RENAL IMPAIRRMENT AND ASSOCIATED FACTORS AMONG TYPE 1 AND TYPE 2 DIABETIC PATIENTS AT JIMMA UNIVERSITY MEDICAL CENTER, SOUTH WEST ETHIOPIA

BY: MOHAMMED ADEM (BSc)

A THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCIENCES; FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH; JIMMA UNIVERSITY FOR PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS INCLINICAL LABORATORY SCIENCE SPECIALITY IN CLINICAL CHEMISTRY

FEBRUARY, 2018

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH, FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

RENAL IMPAIRRMENT AND ASSOCIATED FACTORS AMONG TYPE 1 AND TYPE 2 DIABETIC PATIENTS AT JIMMA UNIVERSITY MEDICAL CENTER, SOUTH WEST ETHIOPIA

BY: MOHAMMED ADEM (BSc)

ADVISORS:-

Mr. AKLILU GETACHEW (MSc)

Mr. SHIFERAW BEKELE (MSc)

ABSTRACT

BACK GROUND: *Kidney disease is far more common in people with diabetes than in people without diabetes .About 20–40% all type of diabetic patients will develop a diabetic renal disease during the course of their disease.*

OBJECTIVE: -*To assess renal impairment and associated factors among type1 and type 2 diabetic patients attending Jimma University Medical Center, South West Ethiopia.*

MATERIALS AND METHODS: - *A hospital based comparative cross–sectional study was conducted among type 1 and type 2 diabetic patients and apparently healthy individuals, aged 18 years and above at Jimma University Medical Center from January 9 to March 22, 2017.*

A total of 234 diabetes (both type1 and 2) and 234 apparently healthy individuals were enrolled by consecutive sampling technique. Socio-demographic and clinical data were collected using a structured questionnaire. Fasting venous blood was collected from each study participant by trained nurses. Fasting blood glucose, serum creatinine and urea were analyzed by using Mindray BS_200E chemistry analyzer. Estimated glomerular filtration rate was calculated by Cockcroft & Gault formula. Descriptive statistics, Pearson's Chi-square, Mann-Whitney test; spearman's correlation coefficient and logistic regression analysis were done using SPSS version20 software.

RESULTS: -*About 21.8% and 13.8 % of diabetic patients had renal impairment (stage 3 CKD-eGFR) by CG-BSA and MDRD respectively, whereas the renal impairment (stage 3 CKD-eGFR) among apparently healthy individuals was 2.6% by CG-BSA and 1.3% by MDRD equation. Age≥50 year (OR 3.12, p=0.014); female sex (OR 3.35, p=0.008), duration of diabetes >10 year (OR 2.42, P=0.043, history of hypertension (OR 8.84, p<0.001), diastolic blood pressure ≥90mmHg (OR 5.48, p=0.004) and high blood glucose ≥130mg/dl (OR 8.44, p=0.003) were significantly associated with renal impairment calculated by CG-BSA in people with diabetes.*

CONCLUSION AND RECOMMENDATION:-*The overall prevalence of renal impairment in diabetes was higher than non-diabetic individuals. Tight glycemic control should be a high priority in reducing the renal impairment burden in the study area.*

ACKNOWLEDGEMENT

I would like to express my deepest sense of gratitude and thank to my advisors Mr. Aklilu Getachew and Mr. Shiferaw Bekele for their valuable constructive comments, suggestions, guidance and unreserved help for the completion this thesis.

I would like to acknowledge the school of medical laboratory sciences, Jimma University for giving me this opportunity to conduct this study. I would like to pass my deepest gratitude to Jimma University Medical Center for sponsoring my education and financial support.

I would also like to thank data collectors and all the respondents who volunteered to participate in the study. Without their participation, this study would not have been successful.

Last but certainly not least I would like to give my heartfelt thanks to my wife w/o Rabiya Seid for her unwavering support and inspire and also to Dr. Tilahun Yemane and Mr. Sintayehu Asaye who were involved in motivating, encouraging and sharing ideas all through my career.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGERS

ABBREVIATIONS

BMI	Body Mass Index
BP	Blood Pressure
BSA	Body Surface Area
CG	Cockcroft Gault
CI	Confidence Interval
CKD	Chronic Kidney Disease
CrCl	Creatinine Clearance
DBP	Diastolic blood pressure
DM	Diabetes Mellitus
DN	Diabetic nephropathy
eGFR	estimated Glomerular Filtration Rate
FBS	Fasting Blood Sugar
IQR	Inter Quartile Range
JUMC	Jimma University Medical Center
MDRD	Modification of Diet in Renal Disease
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SD	Standard Deviation
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus

OPERATIONAL DEFINITIONS

Alcohol consumption: - An individual having 2-3 alcohol drinks per week before data collection period.

Analgesics: -An individual always takes anti-inflammatory drug when he experiences pain.

Cases: -Adult diabetic patients who have chronic illness clinic follow up and participated in the study.

Controls: - Individuals who have no history of DM and other chronic diseases participated in the study.

Current smoker:-An individual who were practicing smoking cigarette for the last 12months before this study.

Hyperglycemia:-Diabetes patient whose fasting blood glucose level was > 130mg/dl.

Non-alcohol consumption:-An individual who not having more than 2 drinks per week.

Non-smoker:-An individual who has no history of smoking.

Normoglycemia: - Diabetes patient whose fasting blood sugar level <130mg/dl

Poor glycemic control:-fasting blood glucose level $\geq$130 mg/dl.

Previous smoker:-An individual who were previous history of cigarette smoking at least one year but stop at the time of study.

Renal impairment: - is defined as an eGFR < 60 mL/min/1.73m^2.

Traditional medicine: An individual who have used traditional medicine at least once per year.

Type 1 diabetes: - A case identified for having initial insulin treatment from diagnosis.

Type 2 diabetes: - A case identified for having initial oral antihyperglycemic treatment from diagnosis

CHAPTER ONE:-INTRODUCTION

1.1 Background

Diabetes mellitus is a chronic, progressive disease characterized by elevated levels of blood glucose. It occurs either when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (1). The abnormalities in carbohydrate, fat, and protein metabolism that are found in diabetes are caused by either lack of insulin secretion or decreased sensitivity of target tissues to insulin (2).

Depending on the etiology of the diabetes mellitus, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production(3). On other hand, hyperglycemia develops as a result of three processes: increased gluconeogenesis, accelerated glycogenolysis, and impaired glucose utilization by peripheral tissues(this is magnified by transient insulin resistance due to the hormone imbalance itself as well as the elevated free fatty acid concentrations)(4).

Normally, the kidneys play multiple roles in the body, including blood filtration, reabsorption, metabolism and excretion of the nonprotein nitrogenous wastes such as creatinine and urea as well as the elimination of exogenous molecules (5).

The hyperglycemic state itself is a strong risk factor for diabetic kidney disease(6). The mechanisms responsible for the development and progression of diabetic kidney disease remains poorly understood. However, it is known that progression of diabetic kidney disease correlates closely with level of high blood sugar. Prolonged hyperglycemia leads to chronic metabolic and hemodynamic changes that modulate various intracellular signaling pathways, transcription factors, cytokines, chemokines, and growth factors(7). The cumulative result of these changes promotes structural abnormalities in the kidney, such as glomerular basement membrane thickening, podocytes injury and loss and mesangial matrix expansion and functional alterations such as increased permeability of glomerular basement membrane or shear stress(8), the occurrence of glomerular sclerosis and tubulointerstitial fibrosis associated with declining glomerular filtration rate (GFR)(9).

