colorimetric method.

Test principle

Serum creatinine was also assessed by COBAS 6000 analyzer. Measurement of creatinine by Jaffe's reaction was done in a wavelength of 500 nm. This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen.

3.14. Data processing and analysis

All data were checked, cleared, coded and entered into Statistical Package for Social Sciences (SPSS) Version 23 for statistical analysis. Descriptive statistics like mean, standard deviation, and percentage were carried out for socio-demographic characteristics. Continuous variables were expressed as the mean ± standard deviation (SD) while categorical variables were presented as frequencies and percentages. Kappa statistics was used for agreement test. In addition, evaluation of differences in means of study groups was evaluated using student's t-test. Pear's correlation and multiple linear regression models were used to assess the relation between outcome variable and different independent variables in the study. Furthermore, P-value <0.05 was used as a cut-off value to include variables for multivariate linear regression and at 95% confidence interval (CI), a P value <0.05 was accepted as statistically significant.

3.15. Ethical Consideration

To conduct this research, ethical approval was obtained from Addis Ababa University Biochemistry Department Ethics and Research Committee (DRERC) and A formal collaboration letter from Department of Biochemistry was provided to Tikur Anbesa specialized hospital, diabetic clinic. Informed consent was obtained from the participants before running the questionnaire and sample collection. All the principles of ethics such as confidentiality, and privacy were kept. The obtained data were used only for the purpose of this research and all study participants read and signed on the informed consent.

4. Result

4.1. The socio-demographic characteristics of the study participants

A total of 120 study participants (60 T2DM cases and 60 health controls) were recruited in this study. The mean ages of T2DM patients and healthy controls were 56.5 and 37.2 years with a minimum age of 32 and 22 years and a maximum age of 90 and 62 years respectively. The detail basic characteristics of the control group and cases with type 2 diabetes mellitus are depicted in Table 2. The number of female participants in the study were 32(53.3 %) in the cases and 35(58.3 %) in healthy controls. Majority of T2DM patients 50(83.3%) and healthy controls 53(88.3%) were living in urban area. 21(35%) T2DM respondents were house wives and most of health controls 22(36.7%) were private workers. Out of 60 cases 23 (38.3%) had an educational status of secondary school and out of 60 health controls 37(61.7%) completed college. Any of the T2DM patients and healthy controls never smoked. However, 17(27.3%) of T2DM patients and 5(8.3%) healthy controls drink alcohol.

Table 2: The general profile of study participants; Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, 2019

Variables		Type-2 DM cases (N=60)	Healthy control (N=60)
Age	(Mean ± SD) in years.	56.5±14.1	37.2 ± 10.9
Sex	Male	28(46.7%)	25(41.7%)
	Female	32(53.3%)	35(58.3%)
Residence	Urban	50 (83%)	53(88%)
	Rural	10(17%)	7(12%)
Educational level	Illiterate	8(13%)	1(1.7%)
	Read and write	1(1.7%)	6(10%)
	Primary school	7(2%)	5(8.3%)
	Secondary school	23 (12%)	11(18.3%)

Occupation	College and above	21(38%)	37(61.7%)
	House wife	21(35%)	11(18.3%)
	Farmer	5(8.3%)	3(5%)
	NGO	3(5%)	6(10%)
	Governmental	14(23.3%)	18(30%)
	Private	17(28.3%)	22(36.7%)
Alcohol consumption	Yes	17(28.3%)	5 (8.3%)
	No	43(71.7%)	55 (91.7%)
Smoking	Yes	0(0%)	0(0%)
	No	60(100%)	60 (100%)
Regular Physical exercise	Yes	38(63.3%)	37(62%)
	No	22(36.7%)	23(38%)

SD= Standard Deviation, %= percentage; Categorical variables are presented in frequency and percentage while continuous variables are presented as mean ± (SD)

4.2. Clinical and biochemical characteristics of the study participants

4.2.1. Duration of T2DM

The mean ± SD of the duration of patients with T2DM was 12.3 ± (8.6) years with the maximum and minimum duration of 41 years and 6 months respectively.

4.2.2 Medications used

Out of 60 T2DM patients 32(53.3%) used oral medication, 18 (30%) used insulin injection and 10 (16.7%) used both oral medication and insulin injection (Fig 3).