Creatinine is an end-product of nitrogen metabolism and is produced in muscle from creatine and phosphate by irreversible, nonenzymatic, spontaneous loss of water (10). It is subsequently excreted principally by way of the kidneys, predominantly by glomerular filtration without tubular reabsorption(11). Thus, creatinine concentration in serum is very useful indexes for evaluating glomerular filtration rate and a general reflection of renal function(12), also it is the most common screening test for chronic kidney disease in current clinical practice. Because, it is not plasma protein bound, not metabolized in the kidney and therefore is freely filtered in the glomerulus, not reabsorbed by renal tubules and a small amount is secreted by the tubules, making it an excellent marker of glomerular filtration(13). It is produced at a relatively constant rate, which is in turn proportional to muscle mass .However, between-person variability in creatinine generation rate related to age, sex, muscle mass, race, exercise and perhaps other factors limits the use of creatinine in the estimation of Glomerular filtration rate(GFR)(14).

Similarly, Urea is a nonprotein nitrogen compound formed in the liver from ammonia as an end product of protein metabolism(15). It diffuses freely into extracellular and intracellular fluid and is ultimately excreted by the kidneys(16).However, about 40-50% filtered urea may be reabsorbed back into the blood by passive transport in the nephron tubules, although the proportion is reduced in advanced renal failure(17). This reabsorption of urea is flow dependent so that more urea is reabsorbed at lower urine flow rates(18).

Serum urea is a less reliable marker of glomerular filtration rate than creatinine and insensitive in detecting renal disease, because its level is increased by other non-nephrogenous factors such as dietary protein, accelerated protein catabolism, dehydration ,hypoperfusion of the kidneys and steroid administration(19). A significantly elevated plasma urea concentration is indicative of impaired glomerular function leads to chronic kidney disease(20).

Glomerular filtration is the physiological process of creating an ultrafiltrate of plasma as it passes through the glomerular capillaries(21).Glomerular filtration rate is traditionally considered as the best overall index of renal function in health and disease and the most widely used renal function test in current clinical practice than serum creatinine alone(22,23).

The most commonly used method to evaluate GFR is creatinine clearance. It is assessed using a timed urine collection and measurement of creatinine excretion over a predefined time period

(often 24hs) and simultaneously in blood (assumes steady state) using the following equation: CrCl (mL/min) = [urinary creatinine (mg/dL) x urine volume (mL/min)] / [SCr (mg/dL)]. However, it is limited in current routine clinical practice due to errors of accurate urine collection inaccurate record of time(24),and systematically overestimates GFR because of tubular secretion of creatinine(25).

As mentioned, the 24-hour urine collection can be difficult to perform accurately. For this reason, the development of formula- based calculations of estimated GFR (eGFR) has offered a very practical and easy approach by combining the patient's serum creatinine with factors such as age, weight, race, and gender)(26). Additionally, estimation of GFR is central to the diagnosis, evaluation, and management of kidney disease(27).

Kidney disease (nephropathy) is far more common in people with diabetes than in people without diabetes; and diabetes is one of the leading causes of chronic kidney disease(CKD)(28).

CKD includes a spectrum of pathophysiologic processes associated with abnormal kidney function and a progressive decline in glomerular filtration rate(29,30).

According to the National Kidney Foundation, different degrees of CKD from the earliest kidney damage to end-stage renal disease have been classified into five stages on the basis of markers of kidney damage and level of kidney function (glomerular filtration rate) (31).CKD stage 1 is kidney damage with normal or increased GFR (>90ml/min/1.73m^2),CKD stage 2 is kidney damage with mild or reduced GFR (60-89ml/min/1.73m^2),CKD stage 3 is kidney damage with moderate reduction in GFR (30-59ml/min/1.73m^2),CKD stage 4 is kidney damage with severe reduction in GFR (15-29ml/min/1.73m^2) and CKD stage 5 is kidney failure (GFR < 15ml/min/1.73m^2 or dialysis)(31).

Renal disease is also not uncommon complication of diabetes in Ethiopia; particularly in the study area amongst diabetic patients. But there are limited studies that determine the prevalence of renal impairment and associated factors among T1DM and T2DM in Jimma, Ethiopia. Therefore, the aim of the study is to assess renal impairment and associated factors in diabetic patients in reference to apparently healthy individuals in Jimma University Medical Center, South West Ethiopia.

1.2 Statement of the problem

Diabetes mellitus has become the mainly common cause of renal function impairment in various parts of the world in general, and in developing countries in particular(32). Diabetic kidney disease is not uncommon complication of diabetes (both type 1 and 2 diabetes) all over the world (33).

The incidence and prevalence of diabetes mellitus has a major impact on development of diabetic kidney disease(34). Diabetic kidney disease is also one of the most frequent and serious complications of both types of diabetes and the leading cause of end-stage renal disease (ESRD), accounting for approximately 50% of cases in the developed world(35). It is estimated that 20–40% of patients with diabetes will develop a diabetic renal disease during the course of their disease(36).

In the United States, the prevalence of diabetic nephropathy increased in direct proportion to the prevalence of diabetes, without a change in the prevalence of DKD among those with diabetes and continues to rise and it remains a strong predictor of morbidity and mortality in diabetic patients(37,38). Among U.S. adults aged 20 years or older with diagnosed diabetes, the estimated crude prevalence of chronic kidney disease (stages 1–4) was 36.5% during 2011-2012(39).

Chronic kidney disease has been suggested to be more frequent among patients with diabetes in Africa as compared to those in the developed world due to late referral to hospital, delayed diagnosis, limited screening and diagnostic resources, limited capacity of health workers for CKD detection and prevention, poor control of blood sugar and other risk factors(include mainly hypertension, obesity, history of heart disease, poor glycemic control and disease duration),inadequate treatment of complications at an early stage and poor awareness of kidney disease in the community(40–42).

Although in Sub-Saharan Africa few studies have described the burden of CKD among adults with diabetes, chronic kidney disease(CKD) is one of the most common complications of diabetes and a serious health threat in this region(43).In Sub-Saharan Africa, people with diabetes, prevalence of CKD in several countries may approximate or exceed that of many high income countries(44). Even though, overall diabetic nephropathy prevalence is between 6%-16%(45),a recent meta-analyses and systematic reviews of observational studies on diabetic

nephropathy in Africa conducted between January 1994 and July 2014,showed that the prevalence rate of DN was as high as 11%-83.7% (46).

In Ethiopia including the study area, poor glycemic control, shortage of drugs and insulin, poor treatment response for hyperglycemia, lack of diabetes team care, lack of awareness of the disease leads to high burden of diabetes-related complications, for instance diabetic kidney disease(47). Retrospective observational studies showed that prevalence of nephropathy in diabetic patients was 6.1%(48),15.7%((49),Proteinurea 13.8% %(50) and microalbuminuria in Type1DM 32% and Type 2DM 37%(51) respectively. But as to the knowledge of the principal investigator, there is paucity of data about the prevalence of renal impairment (nephropathy) and associated factors compared to apparently healthy individuals in the country, specifically in the study area. For that reason, this study was undertaken to fill this gap in the literature. The present study is expected to assess renal impairment and associated factors among diabetic patients attending at Jimma University Medical Center, South West Ethiopia.

1.3 Significance of the study

Diabetes mellitus is one of the primary risk factors for developing renal impairment globally. However, few studies have described the burden of renal impairment among adults with diabetes in South West Ethiopia, particularly in Jimma. Therefore, in this study we aimed to assess renal impairment and associated factors among people with diabetes (both type1and 2) attending our chronic illness clinic in JUMC as compared to apparently healthy individuals. The findings of this study may be used as baseline data for other researchers who have interest to conduct similar study and may be used for national policy makers to develop intervention strategies to reduce morbidity and mortality of diabetic patients from diabetic associated kidney disease and to create awareness with regard to the recent burden of renal impairment among people with diabetes.

CHAPTERTWO:-LITERATURE REVIEW

At present, an estimated 415 million adults are living with diabetes worldwide, aged between 20 and 79 years old, the global prevalence being of 8.8% and by 2040 this number is expected be raised to 642 million, with a prevalence of 10.4%(52).

In 2013 IDF Diabetes Atlas projected that the prevalence of diabetes with economic development is 4.4% in low-income, 5.0% in lower-middle income and 7.0% in upper-middle income countries of Africa region(53).

With the global epidemic of diabetes, diabetic nephropathy has become an important clinical and public health challenge associated with type 1 and type 2 diabetes worldwide, particularly in low and middle-income countries(54).

Diseases of the kidney are a common finding in people with diabetes, with up to half demonstrating signs of kidney damage in their lifetime(55,56). Diabetic nephropathy is the commonest cause of chronic renal failure globally and in the Sub-Saharan Africa and causes renal failure in one third of patients who require dialysis(57,58).

A cross-sectional national medical chart audit study was conducted on the prevalence, determinants and co-morbidities of Chronic Kidney Disease among First Nations adults with diabetes in the year 2012. A total number of 885 FN adults (18 years and older) with type 2 diabetes who lived in First Nations communities across seven provinces in Canada were included. The result of the study showed that, the overall prevalence of CKD-eGFR <60 mL/min/1.73 m^2 was 15.5% calculated by MDRD equation. The independent determinants of CKD-eGFR <60 were increasing age at diabetes diagnosis, diabetes duration and systolic BP(59).

On the other hand, an observational study was conducted on 7596 adults known to have diabetes in primary and secondary care in Salford, Greater Manchester, UK in January 2004. The study revealed that the prevalence of CKD was 27.5% .Moreover; an increased risk of CKD-eGFR MDRD was seen with female sex, older age and long duration of diabetes. But the type of diabetes was not associated with an increased incidence of CKD-eGFR(60).

Similarly, a cross-sectional survey was conducted on a total of 10740 enlisted diabetes and hypertension patients, aged ≥25 years at two Dutch primary health care centers, The Netherlands in October 2006. The study findings indicated that the prevalence of CKD-eGFR calculated by MDRD in people with diabetes was 28 %. On the other hand, decreased eGFR-MDRD was associated with older age and female sex(61).

In addition to this, a population-based cross sectional study was conducted to assess the prevalence of CKD and, and to evaluate any relationship between age, gender, diabetes, obesity, hypertension, and CKD in the Black Sea Region, Turkey. A total of 1,079 persons, aged 18-95 years were selected in this study by stratified sampling technique. The study showed that, the prevalence of CKD by MDRD in the general population, diabetics and nondiabetics was 5.75%, 8.77% and 5.58% respectively. On the other hand, older age, female gender, obesity and hypertension were found to be significant risk factors development of CKD in the study population(62).

Similarly, an institution based cross-sectional study was conducted on a total of 6,045 high-risk adult population in aged ≥ 30 years with hypertension or diabetes who visited health centers as well as primary or secondary private institutions from 2007 in five cities of Korea. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. The study showed that prevalence of reduced renal function, albuminuria, and CKD in the study population were 24.6%, 22.6%, and 39.6 % respectively. Older age, hypertension, higher body mass index, higher systolic and diastolic blood pressure were independently associated with the presence of CKD ,whereas the prevalence of reduced renal function was not significant difference between women and men (25.0% vs. 24.3%, p = 0.552) (63).

Another cross-sectional study conducted on a total of 4,898 persons in Wisconsin, Singapore found that, the prevalence of CKD-eGFR MDRD was 51.8%. Age, male gender, obese persons, use of NSAIDs, presence of hypertension and history of cardiovascular disease were associated with CKD. In addition lower education, current and previous smoking was associated with CKD. On the other hand, current alcohol consumption was not significantly associated with CKD(64).

In addition to this, a cross-sectional screening programme was conducted in Dharan, Nepal on a total of 1330 diabetic and 16623 non diabetic populations, aged ≥ 18 years old. The study

showed that, the prevalence of reduced eGFR in diabetic and non diabetic participants calculated by MDRD equation was 32% and 18% respectively(65).

Similarly, a cross-sectional study was conducted on a total of 6,387 adults, aged 20 to 79 years and residing in the Pudong New Area of Shanghai, China. Study participants were randomly selected through a three-stage sampling process between April and July 2008. The study showed that MDRD Study equation based CKD in known diabetes was 25.8%.Factors associated with CKD were hypertension, female gender, higher BMI and older age while decreased GFR-MDRD was associated with increased age, male sex, and hypertension(66).

Another cross-sectional survey was carried out on a total of 1000 participants, aged from 15 to 65 years selected by a simple random sampling at Dhaka city in Bangladesh over the period from July 2003 to June 2005. The survey revealed that based on MDRD equation, 13.1% of the participants were detected as having CKD) while with Cockcroft-Gault equation 16% had CKD. Increased age and being married had significant association with developing CKD. Conversely, being female sex, housewives and illiterate, and having low income was not associated with CKD (67).

A retrospective analytical study was conducted by reviewing the clinical records of the patients with type 2 diabetes who attended the National Diabetes Centre of Sri Lanka from January 2005 to December 2010. A total of 12517 type 2 diabetic patients aged 20 years or above were included in the study. The study showed that nephropathy was significantly associated with high fasting blood glucose, high systolic blood pressure and diastolic blood pressure and high body mass index(68)

A cross-sectional and a hospital based case control study were conducted on T2 DM patients in Oman, in order to assess the prevalence and the risk factors for diabetic nephropathy respectively. For the prevalence study, 699 diabetic subjects were selected randomly. For the case control study, 215 cases and 358 controls were randomly selected from those who were included in the cross-sectional study. The study findings showed that the overall prevalence of diabetic nephropathy was 42.5%. Male gender , decreased literacy, long duration of diabetes, family history of diabetic nephropathy and poor glycaemic control found to be significantly associated with CKD(69).

An observational cohort study was conducted on a total of 11,409 participants aged between 40 to 75 years randomly selected from Golestan Province of North East of Iran from 2004 to 2008. The study showed that prevalence of CKD-eGFR calculated by MDRD formula was 23.7%. Older age, female sex, urban residence, hypertension and larger BMI were all associated with CKD(70).

On the other hand, an institution based cross-sectional study was conducted to determine the prevalence of chronic kidney disease among adults with diabetes mellitus attended at a public primary health care clinic in southern Chile. One hundred patients with type 2 diabetes mellitus, aged more than 15 years participated in this cross sectional study. The study showed that, the overall prevalence of CKD-MDRD was 34 %.Female sex, insulin treatment, current and previous smoking were not significantly associated with eGFR-MDRD < 60ml/min/1.73 m^2(71).