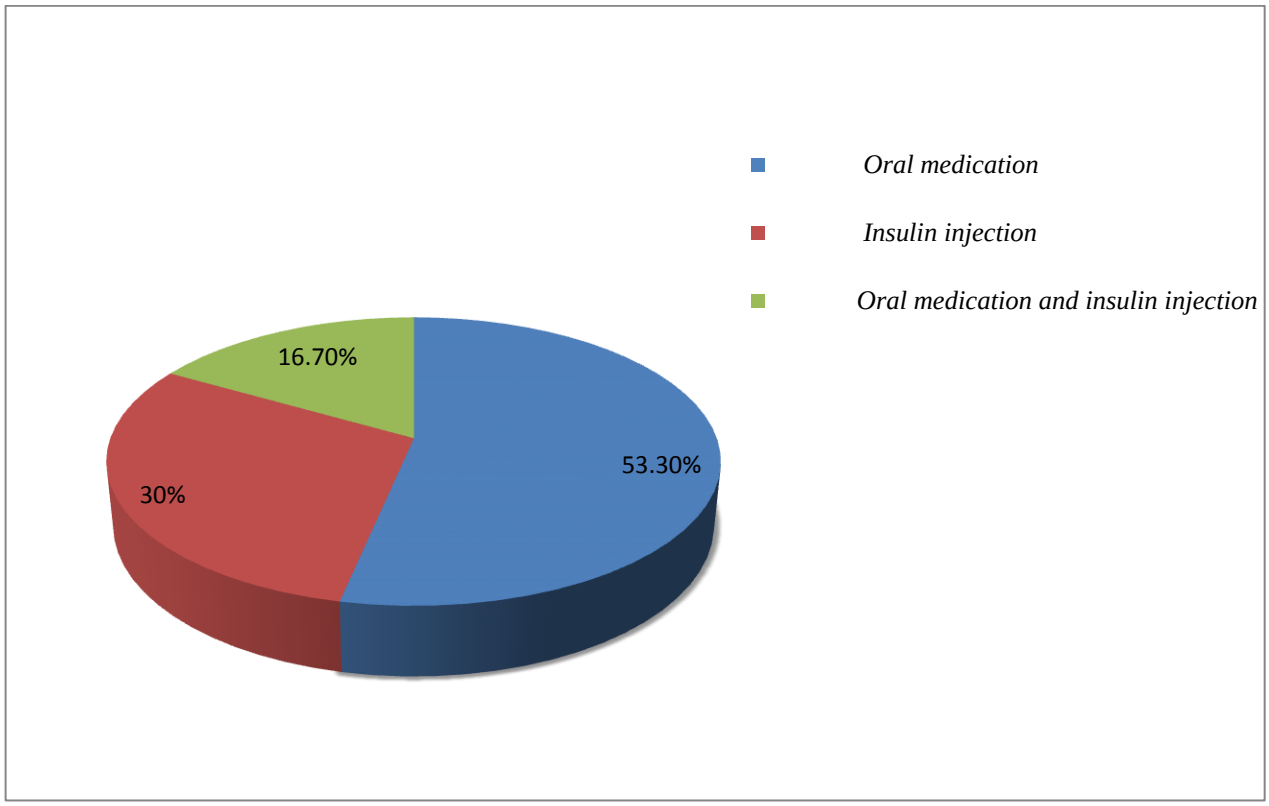

Figure 3: Percentage of Medication used by T2DM patients at Tikur Anbesa hospital, Addis Ababa, Ethiopia, 2019

4.2.2. Body Mass Index

BMI of the study participants was calculated using their weight and height. Based on the calculation, the mean ± SD was found to be 26 ± 4.4 kg/m^2 for cases and 19.7 ± 2.8 kg/m^2 for control group respectively. The mean value of BMI in T2DM patients was found under the category of overweight but the mean value of control groups was found under normal category. The minimum and the maximum values of BMI in cases were 19.1 kg/m2 and 35.2 kg/m^2 respectively. The minimum and the maximum values of BMI in control groups were 15.1 kg/m^2 and 27.8 kg/m^2 respectively. Putting the results categorically, 27 (45%) of the T2DM patients had normal body mass index, 25(41.7%) were overweight and 8 (13.3%) were obese. Among patients none were found underweight but among controls 18(30%) were underweight (Tabe3).

Table 3: Percentage of study participants in each Category of BMI; Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, 2019

BMI category	T2DM cases	Health control groups
Below 18.5	1(1.7%)	18(30%)
18.5-24.9	27(45%)	40(66.7%)
25-29.9	24(40%)	2(3.3%)
30 and above	8(13.3%)	0(0%)

4.3. Comparison of serum Creatinine level between T2DM patients and apparently healthy controls.

The serum creatinine concentration in blood sample was considered as normal when it was found 0.5-0.9 mg/dl for female and 0.7-1.2 mg/dl for male respectively. The serum creatinine concentration of T2DM patients and healthy controls were examined (Table 4). Out of 32 female T2DM patients 28 (87.5 %%) were found in normal range and 4(12.5%) were found above normal range (above 0.9) but none of the female T2DM patients showed a value below the normal range. Out of 28 male T2DM patients 22(78.6%) showed creatinine level in a normal range and 6(21.4%) had values above the normal range (above 1.2mg/dl).

In healthy controls, out of 35 female health controls 22(62.9%) had creatinine level in normal range and 13(37.1%) had values below the normal range. Out of 25 male control groups 17(68%) had serum creatinine level in normal range and 8(32%) had values below the normal range but none of the controls had values above the normal range.

The mean serum creatinine level was higher among cases when compared with controls and it was statistically significant (Table 4).

4.4. Comparison of serum Cytatin C level between T2DM patients and health controls

The serum cystatin C concentration in blood sample was considered as normal when it ranges from 0.61-0.95mg/L for both sexes.

The serum cystatin C concentration of T2DM patients and healthy controls was compared (Table 4). Out of 60 T2DM patients 40(66.7 %%) had normal serum cystatin C level. 15(25%) had values above the normal range and 5(8.3%) of the T2DM patients had values

below the normal range. From the apparently health controls 39(65%) had serum cystatin C level in normal range and 21(35%) %) had values below the normal range but none of the control groups had abnormally higher serum cystatin C level.

The mean serum cystatin C level was higher among cases when compared with controls and it was statistically significant (Table 4).

Age and BMI of the participants were also significantly different between cases and control groups with (p =0.004, p=0.001 respectively).

Table 4: Comparison of age, BMI, serum Creatinine and Sr. Cytatin C levels using Independent sample t-test between T2DM patients and health controls: Tikur Anbessa specialized teaching hospital, 2019

Variables	T2DM patients (N=60, mean ±SD)	Control group (N=60, mean ±SD)	p-value
Age in years	56.5±14.1	37.2 ± 10.9	0.004
BMI in Kg/m^2	26 ± 4.4	19.7 ± 2.8	0.001
Creatinine level (mg/dl)	0.87 ± 0.44	0.63 ± 0.27	0.001
Cystatin C level (mg/L)	0.92 ± 0.38	0.52 ± 0.20	0.0001

*Values are expressed as Mean ± SD. *P-values is significant at <0.05.*

Table 5: Comparison of serum Creatinine and Sr. Cytatin C levels between males and females using Independent sample t-test in T2DM patients: Tikur Anbessa specialized teaching hospital, 2019

Variables	Males (n=28, mean ±SD)	Females (n=32, mean ±SD)	P-value
Serum creatinine level (mg/dl)	1.07 ± 0.56	0.68 ± 0.16	**<0.0001**
Serum cystatin C level (mg/L)	1.03 ± 0.48	0.89 ± 0.23	0.29

* *P-values is significant at <0.05.*

As shown in table (5) the mean value of serum creatinine was significantly higher in males than in females. This is may be due to muscle mass variation between males and females. But the mean value of serum cystatin C in males was not significantly higher than females.

Table 6: Pearson's correlation analysis between serum creatinine and different studied variables, Tikur Anbessa teaching hospital, 2019

	Serum creatinine	
variables	r	p-value
Age	0.422	**0.001**
Duration of DM	0.122	0.353
BMI	0.594	**0.0001**

*DM, diabetes mellitus, BMI, body mass index, * *P-values is significant at <0.05.*

Correlation analysis showed that the level of serum creatinine positively and significantly correlated with the age and BMI of T2DM patients but no significantly correlated with duration of DM. The serum creatinine correlated with BMI than age. This is graphically depicted using scatter plot (Fig 3).