An analytical cross-sectional study was conducted on 344 diabetic patients, aged ≥18years who had attended an outpatient clinic at Parirenyatwa Hospital, Harare, Zimbabwe, between October 2013 and July 2014.The prevalence of decreased renal function in diabetic patients was 42.9% , while 28.6% in T1 and 43.5% in T2 DM patients respectively(72).

In addition to this, a population-based survey was conducted on a total of 1037 adults (≥ 18 years) who resided in Saint-Louis, Senegal from January to May 2012.The Survey results showed that the overall prevalence of CKD estimated by MDRD equation was 4.9%(73).

Another community-based survey conducted on 1095 randomly selected Tanzanian adults (≥18 years) between May 2012 and April 2013 showed that the prevalence of reduced eGFR calculated by MDRD was 5.1%, 11.0% and 9.4% in Mwanza city, district towns and rural areas, respectively. The study also indicated that decreased renal function was associated with district town residence, older age, lower income and hypertension (74).

An institution based cross-sectional study was conducted on a total of 4815 participants (421 cases and 4394 controls) ,aged≥18 years from university medical centers in Ibadan, Enugu, and Lagos in Nigeria, Accra, and Kumasi in Ghana and Eldoret in Kenya as part of a genetic epidemiology study of T2D in sub-Saharan Africans. According to the result of the study, 13.4% impaired kidney function was found in T2diabetes compared to 4.8% of impaired kidney function in individuals without T2 diabetes. This race and gender-specific eGFR was calculated

by using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations(75).

In addition to this, a cross-sectional study was conducted on a total of two hundred eight diabetic, hypertensive and both diabetic and hypertensive patients (aged ≥18 years) enrolled consecutively at the outpatient diabetes and hypertension clinics of the Government hospitals in South Western Ghana. The study revealed that the prevalence of CKD by CKD-EPI and urine albumin-creatinine ratio was 27 % in patients with diabetes, 22 % in patients with hypertension only and 74 % in patients with both diabetes and hypertension. High systolic and diastolic blood pressure was associated with CKD(76).

Moreover, a pilot cross-sectional community-based survey was conducted on 273 individuals at police housing complexes in Greater Khartoum, Sudan. Out of 436 individuals approached, 426 responded to respective questionnaire and had their anthropometric measures and blood pressure recorded. However, because of lack of some subjects' consent and .The study revealed that, the overall prevalence of CKD was 11% using the standardized Cockcroft-Gault equation and 7.7% using the four variable MDRD equations. Moreover, older age and low educational level were significantly associated with CKD(77).

A cross sectional descriptive study was conducted on 100 known diabetic patients whom are on regular follow up at diabetic clinic of Halibet and Hazhaz National Referral Hospital in Asmara, Eritrea from Oct 2014 to Jan 2015.The study showed that 75% of the patients were in stage 1 CKD with normal or elevated GFR, and 21% of the patient were in stage 2 CKD with mild GFR reduction whereas only 4% of the patients were in stage 3 with moderate decreased GFR, and no patients in stage 4 with severe GFR reduction using (CKD-EPI 2009) equation(78).

A facility based across sectional study was conducted on 214 diabetic patients in Butajira hospital of Southern Ethiopia over a period of two months (September 2013 to October 2013). The result of the study indicated that 18.2 and 23.8 % of the study participants were found to have reduced renal function, according to the MDRD and Cockcroft-Gault equations, respectively. Moreover, reduced renal function was found in 18.2 and 23.8% of participants with normal serum creatinine using the MDRD and C-G equations, respectively. The result of the study also indicated that the major risk factors for the development of CKD were older age,

longer duration of diabetes and family history of kidney disease, poor glucose control and obesity(79).

Comparisons of renal function parameters and renal impairment associated factors in diabetic and non-diabetic subjects

A cross-sectional study conducted on 121 consecutive patients with diabetes referred for coronary angiography due to coronary heart disease to the Department of Invasive Cardiology of the Medical University Hospital in Bialystok, Poland in 2008,and a reference group consisting of 64 patients without diabetes also referred for coronary angiography showed that, body mass index, serum creatinine concentration were significant higher in diabetes compared to non-diabetes($p<0.001$ for each) while systolic and diastolic blood pressure were not significant between two groups($p>0.05$)(80).

The Korea National Health and Nutrition Examination Survey (KNHANES) V conducted a cross sectional, nationwide survey on a total of 3,992 non diabetics and 660 with diabetic subjects aged >30 years in 2011. The study showed that age, male sex, BMI, SBP, FBG,BUN, creatinine and eGFR were statistically significant($p<0.05$) in diabetic subjects compared to apparently healthy subjects(81).

An institution based cross sectional study was conducted on Type1 and Type 2 DM patients who were attending the diabetic clinic and non-diabetic subjects who were selected from the general population as a control group in India found that, there was statistically significant increase in serum urea and creatinine levels in both Type 1 and Type 2 diabetic subjects compared to non-diabetic subjects (82).

A hospital based cross- sectional study carried out in Nepal showed that the level of blood urea ($P<0.0001$, 95%Cl) and serum creatinine ($P\approx0.0004$, 95%Cl) were significantly higher in type 2 diabetics as compared to non-diabetics in both males and females. In addition to this, there was statistical significant increase in urea level with increased in blood sugar levels(83).

Conversely, an institution based cross- sectional study conducted in Nepal ,showed that significant correlation between urea and blood sugar levels($p <0.05$),whereas there was no

significant correlation between blood sugar level and serum creatinine levels($p > 0.05$) in diabetic subjects(84).

A cross-sectional study was conducted on diabetic patients and age and sex matched apparently healthy controls in Bangladesh. The findings indicated that, fasting blood sugar showed very high correlations with urea ($p=0.036$) and creatinine ($p=0.05$) than non diabetic subjects ($p>0.05$)(85).

A cross-sectional descriptive and analytical study was conducted on a total of 283 type 2 diabetes patients in Brazil showed that diabetic patients with reduced renal function were older, had long-term disease duration, higher systolic blood pressure and higher levels of fasting glucose, compared to diabetics with normal renal function(86).

An epidemiological study was conducted on a random population sample of 1016 individuals, aged $\geq$15 years to investigate BP, renal disease and other risk factors in the city of San Andres de Giles, Argentina between September and December 2007. The study revealed that the eGFR-MDRD decreased with age. Additionally, eGFR-MDRD correlates inversely with age, SBP, DBP, BMI, fasting serum glucose, whereas the reciprocal of serum creatinine correlates inversely with age, SBP, DBP in both sexes(87).

Another study conducted in Nigeria, showed that in addition to elevated blood sugar level in Type 2 diabetes mellitus, plasma creatinine and urea concentration are also significantly increased($p<0.05$) in male and female diabetics compared with their levels in apparently non-diabetic male and female controls(88).

A cross- sectional study conducted in Ghana on diabetic patients and age-matched non-diabetics individuals showed that there was a weak positive correlation between age of the diabetics with their plasma urea and creatinine levels ($r<0.5$, $p=0.018$),($r=0.375$, $p= 0.029$) respectively. There was also a strong positive correlation between their Fasting Blood Sugar (FBS) and the plasma levels of the measured urea and creatinine($r >0.5$, $p<0.05$) in diabetic patients(89).

A quantitative, descriptive, analytic, cross- sectional and hospital-based study was conducted on a total of 100 patients with type 2 diabetes mellitus and fifty age and gender matched healthy controls at Jabir-Abulizz Khartoum, Sudan Diabetes Centre during the period between February

and May 2012. The study showed that urea and creatinine were significantly elevated with significant positive correlation with each of body mass indexes, duration of diabetes, and fasting blood sugar(90).