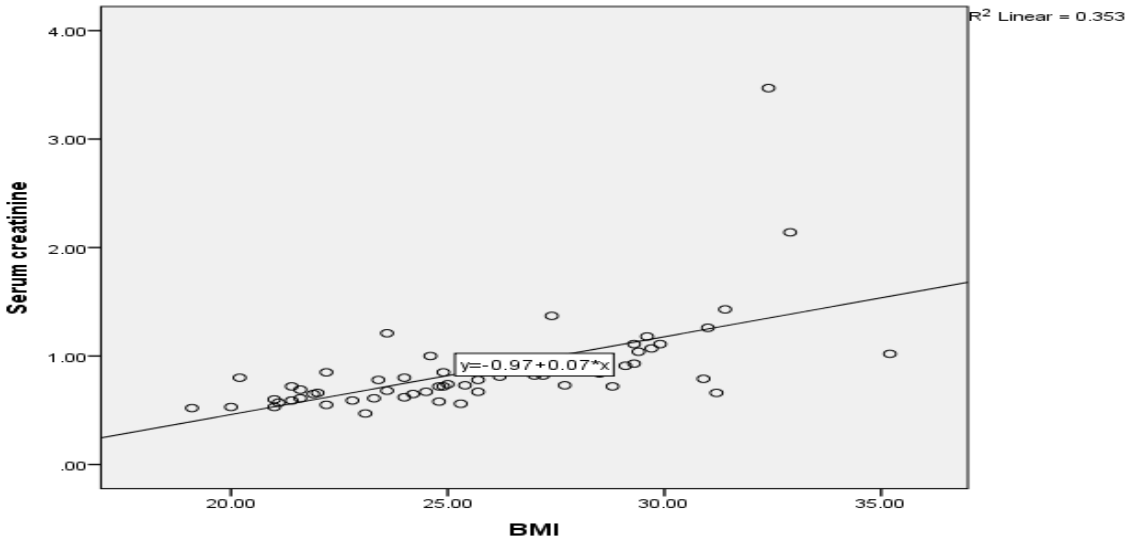

Figure 4: Scatter plot correlation between serum creatinine level and BMI of T2DM patients

Table 7: Pearson's correlation analysis between serum cystatin C and different variables of the T2DM patients, Tikur Anbessa teaching hospital, 2019

variables	Serum cystatin C	
	r	p
Age	0.302	**0.020**
Duration of DM	0.175	0.182
BMI	0.369	**0.021**

* *P-values is significant at <0.05.*

The level of serum cystatin C positively and significantly correlated with the age and BMI of T2DM patients (r = 0.302, p =0.02), (r = 0.369, P = 0.021) respectively. But unlike serum creatinine, it correlated weakly with BMI and age of the patients.

Table 8: Correlation analysis between serum cystatin C and creatinine levels, Tikur Anbessa specialized teaching hospital, 2019

Variable	Serum Cystatin c	
	r	p-value
Serum creatinine	0.90	0.0001

There was a strong and positive correlation between serum cystatin C and serum creatinine among T2DM patients (r = 0.90, P= <0.0001). This is graphically depicted using a scatter plot (Figure 5).

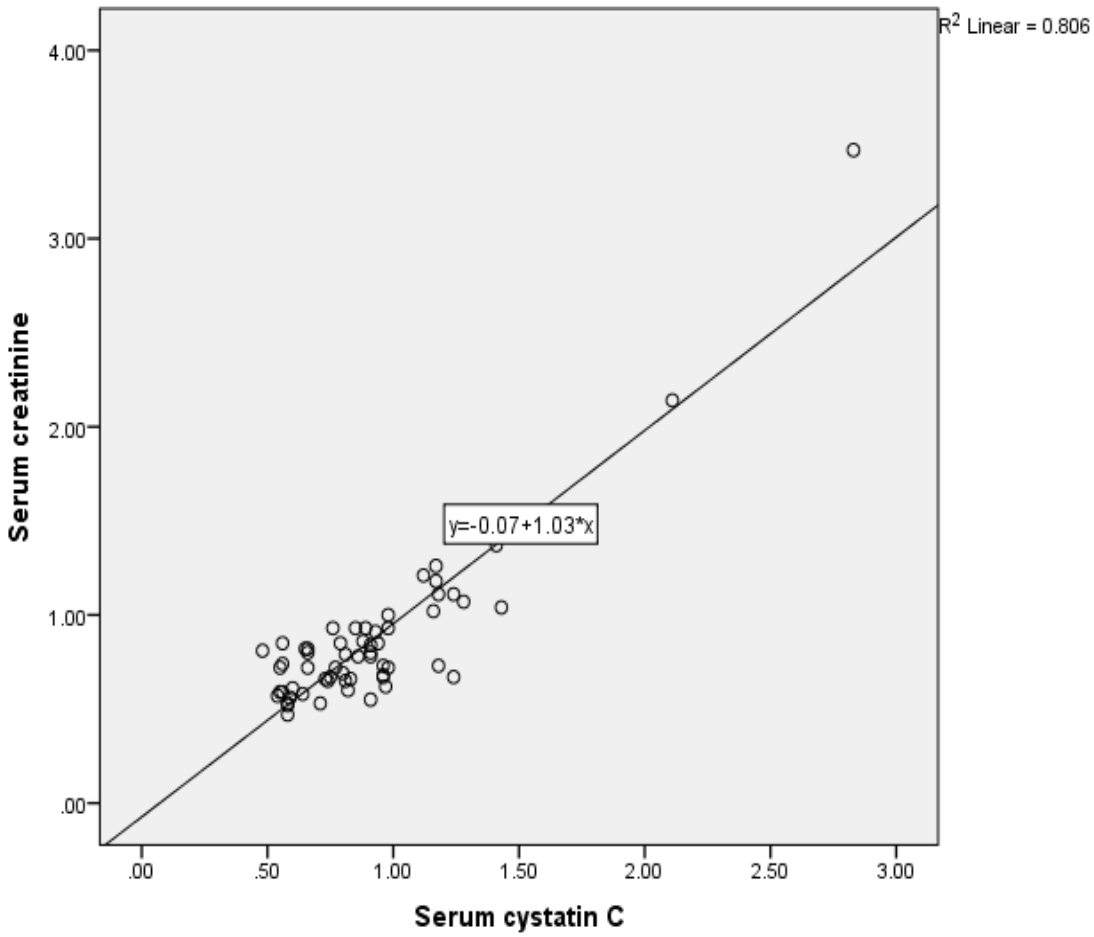

Figure 5: Scatter plot depicting correlation between serum levels of cystatin C and creatinine among T2DM patients, Tikur Anbessa teaching hospital, 2019

4.5. Multiple linear regression analysis of serum creatinine in type 2 diabetic patients

All predictor variables were checked by bivariate analysis and the result was that sex, age and BMI of the T2DM participants were found significantly related with serum creatinine (Table 9). This implies being female decreases the serum creatinine by 0.388; one unit change of age increases/decreases the serum creatinine by 0.013 and a unit change of BMI increases/decreases the serum creatininen by 0.221.

Table 9: multiple linear regression analysis of serum creatinine in type 2 diabetic patients, Tikur Anbessa teaching hospital, 2019

Variables	Serum creatinine	
	β (95% CI)	P-value
Sex of participants	-.388	**< 0.001**
Age of participant	.013	**0.001**
Duration of DM	.006	.353
BMI	.221	**< 0.001**

∗ Dependent variable "serum cystatin C" * *P-values is significant at <0.05.*

Table 10: Multiple linear regression analysis of serum cystatin C in type 2 diabetic patients, Tikur Anbessa teaching hospital, 2019

Variables	Serum cystatin C	
	β (95% CI)	P-value
Sex of paticipant	-.061	.481
Age of participant	.009	**.015**
Duration of DM	.006	.303
BMI	.011	**.013**

∗ Dependent variable "serum cystatin C" * *P-values is significant at <0.05.*

Even though the age and BMI of T2DM participants significantly related with serum cystatin C level, the relation was not strong like serum creatinine. Unlike serum creatinine serum cystatin C was not significantly affected by sex.

4.6. eGFR measurements

For Estimating GFR, three equations (Creatinine Equation (CKD-EPI 2009), Cystatin C Equation (CKD-EPI 2012), and Creatinine–Cystatin C Equation (CKD-EPI 2012)) were calculated (Table 11).

Table 11: The formula of three equations for Estimating GFR, Expressed for Specified Sex, Tikur Anbessa Specialized Teaching Hospital, 2019

Equations and Sex	Serum creatinine mg/dl	Serum Cystatin C *mg/L*	Equation for Estimating GFR
CKD-EPI creatinine equation			
Female	≤0.7		$144 \times (Scr/0.7^{)-0.329} \times 0.993^{Age}$ [× 1.159 if black]
Female	>0.7		$144 \times (Scr/0.7)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
Male	≤0.9		$141 \times (Scr/0.9)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
Male	>0.9		$141 \times (Scr/0.9)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
CKD-EPI cystatin C equation			
Female or male	≤0.8		$133 \times (Scys/0.8)^{-0.499} \times 0.996^{Age}$ [× 0.932 if female]
Female or male	>0.8		$133 \times (Scys/0.8)^{-1.328} \times 0.996^{Age}$ [× 0.932 if female]
CKD-EPI creatinine–cystatin C equation			
Female	≤0.7	≤0.8	$130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	

Female	>0.7	≤0.8	$130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
male	≤0.9	≤0.8	$130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
male	>0.9	≤0.8	$135 \times (Scr/0.9)^{-0.601} \times (Scys/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$135 \times (Scr/0.9)^{-0.601} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]

4.7. Characteristics of the outcome variables in T2DM patients

Table 12 shows the descriptive statistics of the outcome variables in T2DM patients. The mean ±SD Serum Creatinine value of the T2DM patients was 0.87 ± 0.44 mg/dl. The mean ± SD value of Serum Cystatiin C was 0.92 ± 0.38 mg/dl. The means ± SD of eGFR in three equatios (Creatinine Equation (CKD-EPI 2009), Cystatin C Equation (CKD-EPI 2012), and Creatinine–Cystatin C Equation (CKD-EPI 2012) were 105.7±27.5 ml/min/m^2, 90.4 ± 28.2 ml/min/m^2 and 100 ± 29.5 ml/min/m^2 respectively.

Table 12: Descriptive statistics of the outcome variable in T2DM patients: Tikur Anbessa teaching hospital, Addis Ababa, Ethiopia, 2019

Measured parameters	Minimum	Maximum	Mean	Std. Deviation
Serum cystatin C	.48	2.83	.92	.38
Serum creatinine	.47	3.47	.87	.44
eGFR – Creatinine Eq (CKD-EPI 2009)	20.00	143.00	105.72	27.5
eGFR - cystatin C Eq (CKD-EPI 2012)	19.00	130.00	90.3500	28.2
Both eGFR Creatinine–Cystatin C Eq (CKD-EPI mix)	19.00	142.00	100	29.5

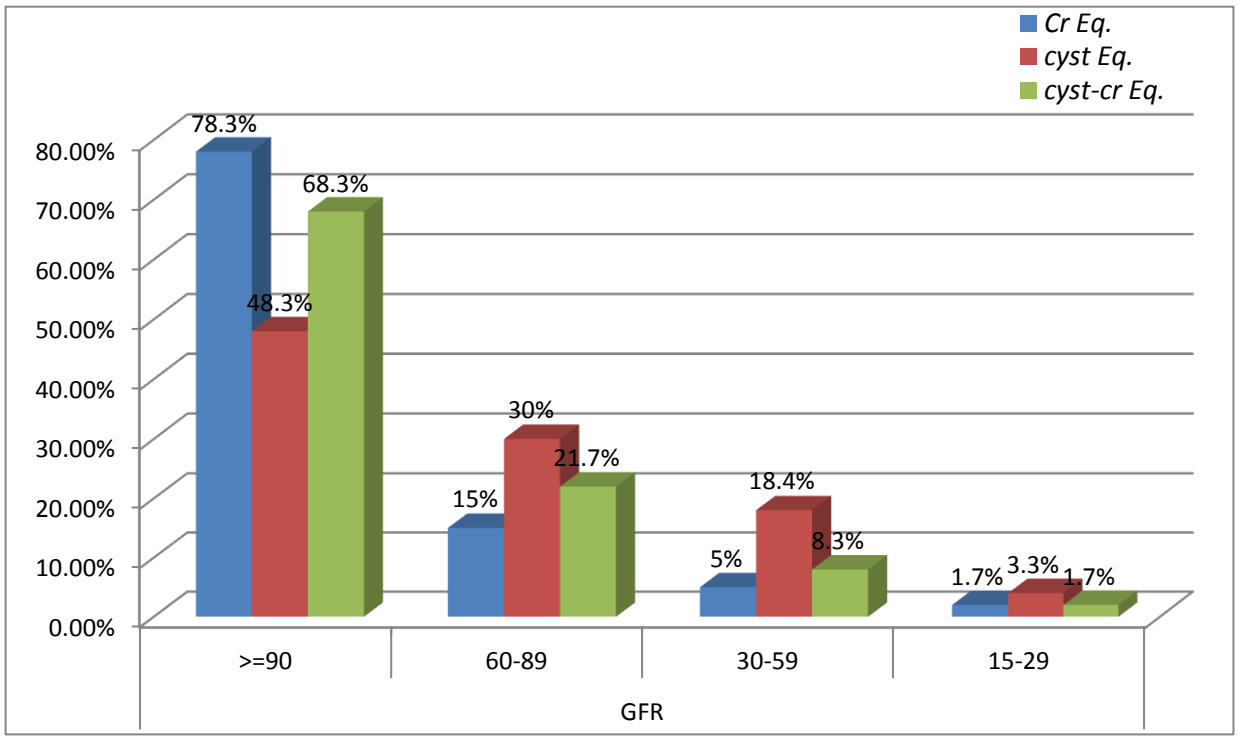

Figure 6: Percentage of participants' verses eGFR category in ml/min/1.73m2 from 15 to 90 calculated with the 3 equations, Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, 2019

Based on the eGFR calculated using the serum creatinine, 47(78.3%) of the T2DM patients were in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m^2), and 9(15%) of the patient were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2). While 3(5%) of the T2DM patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73 m^2), and only one T2DM patient was in stage 4 CKD with severe GFR reduction (GFR=15-29 mL/min/1.73 m^2) (Fig 6).