Thus, this study is aimed at assessing renal impairment and associated factors among people with diabetes as compared to apparently healthy individuals.

Conceptual frame work

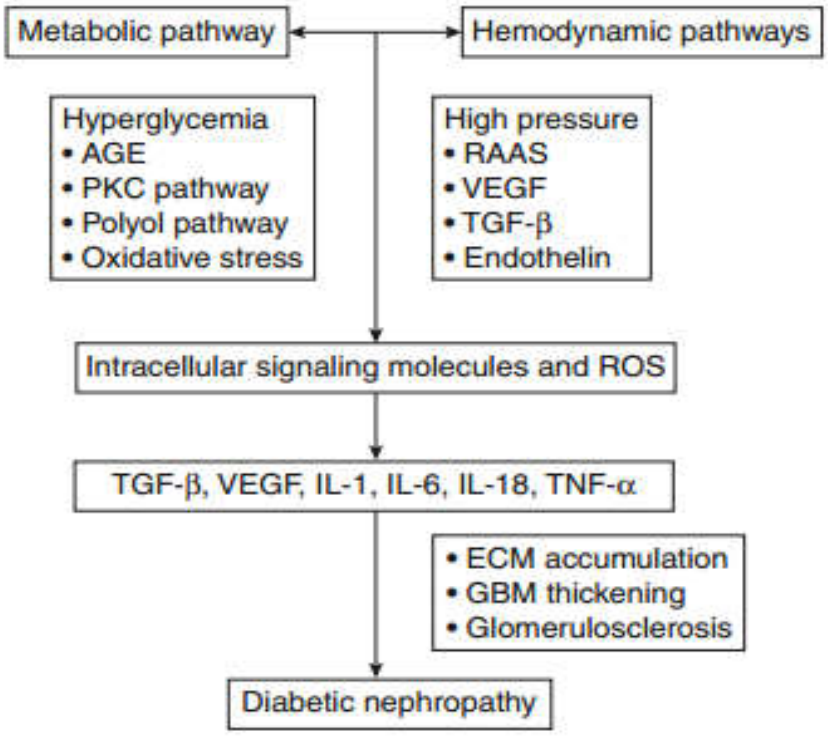

Figure 1. Biochemical Pathways involved in the development of diabetic kidney disease.

Adopted from: Vinod, Clin Queries Nephrol; 0102 (2012) 121–126.

CHAPTER THREE:-OBJECTIVE

3.1 General Objective

- To assess renal impairment and associated factors among diabetic patients and apparently healthy individuals at Jimma University Medical Center, South West Ethiopia.

3.2 Specific Objectives

1. To assess the prevalence of reduced renal function in patients with diabetes and apparently healthy individuals.
2. To compare clinical characteristics, serum levels of FBS, creatinine, urea and eGFR in diabetic patients in reference to apparently healthy individuals.
3. To assess the association between renal impairment and associated factors in people with diabetes.

CHAPTER FOUR: - MATERIALS AND METHODS

4.1 Study area

This study was conducted at chronic illness clinic of Jimma University Medical Center (JUMC) from January 9 to March 22, 2017. JUMC is found in Jimma city 352 km to South West of Addis Ababa, the capital city of Ethiopia.

JUMC is one of the oldest public hospitals in the country. It was established in 1930 E.C by Italian invaders for the service of their soldiers. Currently it is the only teaching and referral hospital in the Southwest part of the country, providing service to over 15 million inhabitants in South-Western part of Oromia, part of Southern People Nations and Nationalities, as well as Gambella region(91). The hospital has a total of 2500 registered DM patients on follow up at the chronic illness clinic. Currently the hospital has total beds of 523, more than 600 health professionals and 500 supportive staffs giving service to the community.

Chronic illness clinic is one of the components of JUMC which DM patients have been appointed twice weekly (Monday and Tuesday). Different professionals such as internists, medical residents, general practitioners, medical interns and 10 staff nurses (7BSc & 5 diploma clinical nurses) are giving service in this clinic.

4.2 Study design and period

A hospital based cross -sectional comparative study was conducted from January 9 to March 22, 2017.

4.3 Source population

- All DM patients who were attending chronic illness clinic of JUMC were used as cases.
- All healthy subjects who were giving service in JUMC and healthy patient attendants.

4.4 Study population

- All adult DM patients who were registered and attending JUMC chronic illness clinic during the specified study period.

- All apparently healthy JUMC employees and patient attendants who were visiting the outpatient department.

4.5 Eligibility criteria

4.5.1 Inclusion criteria

- Adult diabetic patients who were attending chronic illness clinic of JUMC for their diabetic follow up at the time of data collection and who were fulfilling the inclusion criteria and willing to participate in the study were included.
- Apparently healthy individuals who were working in JUMC and patient attendants who were visiting the outpatient department of Jimma University Medical center at the time of data collection period.

4.5.2 Exclusion criteria

Cases

Pregnant women, taking nephrotoxic drugs, DM patients who were admitted and critically ill were excluded from the study.

Controls

Pregnant women, those who have previous history of chronic diseases, two consecutive fasting blood glucose values ≥126mg/dl and individuals known to have diabetes mellitus were excluded from the study.

4.6 Sample size determination and sampling technique

4.6.1 Sample size determination

$$n = \left(1+\frac{1}{r}\right)\left(\frac{Z\alpha}{2}+Z\beta\right)^2 \frac{\sigma^2}{\left(\bar{X}1-\bar{X}2\right)^2}$$

This two population means formula was used to calculate the sample size using OpenEpi, version 2, open source calculator by considering the following assumptions:

n=sample size in each group (assume equal sized groups)

r=the ratio of cases to controls (1:1)

$Z\alpha/2$=desired level of statistical significance (two-tailed=1.96)

Z_β=desired power (typically 0.84 for 80%power)

σ=common standard deviation (pooled standard deviation)

$\bar{X}1-\bar{X}2$= the difference in means of the two groups

Taking the mean and standard deviation (SD) of eGFR from a study conducted in south Africa in T2DM patients(92).For DM patients, the mean and standard deviation of eGFR were 101.6 and 22.4 respectively ; for control group, the mean and standard deviation were 95.7 and 23.1 respectively. The sample size was determined to be 234 for each group.

4.6.2 Sampling technique

Consecutive sampling technique was used to include 234 diabetic patients who had been visiting the chronic illness clinic during the study period and 234 apparently healthy JUMC employees and patient attendants who had been visiting the outpatient department.

4.7 Study variables

4.7.1 Dependent variable

- Renal impairment

4.7.2 Independent variables

- Age
- Sex
- Place of residence
- Marital status
- Body mass index
- Educational status
- Monthly income

- Alcohol consumption
- Cigarette smoking status
- Type of anti-diabetic medication
- Duration after being diagnosed to be of diabetes
- Type of DM
- Blood pressure
- Fasting blood glucose
- Traditional medicine
- Analgesics

4.8 Data collection instruments

Table 1.Data collection instruments used.