On the other hand according to the eGFR using the serum cystatin C equation, 29(48.3%) of the T2DM patients were in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m^2), 18(30%) of the patients were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2 and 11(18.4%) of the patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73), and 2(3.3%) patients were in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m^2) (Fig 6).

Based on eGFR using both serum cystatin C-creatinine Eq., 41(68. 3%) of the T2DM patients were in stage 1 CKD with normal or elevated eGFR (≥90 mL/min/1.73 m^2), 13(21.7%) of the patients were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2, 5(8.3%) of the patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73) and only 1(1.7%) patient was in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m2) (Fig 6).

4.8. Agreement test between estimated GFR- Creatinine based Equation and estimated GFR-Cystin C based Equation.

We investigated whether there is an agreement between the results of estimated GFR-Creatinine based Eq. and estimated GFR-Cystin C based Eq. by using the Cohen's Kappa statistical analysis. The results are presented in Table 13 below and p-value was found to be 0.003 and the kappa result was 0.24 suggesting that there is a poor agreement between the two equations.

In eGFR of 90 ml per minute per 1.73 m^2 and above, the two equations agreed for 29 participants. 18 patients were reclassified to a cystatin C–based eGFR of less than 90 ml per minute per 1.73 m^2.

In terms of 60 to 89 ml per minute per 1.73 m^2, the two equations agreed in only 2 T2DM patients. From the total of 18 T2DM patients who were classified as stage 2 CKD (60-89 ml per minute per 1.73 m^2) in estimated GFR-cystatin based equation, 16 patients were reclassified in 90 ml per minute per 1.73 m^2 and above in terms of creatinine-based eGFR Eq (table 13).

Within the category of a cystatin C-based eGFR Eq. of 30 to 59 ml per minute per 1.73 m^2 9 T2DM Patients were reclassified above 60 ml per minute per 1.73 m^2 by creatinine-based eGFR Eq. In two patients of stage 4 CKD (15-29 ml per minute per 1.73 m^2) based on cystatin C-eGFR equation, one patient was reclassified as above 30 ml per minute per 1.73 m^2 in creatinine-based eGFR Eq. In general accurate detection and staging of chronic kidney disease is integral components of clinical medicine, since such evaluations have a major effect on disease labeling, interventions, drug doses, and risk stratification for clinical procedures.

Table 13: eGFR- Creatinine versus eGFR-cystatin C cross-tabulation, Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia, 2019

eGFR-creatinine-based equation	eGFR-cystatin C- based equation				
	15-29	30-59	60-89	90 and above	Total
15-29	1	0	0	0	1
30-59	1	2	0	0	3
60-89	0	7	2	0	9
90 and above	0	2	16	29	47
Total	2	11	18	29	60
K= 0.24			P=0.003		

The kappa **(k)** value is interpreted as: <0.4 = poor; 0.4 -0.75 = fair to good; >0.75 = strong.

5. Discussion

This study was conducted in Tikur Anbessa specialized teaching hospital with the aim of assessing renal damage status of known type 2 diabetic patients. These patients were on regular follow up at the diabetic clinic, and with no previous documented impaired renal function. For early detection of diabetic-nephropathy, renal function was assessed using both serum creatinine and Cystatin C. Based on the serum creatinine and Cystatin C, the eGFR was also calculated using epidemiology collaborated equations.

This study was conducted on total of 120 study participants (60 T2DM cases and 60 health controls). The mean ages of cases and controls were found to be 56.5 and 37.2 years respectively. The number of female participants of the study were 32(53.3 %) in the cases and 35(58.3 %) in controls. The mean ±SD of the duration of patients with T2DM was 12.3 ± (8.6) years with the maximum and minimum duration of 41 years and 6 months respectively.

We calculated the BMI of the study participants using their weight and height. The mean ± SD was found to be 26 ± 4.4 kg/m^2 in cases and 19.7 ± 2.8 kg/m^2 in control groups respectively. Independent sample t-test was done and it showed that there was a statistically significant difference in terms of age and BMI between cases and control groups with (p =0.004, p=0.001 respectively).This study agreed with study conducted by Ashwin-Kumar *et al* (2014).

The serum creatinine and cystatin C concentration of T2DEM patients and healthy control groups were examined in this study. The study showed significant increase in serum creatinine level in type 2 diabetics compared to controls. The serum creatinine was found to be significant (p=0.001) higher in cases ((0.87 ± 0.44) compared to controls (0.63 ± 0.27).The serum cystatin C was also found to be significantly (p=0.0001) higher in cases (0.92 ± 0.38) compared to controls (0.52 ± 0.20). These findings are similar to studies conducted earlier (Borges *et al*,2010; Rahul-Singh *et al*,2017 and Ashwin-Kumar *et al*,2014).

Many previous studies have provided evidences that the serum cystatin C has superiority over serum creatinine as a marker of renal impairment. This can be explained by the fact that the serum creatinine as marker of GFR has drawbacks because the endogenous production

of creatinine is affected by age, sex muscle mass and dietary intake. This is probably the reason for different serum creatinine levels observed in various population groups (Shamseldin*et al*2015)

The inability of creatinine to detect early decline in GFR is due to the fact that serum creatinine (SCr) levels only begin to rise above the normal range when approximately 50% of renal function is already lost, suggesting that GFR can change before SCr becomes abnormal (Shemesh *et al*, 1985). Many evidences suggest that serum cystatin c may not be influenced by age, sex muscle mass and dietary intake and as a result its levels rise faster than creatinine after a fall in GFR and is a reliable endogenous marker for assessing renal function in type 2 diabetic patients. In a study, including 52 type 2 diabetic patients, an early and more significantly increased levels of serum cystatin C than SCr was observed as GFR decreases, which indicated that serum cystatin C might be a useful marker for detecting early renal impairment in diabetic patients (Hamed *et al*, 2011).

To confirm this we have done the correlation and regression analysis for serum creatinine and cystatin C. The correlation analysis between ages, BMI and serum creatinine showed that the level of serum creatinine was positively and significantly (p=0.001, p=0.000) correlated with the age and BMI of T2DM patients respectively. In our study regression analysis also showed that being female decreases the serum creatinine by 0.388, one unit change of age increases/decreases the serum creatinine by 0.013 and a unit change of BMI increases/decreases the serum creatininen by 0.221. This was similar with a study conducted by Nabil *et al.,* (2013) but unlike our study the correlation between age and serum creatinine was insignificant in this previous study. This may be due to the influence of serum creatinine by the extreme ages.

We also conducted the pearson's correlation analysis between serum cystatin C and age and BMI variables of the T2DM patients. We found that the level of serum cystatin C positively and significantly correlated with the age and BMI of T2DM patients (r = 0.302, p =0.02), (r = 0.369, P = 0.021) respectively. But unlike serum creatinine, it weakly correlated with BMI and age of the patients. This was in line with Nabil *et al.,* (2013). However, the regression analysis showed that even though the age and BMI of T2DM participants significantly related with serum cystatin C level, relation was not strong like serum creatinine. Unlike

serum creatinine serum cystatin C was not significantly affected by sex. These findings agreed with many reports (Alehagen *et al.,* 2009; Mcnamara *et al.,* 2009 and Krishan Pandey *et al.,* 2015).

The correlation analysis also showed that there was a strong and positive correlation between serum cystatin C and serum creatinine among T2DM patients (r = 0.90, P= <0.0001). These findings agreed with the study conducted by Afshari *et al* (2017).

For Estimating GFR, as detection of renal impairment in T2DM patients we also calculated the three equations (Creatinine Equation (CKD-EPI 2009), Cystatin C Equation (CKD-EPI 2012), and both Creatinine–Cystatin C Equation (CKD-EPI 2012)). After determination of serum creatinine and cystatin C levels, the means ±SD of eGFR in three equations were 105.7±27.5 ml/min/m^2, 90.4 ± 28.2 ml/min/m^2 and 100 ± 29.5 ml/min/m^2 respectively. This finding was consistent with the cohort study conducted by Michael*et al.*,(2013) and a cross sectional study conducted by Shima *et al., (2011).*

Concerning the variation due to gender, we found the mean serum creatinine level was significantly higher in males compared to females (p<0.0001). This is an expected finding and agrees with many previous studies. However, in case of mean value of serum cystatin C, we did not find significant difference between males and females. This finding was agreed with many other studies (Mangge *et al*., 2000; Galteau et *al*., 2001; Jayasekara *et al*., 2018). They usually defined a reference value common for both sexes. In contrast to this Finney *et al*., (2000) and Wang *et al., (*2011) found a significant difference in cystatin C levels, which were higher in men compared to women.

Based on the eGFR calculated using the serum creatinine, we found that 47(78.3%) of the T2DM patients were in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m^2), and 9(15%) of the patient were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2). While 3(5%) of the T2DM patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73 m^2), and only one T2DM patient was in stage 4 CKD with severe GFR reduction (GFR=15-29 mL/min/1.73 m^2). Our finding was similar with other studies which were conducted by (Lujambio *et al.,* 2014 and Shamseldin*et al* (2015).

We also calculated the serum cystatin C derived eGFR equation and found that, 29(48.3%) of the T2DM patients were in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m^2), 18(30%) of the patients were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2 and 11(18.4%) of the patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73), and 2(3.3%) patients were in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m^2).Based on eGFR using both serum cystatin C-creatinine Eq., 41(68. 3%) of the T2DM patients were in stage 1 CKD with normal or elevated eGFR (≥90 mL/min/1.73 m^2), 13(21.7%) of the patients were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2, 5(8.3%) of the patients were in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73) and we found only 1(1.7%) patient was in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m2). This was consistent with other studies conducted by Shamseldin *et al*., (2015) andMichael *et al.*, (2018).

Based on eGFR using both serum cystatin C-creatinine Eq. we found that 41(68. 3%) of the T2DM patients were in stage 1 CKD with normal or elevated eGFR (≥90 mL/min/1.73 m^2), 13(21.7%) of the patients were in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m^2, 5(8.3%) of the patients were in stage 3 with moderately decreased GFR (GFR= 30-59 mL/min/1.73) and only 1(1.7%) patient was in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m2).This finding was consistent with a study conducted by Shamseldin *et al*., (2015).

We also investigated whether there is an agreement between the results of estimated GFR-Creatinine based Eq. and estimated GFR-Cystin C based Eq. by using the Cohen's Kappa statistical analysis. We found that the p-value of 0.003 and the kappa value of 0.24 which indicates the overall agreement between these two equations were poor. This finding was similar to other studies conducted by Michael *et al.,* (2018) and Tsai et *al* (2014). But in contrast to this study, Shima *et al., (2011)* showed the Kappa (k) value of 0.74 that indicates good agreement. The possible reason for this controversy may be due to sample size difference.

In eGFR of 90 ml per minute per 1.73 m^2 and above, we found that the two equations agreed for 29 participants. From total 47 T2DM patients 18(37.3%) T2DM patients were reclassified to a cystatin C–based eGFR of less than 90 ml per minute per 1.73 m^2. In terms of 60 to 89 ml per minute per 1.73 m^2, the two equations agreed for only 2 T2DM patients. From the total of 18 T2DM patients who were classified as stage 2 CKD (60-89 ml perminute per 1.73 m^2) in estimated GFR-cystatin based equation, 16 patients were reclassified 90 ml per minute per 1.73 m^2 and above in creatinine-based eGFR Eq. This study agreed with studies done by Martin *et al* (2008) Ching-Wei Tsai et *al* (2014).

Within the category of a cystatin C-based eGFR Eq. of 30 to 59 ml per minute per 1.73 m^2 from the total of 11 T2DM Patients 9 patients were reclassified above 60 ml per minute per 1.73 m^2 by creatinine-based eGFR Eq. In two patients of stage 4 CKD (15-29 ml per minute per 1.73 m^2) based on cystatin C-eGFR equation, one patient was reclassified as above 30 ml per minute per 1.73 m^2 in creatinine-based eGFR Eq. This in line with other studies which were conducted by Inés Lujambio *et al.,* (2014) and Shamseldin*et al* (2015).In general accurate detection and staging of chronic kidney disease is integral components of clinical medicine, since such evaluations have a major effect on disease labeling, interventions, drug doses, and risk stratification for clinical procedures.