Items	**Description**
Mindray BS-200E automated clinical chemistry analyzer(Shenzhen Mindray Bio-Medical Electronics Co., Germany	To analyze biochemical tests
Glucose liquicolor kit(Human)	Glucose reagent for serum glucose determination
Creatinine liquicolor kit(human)	Creatinine reagent for serum creatinine determination
Urea liquiUV kit(Human)	Urea reagent for serum urea determination
Autocal(Human)	A calibrator for calibrating the clinical chemistry analyzer
Controls (Both Humatrol N and Humatrol P)	Normal and pathological controls for checking the analyzer before processing the test samples
Plastic bulb pipette	For pipetting serum after centrifuged the sample
Serum separator tube (plain tube)	Used to blood sample collection for clotting
Eppendorf tubes	For preservation of serum samples
Cuvette	For sample and reagent mixing, incubation and absorbance reading in the analyzer
Questionnaires	For socio-demographic and clinical data

	collection
Blood pressure apparatus	For measuring blood pressure of the respondents
Bench top centrifuge	For separating serum from whole blood
70% alcohol, disposable syringe with needle, cotton, tourniquet, examination glove and	For venous blood collection from each study participant
Seca weight floor scale	Used for measuring weight
Seca stadiometer	Used for measuring height
Safety box	For disposing used syringes and gloves
Sample rack	For transporting & placing of samples

4.9 Data collection techniques

Diabetes mellitus patients and apparently healthy individuals were interviewed to obtain socio-demographic data, risk factor variables by using structured questionnaires and anthropometric measurements by trained clinical nurses. In addition, medical records /charts of DM participants were reviewed to obtain clinical data such as duration of diabetes, type of diabetes, treatment modifications and type of hypoglycemic medications.

Blood pressure measurement:-Blood pressure (BP) was measured using an aneroid sphygmomanometer (Henry Schein inc. Melville, NY, USA) and a stethoscope from the left upper arm. The study participant seated comfortably and on a wakeful state in sitting position, with back supported, legs uncrossed, the feet on the ground and upper arm was bared and positioned at the heart level. After 5 minutes of rest, two consecutive blood pressure readings were taken at an interval of at least one minute and the average taken as mean BP(93).

Hypertension diagnosis for adult DM patient was made when the average of the two blood pressure measurements of systolic pressure ≥140 mm Hg and /or diastolic blood pressure ≥ 90 mm Hg in accordance with the World Health Organization (WHO) hypertension diagnostic criteria(94).

Anthropometric measurement:-Height was measured using a seca stadiometer (GMPH &CO.KG, model 813, Germany) which was placed on a hard, flat and uncarpeted surface. During the height measurement, the study participant's shoes and any hats or hair ornaments

were removed. The subject faced away from the stadiometer with the heels together and the back as straight as possible. With the subject looking straight ahead, the head projection was placed at the crown of the head and with the reader's eye at the level of the head piece .Then the height measurement was recorded(95).

For the measurement of weight, the participant asked to remove his/her shoes and any bulky clothing and stepped up backwards onto a Seca weight floor scale(GMPH &CO.KG, model813, Germany).The participant's arms should be hanging freely by the sides of the body, with palms facing the thighs. The participant should hold his/her head up, and face forward. Then weight was recorded(96).

Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meter squared (kg/m^2).The WHO definition of obesity is based on various categorical cut-points based on the body mass index (BMI) of weight-for-height: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity (≥30 kg/m^2)(97).

4.10 Blood sample collection and laboratory investigation

After obtaining a written consent from all subjects who were included in the study and by giving detail information about the study, instruct the study subject about the procedure and the site was selected preferably at the antecubital area. Warming the arm with hand or hanging the hand down made it easier to see the veins. The area was palpated to locate the anatomic landmarks. Applied a tourniquet, about 4–5 finger widths above the selected venepuncture site. The site was disinfected using 70% alcohol swaps for 30 seconds in a circular motion from inside to outside fashion and allowed to dry completely for 30 seconds. Then, the needle was entered swiftly at a 30 degree angle.

About 5 ml of venous blood was collected aseptically from the antecubital vein from each study participant by trained nurses in the morning after ≥8hrs of overnight fast. The blood sample was dispensed into jell coated serum separator test tube or plain tube labelled with unique ID number. The collected blood sample was left for 30 minutes to facilitate clotting at room temperature. Then the clotted blood samples were centrifuged for 10minutes at 2000 revolution per minutes (rpm) to separate serum from formed elements. The serum glucose was analyzed within the same

day of sample collection. However, the serum was kept in refrigerator at -20^{o}c by Eppendorf tubes still the time of analysis greater than one week.

4.11 Methods of Biochemical Analysis

Biochemical tests such as fasting blood sugar; serum creatinine and urea levels were analyzed by using Mindray BS-200E automated clinical chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Germany) according to the manufacturer's instructions and procedures in JUMC laboratory. The instrument Mindray BS-200E chemistry analyzer was calibrated using Autocal and quality control samples both normal (Humatrol N) and pathological (Humatrol P) were run each day before running samples for testing.

Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine, age, sex and weight of the study participants by using the Cockcroft-Gault prediction formula with adjustment of black ethnicity. Patients were then classified according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiatives (NKF KDOQI) CKD classification(31).

Determination of fasting blood glucose

Serum glucose was measured by GOD-PAP enzymatic method with deproteinization on a fully automated Mindray BS-200E clinical chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Germany). Glucose present in the serum is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured colorimetrically at 500nm.

The American Diabetic Association recommends a premeal fasting blood glucose target of 80–130 mg/dL for diabetes patients on diabetic follow up clinic(98).

Determination of Creatinine by Jaffe Reaction

Serum creatinine level was determined using a Jaffe reaction without deproteinization on a fully automated Mindray BS-200E clinical chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Germany). Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine

concentration in the sample. The result was reported in mg/dl and interpreted based on the manufacture's reference range.

Determination of UREA by GLDH Method

Serum urea level was determined by enzymatic kinetic method on a fully automated Mindray BS-200E clinical chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Germany).Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia from this reaction combines with 2-oxaloglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD^+. There has been optimized so that the GLDH is the rate limiting enzyme. The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the urea concentration. The result was reported in mg/dl and interpreted based on the manufacture's reference range.

Determination of eCrCl by Cockcroft-Gault and MDRD equation

The CG equation in adults is popular because it is easy to calculate estimated glomerular filtration rate from serum creatinine. The equation is named after the scientists who first published the formula was Cockcroft and Gault(99).

Calculation: eGFR= [(140 age) × weight in kg]/72× serum creatinine × (0.85 if female); where GFR = Glomerular Filtration Rate in mL/min, Age is in years, SCr is serum creatinine in mg/dL.

Where: eGFR (ml/min/1.73m^2)=$\frac{(140-age)\times(Wt)\times 0.85(if\ female)\times 1.73}{(serum\ creatinine\times 72\times BSA)}$

BSA (m^2) is calculated by Mosteller formula(100):$\sqrt{\frac{Ht(cm)\times Wt(kg)}{3600}}$

The most recently advocated formula for calculating the GFR is the one that was developed as a result of the modification of diet in renal disease study (MDRD)(101), which is more accurate to estimate impaired renal function in people with diabetes and in general population than C-G equation(102).

Calculation: eGFR=186 × (SCr) $^{1.154}$ × (Age) $^{0.203}$ × (0.742 if female) × (1.212 if black). Where GFR = Glomerular Filtration Rate in mL/min 1.73 m2, Age is in years, SCr is serum creatinine in mg/dL.

4.11 Data quality assurance and management

The data quality starts with training of data and sample collectors. After completion of each questionnaire, cross checking was done to assure the completeness of the information gathered. The label on the test tube and subject's unique identification number on questionnaire were checked for similarity.