6. Strength and Limitation of the Study

6.1. Strength of the Study

- This is the first study in Ethiopia. Therefore, this will give the baseline information for further studies.
- This study also attempts to test the agreement between creatinine derived eGFR and cystatin C derived eGFR for early detection of renal impairment in patients with T2DM.
- The study involved healthy control samples to clearly show the contrasting features

6.2. Limitation of Study

- The study was cross sectional based and we measured the serum parameters only once for each participant which could overestimate or underestimate the results..
- We did not have a "*gold standard*" marker due to performing inulin or iothalamate clearance that needs invasive and tedious procedures and not suitable for study subjects.
- We didn't include other parameters like albuminuria in this study due to the limitation of reagent availability.

7. Conclusion

From our study, we conclude that cystatin C is an excellent marker of renal function and correlates better to measures eGFR more precisely than creatinine, because unlike serum creatinine its serum concentration is not that much influenced by sex, muscle mass and age.

The results of this study suggest that cystatin C measurement is a useful, practical tool for the evaluation of renal involvement in T2DM patients. Serum cystatin C can detect mild-to-moderate decreases of GFR that may not detected with serum creatinine-based measurements. To compare the performance of eGFR equations based on cystatin C and creatinine, we confirmed discrepancies in eGFR using equations for serum creatinine and cystatin C based biomarkers and found that most T2DM patients in cystatin C based equation, compared with creatinine based equation, were in lower eGFR values.

8. Recommendations

- This study is small in size and cross sectional. Therefore, more studies need to be done in larger cohort of participants for early detecting of diabetic-nephropathy.
- Further studies can be done with higher sample size using other parameters like urine albumin and gold standard methods (inulin) of GFR measurement for better evaluation of cystatin C as a marker of renal impairment in T2DM patients.
- Serum cystatin C as a screening test and as an early indicator and predictor of the development of diabetes complication like renal failure has to be considered during treatment of patients with T2DM.
- It is recommended that more studies should be undertaken in Ethiopia because more renal complications of diabetes patients are increasing.
- Due to limitation of reagent availability, albuminuria was not done on the study participants. There is a necessity for measurement of albuminuria to detect kidney damage. So further study may be needed to show both the functional status and cellular damage of kidney by assessing both glomerular filtration rate and albuminuria.

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10. ANNEXES

10.1. Annex I: Information sheet (English version)

Addis Ababa University, School of Medicine, Department of Medical Biochemistry

Information sheet for the participants of the study entitled on evaluation of serum cystatin c versus serum creatinine levels for the detection of early renal impairment among patients with type-2 diabetic mellitus, attending tikur anbessa specialized teaching hospital Addis Ababa, Ethiopia.

Principal Investigator: Tadesse Asmamaw Dejenie

Advisors:

1. Dr. Solomon Genet,
2. Dr. Menakath Menon,

Sponsoring organization: - Federal Ministry of Education in collaboration with Addis Ababa University

Introduction: - My Name is ----------; I am working in ----------. I am working as data collector in a study conducted by the research team member of Addis Ababa University College of Health Sciences. I would like to inform you that I would have a short interview concerning this study. Before we go to our discussion, I will ask you to listen carefully to what I am going to tell you about the purpose and general condition of the study and tell me whether you agree or disagree to participate in this study.

The objective of the study is to evaluate the serum cystatin c versus serum creatinine level and compare them for early detection of diabetic nephropathy which is one of the common chronic complication in diabetic mellitus patients and better one will be used as early screening and diagnosis marker of this complication.

Procedure and things that will be expected from you for participation

If you agree to take part in this study, you will be given the consent form to sign, and interviewed by health professional to assess whether you qualify to participate in the study or not. If you are fit for the study, the data collector will ask some questions which are important for our study like socio-demographic. Physical measurements like weight, height, and blood pressure will be taken. 5mL of blood sample will be also collected for laboratory

examination of serum cystatin C and serum creatinine. eGFR will be calculated based on MDRD and CKD-EPI equation.

3. Potential Risks

Participating in this project will not cause more discomfort than the routine examination you required during clinic visit. But minor pain and skin color change may be occurred following blood drawing, which will disappear within short duration. The amount of blood required in the study is 5mL, which will not affect your health. The whole procedure will be carried out by health professionals through standard clinical practice, so participating in this study has no major risks. If anything happened, appropriate medical care will be provided for you.

4. Potential Benefits

you will not receive any payment during participation in this study as compensation. But You will have the chance to know your general health status from the project without any direct incentive because the cost will be covered by the project. In addition, the result of the study will be beneficial for the early diabetic nephropathy. Hence, you are indirectly benefiting other patients and the society in this respect.

5. Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. Logbooks used in the laboratory will have no names but codes. The information sheet that links the coded number to participant name will be locked inside a box.

6. Right to refuse

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind.

Annex II: Informed consent (English Version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project.

Code number_____

Study participant name_____

Study participant signature_____

I have been informed about the study, that aims to evaluate the serum cystatin c versus serum creatinine levels for the detection of early renal impairment among patients with type-2 diabetes mellitus, attending Tikur Anbessa specialized hospital. The objective and the application of the study were explained to me and I have been told that the results obtained will help me and the community. I am also informed that all information about the participant will be kept confidential and the right to withdraw from the study any time. I had also the right to ask a question and suggestion about the study. I was also told that the result of the laboratory test would be reported to me if I need it. therefore, I voluntarily agreed the informed consent with full understanding of the research and answered the prepared question and give my blood for the aforementioned study. I, the under signed, confirm that, as I give consent to participate in the study; it is with a clear understanding of the objectives and the study conditions.

I _____ do hereby give consent to Dr./Mr./Mrs./Miss_____ to include me in the proposed research.

Signature Date

Participant: _____ _____

Interviewer: _____ _____

10.2. Annex II: Questionnaires

Dear respondents the aim of this questionnaire is to collect the necessary data to evaluate serum cystatin C and serum creatinine among diabetic patients. It is a part of the study for the masters of degree in medical biochemistry at Addis Ababa university school of graduate studies. This study is purely academic and all your responses will be used for accomplishing the requirements of this study. Therefore, you are kindly requested to give correct information accordingly. Thank you for your time and kindness.