The blood sample was taken in aseptic techniques and the quality of test result was tried to be maintained strictly starting from the pre-analytic phase of blood collection up to post-analytical phase of result interpretation adhered with clinical chemistry standard operation procedures (SOP).

After checking the expiry date of both the reagents and controls, Mindray BS-200E chemistry analyzer was checked for delivering correct result by using normal and pathological controls. Normal control which is low levels of known concentration and high (pathological) control which is high levels of known concentrations for measured parameters were used. Before any test sample processed, dual quality controls (normal and pathological) were performed and the test result was taken after the controls passed. All necessary procedures and steps were followed based on the clinical chemistry SOP. Laboratory results were rechecked repeatedly for completeness on daily basis by the principal investigator.

4.12 Statistical analysis

All questionnaires were checked daily for completeness by the investigator and pre-coded data were entered into computer using EpiData version 3.1, and then data were transferred to SPSS (Statistical Package for Social Science) version 20 software (IBM Corporation, Armonk, NY, USA) for further data cleaning so that to allow consistence and eliminate discrepancies, categorizing of continuous variable and finally analysis.

The data were tested for normality with the help of histograms and Kolmogorov–Smirnov tests. All continuous variables with non-normally distributed expressed as a median (interquartile range),and frequencies or percentages for categorical variables .The significance median differences between diabetes and controls were determined by Mann-Whitney U test for skewed distributions for continuous variables. Chi-squared test was utilized for comparing categorical variables.

Additionally, spearman's coefficient correlation was performed to determine the correlation between quantitative variables. Multivariate logistic regression analysis was calculated to identify risk factors associated with reduced renal function. All Variables with a p-value of more than 0.25 in the bivariate analysis were excluded in multivariate logistic regression. Fitness of goodness of the final model will be checked by Hosmer and Lemeshow. Results were presented as the odds ratio (OR) and 95% confidence interval (CI). Finally, a p-value < 0.05 was considered statistically significant.

4.13 Ethical consideration

Ethical clearance was obtained from Ethical Institutional review board (IRB) of Jimma University; Institute of Health Science. After Ethical Clearance received, permission to conduct the research was obtained from the clinical director of JUMC and the head of the chronic illness clinic of JUMC. Information sheet was prepared and read to all eligible participants of the study. All participants were informed the purpose of the study and their participation was on voluntary basis. Verbal informed consent was received from all participants. Name of the participant was omitted from the questionnaire; instead we used ID number to ensure confidentiality. Any abnormal findings were communicated with the physician for better management of patients.

4.14 Dissemination of the result

The result of this study will be presented to Jimma University, Institute of Health Science, School of Medical Laboratory Science, as partial fulfillment of the requirement of master's degree in clinical chemistry. Furthermore the result will be shared with department of internal medicine of JUMC and also the manuscript of the research will be prepared and submitted to appropriate scientific journals for possible publication.

CHAPTER FIVE: - RESULT

5.1 Socio demographic characteristics of the study participants

A total of 468 (234 diabetic patients and 234 controls) were included in this study. Of the total diabetic patients, 130(55.6%) were males while 104(44.4%) were females.

The age ranged from 18 -83 years and the median (IQR) age was 50(40-60) years. One hundred seventeen (50.0%) of diabetic participants were age below 50 years and 117(50.0%) were age 50years and above, the majority 159(67.9%) from urban areas, 171(73.1%) married, 71(30.3%) no formal education, 143(61.1%) Oromo by ethnic group, 138(59.0%) Muslims by religion, 84(35.9%) Government/private employed and, 92(39.3%) had monthly income below 500ETB (Table-2).

Similarly, of the total 234 control groups, 126(53.8%) were males while 108(46.2%) were females. The age ranged from 18 - 70 years and the median (IQR) age was 32(27-44). The majority of participants 211(90.2%) were age less than 50 years, 141(60.3%) urban dwellers, 140(59.8%) married, 65(27.8%) had high level of education (College/University), 134(57.3%) Oromo by ethnic group, 120(51.3%) were Muslims by religion, 131(56.0%) government/private employed and, 63.9% participants had monthly income below 1500ETB (Table-2).

Table 2. Socio-demographic characteristics of all study participants at JUMC, south West Ethiopia, from January 9 to March 22, 2017.

Variables	Type of study participants		
	All participants No. (%)	**Diabetes No (%)**	**Controls No (%)**
Total	468(100%)	234(50%)	234(50%)
Sex			
Male	256(54.7)	130(55.6)	126(53.8)
Female	212(45.3)	104(44.4)	108(46.2)
Age in years			
<50	392(83.8)	117(50.0)	211(90.2)
≥50	76(16.2)	117 (50.0)	23(9.8)
Place of residence			
Urban	300(64.1)	159(67.9)	141(60.3)
Rural	168(35.9)	75(32.1)	93(39.7)
Marital status			
Single	120(25.6)	37(15.8)	83(35.5)
Married	311(66.5)	171(73.1)	140(59.8)
Divorced	15(3.2)	12(5.1)	3(1.3)
Widowed	22(4.7)	14(6.0)	8(3.4)
Educational level			
No formal education	130(27.8)	71(30.3)	59(25.2)
Primary School	124(26.5)	70(29.9)	54(23.1)
Secondary School	114(24.4)	58(24.8)	56(23.9)
College/University	100(21.4)	35(15.0)	65(27.8)
Ethnicity			

Oromo	277(59.2)	143(61.1)	134(57.3)
Amhara	94(20.1)	46(19.7)	48(20.5)
Kefa	28(6.0)	10(4.3)	18(7.7)
Gurage	28(6.0)	11(4.7)	17(7.3)
Tigrie	11(2.4)	4(1.7)	7(3.0)
Others	30(6.4)	20(8.5)	10(4.3)
Religion			
Muslim	258(55.1)	138(59.0)	120(51.3)
Orthodox	161(34.4)	77(32.9)	84(35.9)
Protestant	46(9.8)	18(7.7)	28(12.0)
Others	3(0.6)	1(0.4)	2(0.8)
Monthly income(ETB)			
<500	158(30.1)	92(39.3)	66(28.2)
≥500	310(59.0)	142(60.7)	168(71.8)
Occupational status			
Unemployed	46(9.8)	31(13.2)	15(6.4)
Government/Private	215(45.9)	84(35.9)	131(56.0)
Farmer	135(28.8)	59(25.2)	76(32.5)
Pensioner	19(4.1)	18(7.7)	1(0.4)
House Maker	53(11.3)	42(17.9)	11(4.7)

Regarding life style factors of diabetic study participants, majority 232(99.1%) had never smoked currently, only 2(0.9%) were current smokers at the time of data collection. 27(11.5%)

were smokers previously while 207(88.5%) were not. Twenty one (9.0%) of the respondents used to consume alcohol before data collection period while 213(91.0%) did not. On the other hand, 22(9.4%) of diabetic participants had habits of using analgesics while 212(90.6%) did not. Concerning traditional medicine, only 17(7.3%) of diabetes used traditional medicine for a long period of time (Table -3).

Regarding lifestyle factors in apparently healthy controls, majority of participants were found to have never smoked 222(94.9%) currently and 232(99.1%) previously, 219(93.6%) never used to consume alcohol. On the otherhand, nine (3.8%) of apparently healthy participants had habits of using analgesics while 225(96.2 %) did not. Only two (0.9%) used traditional medicines for a long period of time, while 232(99.1%) did not (Table-3).