Signature Date

Participant: _____ _____

Interviewer: _____ _____

10.2.1. Annex III: English Version Questionnaires

Questionnaire Code

_____

Part-1 Socio demographic characteristics		
No	Variables	Response
101	Sex	1. Male 2. Female
102	Age	_____years
103	Educational status	1. Illiterate 4. Secondary education 2. Read and write 5. College and above 3. Primary education 6. other
104	Occupational status	1. Housewife 4. Governmental 2. Non-governmental 5. Private 3. student 6. other
105	Residence	1. Rural 2. Urban
Part-2. Behavioral characteristics		
201	Smoking status	1. Never smok 2. Former smoke 3. Currently smoke
202	If you smoke currently, how long ago you first started smoking?	_____
203	On average, how many packs do you smoke each day?	_____

204	If you were former smoker, how long ago did you stop smoking?	_____
205	Do you drink alcoholic	1. yes 2. No
Part-3. Clinical variables		
301	Duration of DM	_____years
302	Medication use	1. oral medication 2. insulin injection 3. oral medication and insulin injection

10.2.2. Annex IV: Anthropometric and biochemical measurements

Anthropometric measurements

1. Height _____cm

2. Weight _____kg

3. BMI _____kg/m2

Biochemical measurements

1. Serum cystatin C _____ mg/dL

2. Serum creatinine _____mg/dL

3. eGFR

Using the Creatinine Equation (CKD-EPI 2009) _____mL/min/1.73m2

Using Cystatin C Equation (CKD-EPI 2012) _____mL/min/1.73m2

Using Creatinine–Cystatin C Equation (CKD-EPI mix) _____mL/min/1.73m^2

10.3. **Annex VI**: Information sheet (Amharic version)

<u>የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ</u>

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ ሕክምና ት/ቤት በባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡
እባክዎን በመጠይቅ ላይ ላሉት ጥያቄዎች አስፈላጊዉን መረጃ በክፍት ቦታዉ ላይ ይሙሉ፡፡

የጥናቱ ርዕስ፡- በሁለተኛው ኣይነት የስኮር በሽተኞቸ ደም ውስጥ ያለውን cystatin c እና creatinine የተባለውን marker በመመርመር እና ምንም በሽታ ከለለባቸው ሰዎች ጋር በማዎዳደር ይህ የስኮር በሽታ ሊያስከትለው የሚችለውን ተጨማሪ የኩላሊት በሽታ ቀድሞ ለማወቅ የትኛው የመርመሪያ ዘዴ ሊጠቅም እንደሚችል በመለየት የተሻለውን በመጠቀም ከስኮር በሽታ ጋር ተያይዞ የሚመጣውን የኩላሊት በሽታ ቀድሞ ለማውቅ እና ለመከላከል በሚያስችል የምርመራ ዘዴ ላይ የሚደረግ ምርምር ነው፡፡

እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት ኣይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት፡፡

በዚህ ጥናት መሳተፍ ሊያስከትላቻው የሚችሉ ቸግሮች

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

የመረጃ በሚስጥር አጠባበቅ ሁኔታ

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ::

በዚህ ጥናት መሳተፍ ሊገኙ የሚችሉ ጥቅሞች

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት፡፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎን የስኮር በሽታ እና ሊያስከትላቸው የሚችለውን ተዛማጅ በሽታዎች ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ለመሳተፍ ወይም ላለመሳተፍ ያለዎት መብት

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ::ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካልዎት ወይም ችግር ካጋጠመዎት

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ችግር ከገጠምዎት ወይም ማንኛም ጥያቄ ካለዎት የሚከተለውን አድራሻ ይጠቀሙ፡፡

<u>አድራሻ፡-</u>

ታደሰ አስማማው ደጀኔ

ስልክ ቁጥር 0909045760/0967913355

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Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡

በተጨማሪም የምሰጠዉ የደም ናሙና ለ cystatin C እና Creatinine ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለህኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤአለሁ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

እኔ_____የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን_____

ተሳታፊ _____ _____

10.3.1 **Annex III**: Amharic Version Questionnaire

ፊርማ ቀን

1. የጠያቂው---------- ----------

የተጠያቂው---------- ----------

-

መለያ ቁጥር ----------

ክፍል-1 የማህበረሰብና ስነህዝብ ባህሪያትን በተመለከተ		
ተ.ቁ	ጥያቄ	የተጠያቂዉ መልስ
101	ፆታ	1. ወንድ 2. ሴት
102	ዕድሜ	_____
103	የትምህርት ትደረጃ	1. አልተማርኩም 2. ማንበብ መፃፍ እችላለሁ 3. አንደኛ ደረጃ ያጠናቀኩ 4. ሁለተኛ ደረጃ ያጠናቀኩ 5. ኮሌጅ እና ከዚያ በላይ

		6. ሌላ ካለ
104	ስራ	1. የቤት እመቤት 2. የመንግስት ሠራተኛ 3. መንግስታዊ ያልሆነ ድርጅት ሠራተኛ 4. ተማሪ 5. የግል ስራ 6. ሌላ ካለ ይግለጡ _____
105	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ
ክፍል-2 ባህሪን የሚመለከቱ ጥያቄዎች		
201	ሲጃራ ያጨሳሉ?	1. በፍጹም አጭሽ አላውቅም 2. አጨስ ነበረ አሁን ግን አቁሚያለሁ 3. አዎ አጨሳለሁ
202	የሚያጨሱ ከሆነ ለስንት ጊዜ አጨሱ	_____
203	ባማካይ በቀን ስንት ፓኮ ሲጋራ ያጨሳሉ	_____
204	ሳጨስ ነበር አሁን ግን አቁሚያለሁ ካሉ፤ ካቆሙ ምን ያህል ጊዜ ሆኑዎት?	_____
205	አልኮል ይጠጣሉ?	1. አዎ እጠጣለሁ 2. አልጣም
ፍክል-3 የስኳር በሽታ የሚመለከት ጥያቄ		
301	የስኳር በሽታ እንዳለብዎ ካወቁ ስንት ጊዜ ሆነዎት?	_____ዓመት
302	ለስኳር በሽታ የሚወስዱት ሕክምና	1. የሚዋጥ መድሐኒት 2. በመርፌ የሚዎጋ (ኢንሱሊን) 3. የሚዋጥመድሐኒት+ኢንሱሊን

ክፍል-4 የአካል መጠን መለከኪያ እና የላቦራቶሪ ውጤቶች		
401	ቁመት	በሳ.ሜትር_____
402	ክብደት	በኪ..ግራም_____
403	Serum cystatin C	_____mg/dL
404	Serum creatinine	_____mg/dL
405	eGFR	Using the Creatinine Equation (CKD-EPI 2009) _mL/min/1.73m2 Using Cystatin C Equation (CKD-EPI 2012) ____mL/min/1.73m2 Using Creatinine–Cystatin C Equation (CKD-EPI mix) _____mL/min/1.73m^2