Table 3.Life style factors of all study participants at JUMC, South West Ethiopia, from January 1 to March 22, 2017.

Characteristics	Type of study participants		
	All participants No (%)	Diabetes No (%)	Controls No (%)
Total	468(100)	234(50)	234(50)
Current smoker			
Yes	14(3.0)	2(0.9)	12(5.1)
No	454(97.0)	232(99.1)	222(94.9)
Previous smoker			
Yes	29(6.2)	27(11.5)	2(0.9)
No	439(93.8)	207(88.5)	232(99.1)
Alcohol consumption			
Yes	36(7.7)	21(9.0)	15(6.4)
No	432(92.3)	213(91.0)	219(93.6)
Analgesics users			
Yes	31(6.6)	22(9.4)	9(3.8)
No	437(93.4)	212(90.6)	225(96.2)
Use of traditional medicines			
Yes	19(4.1)	17(7.3)	2(0.9)
No	449(95.9)	217(92.7)	232(99.1)

5.2 Clinical characteristics of the study participants

Among diabetic participants, the majority 62.4% were T2DM patients and the rest of 37.6 % were T1DM patients. For the majority people with diabetes, 74.4% had poor blood glucose

control, 42.7% their duration of disease <5 years and 45.7% of them treated by oral diabetic medications (Table-4).

Moreover,49(20.9%) of diabetic patients had known hypertension, about 24.8% had systolic blood pressure ≥140mmHg, while 23.9% had diastolic blood pressure ≥90mmHg. Body mass index study categorized, 50.4% as normal, 10.3% as underweight, 32.1%) as overweight, and 7.3% as obese (Table-4).

On the other hand, the majority of healthy individual participants (95.7%), and (92.3%) were their systolic and diastolic blood pressure <140mmHg and <90mmHg respectively. 162(69.2%) of controls had normal BMI, and the rest 15.8%, 3.0 % and 12.0% were overweight, obese and underweight respectively(Table-4).

Table 4.Clinical characteristics of all the study participants at JUMC, South West Ethiopia, from January 9 to March 22, 2017.

Characteristics	**Type of study participants**		
	All participants No (%)	**Diabetes No (%)**	**Controls No (%)**
Total	468(100)	234(50)	234(50)
Type of diabetes			
Type 1	88(37.6)	88(37.6)	-
Type 2	146(62.4)	146(62.4)	-
FBS levels			
High	174(74.4)	174(74.4)	-
Normal	60(25.6)	60(25.6)	-
Duration of diabetes (years)			
<5	100(42.7)	100(42.7)	-
5-10	71(30.3)	71(30.3)	-

11-20	57(24.4)	57(24.4)	-
>20	6(2.6)	6(2.6)	
Types of Hypoglycemic agents			
Oral	107(45.7)	107(45.7)	-
Insulin	96(41.0)	96(41.0)	-
Oral and insulin	31(13.2)	31(13.2)	-
Known hypertension			
No	185(79.1)	185(79.1)	-
Yes	49(20.9)	49(20.9)	-
Systolic blood pressure(mmHg)			
Normal(<140)	400(85.5)	176(75.2)	224(95.7)
Abnormal(≥140)	68(14.5)	58(24.8)	10(4.3)
Diastolic blood pressure(mmHg			
Normal(<90)	394(84.2)	178(76.1)	216(92.3)
Abnormal(≥90)	74(15.8)	56(23.9)	18(7.7)
BMI(kg/m^2)			
Underweight	52(11.1)	24(10.3)	28(12.0)
Normal	280(59.8)	118(50.4)	162(69.2)
Overweight	112(23.9)	75(32.1)	37(15.8)
Obese	24(5.1)	17(7.3)	7(3.0)

The median and IQR of fasting blood sugar, serum creatinine, serum urea and estimated creatinine clearance(CG) in diabetic patients were 175(127-269)mg/dl,0.93(0.78-1.16)mg/dl,24(18-28)mg/dl and 83(64-99)ml/min/m^2 respectively(Table-5).

Conversely, the median and IQR of fasting blood sugar values were 84(77-92) mg/dl, serum creatinine 0.82(0.73-0.94) mg/dl, serum urea 19(15-24) mg/dl and the estimated creatinine clearance by CG were 102(90-118) ml/min/m^2 in apparently healthy control groups (Table-5).

Table 5.Laboratory tests of the study participants at JUMC, South West Ethiopia, from January 9 to March 22, 2017.

Variable	**All participants** **Median(IQR)**	**Diabetes** **Median(IQR)**	**Controls** **Median(IQR)**	**Laboratory reference range**
Fasting blood sugar (mg/dl)	102(82-175)	175(127-269)	84(77-92)	70-110
Serum creatinine(mg/dl)	0.87(0.75-0.99)	0.93(0.78-1.16)	0.82(0.73-0.94)	0.6-1.1(male) 0.5-0.9(female)
Serum urea (mg/dl)	21(16-26)	24(18-28)	19(15-24)	10-50
eGFR(ml/min/1.73m^2)	91(73-111)	83(64-99)	102(90-118)	-

5.3 Prevalence of reduced renal function stratified by study participants

As it was mentioned earlier, for the rational of this study renal impairment is defined as a condition where eGFR of respondents is less than 60/ml/min/1.73m^2. And also this definition works for both BSA adjusted Cockcroft Gault and MDRD equations used to calculate eGFR.

Based on this, of the 468 participants, the overall prevalence of renal impairment in diabetic study participants calculated by CG-BSA equation was 21.8 % (51/234) [95%CI, 16-27] and 13.8% (32/234) [95%CI, 9-18)] by MDRD equation respectively.

The prevalence of renal impairment classified by stages as per adjusted Cockcroft Gault equation was 37.2% stage 1, 41.0 % stage2 and 21.8% stage 3, 0.0%,stage 4 and 0.0% stage 5

respectively. According to MDRD equation 60.7% of the diabetic participants had stage 1, 25.6 % stage2 and 13.8 % stage 3 renal impairment.

Similarly, the over prevalence of renal impairment in apparently healthy controls calculated by CG-BSA equation was 2.6% [95%CI; 1- 5] (5/234), and 1.3% (3/234) [95%CI, 0-3] by MDRD equation respectively. The prevalence of individuals with estimated GFRCG -BSA in between 60 & 89ml/min/1.73m^2 are greater in diabetic study participants 96(41.0%) than that of apparently healthy controls 60(25.6%).There is greater proportion of individuals with estimated GFR ≥90ml/min/m^2 in apparently healthy controls 182(77.8 %) and 212(90.6%) than that of diabetic participants 87(37.2%) and142 (60.7) by CG-BSA and MDRD equation respectively (Table-6).

Table 6.Stratification of study participants based on renal function by stage at JUMC, South West Ethiopia, from January 9 to March 22, 2017

Renal impairment		**Diabetes(n=234)**		**Controls(n=234)**	
		eGFR CG-BSA	**MDRD**	**eGFR CG-BSA**	**MDRD**
	stage	N (%)	N (%)	N (%)	N (%)
≥90	1	87(37.2)	142(60.7)	182(77.8)	212(90.6)
60-89	2	96(41.0)	60(25.6)	46(19.7)	19(8.1)
30-59	3	51(21.8)	32(13.7)	6(2.6)	3(1.3)
15-29	4	0(0.0)	0(0.0)	0(0.0)	0(0.0)
<15	5	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		234(100)	234(100)	234(100)	234(100)

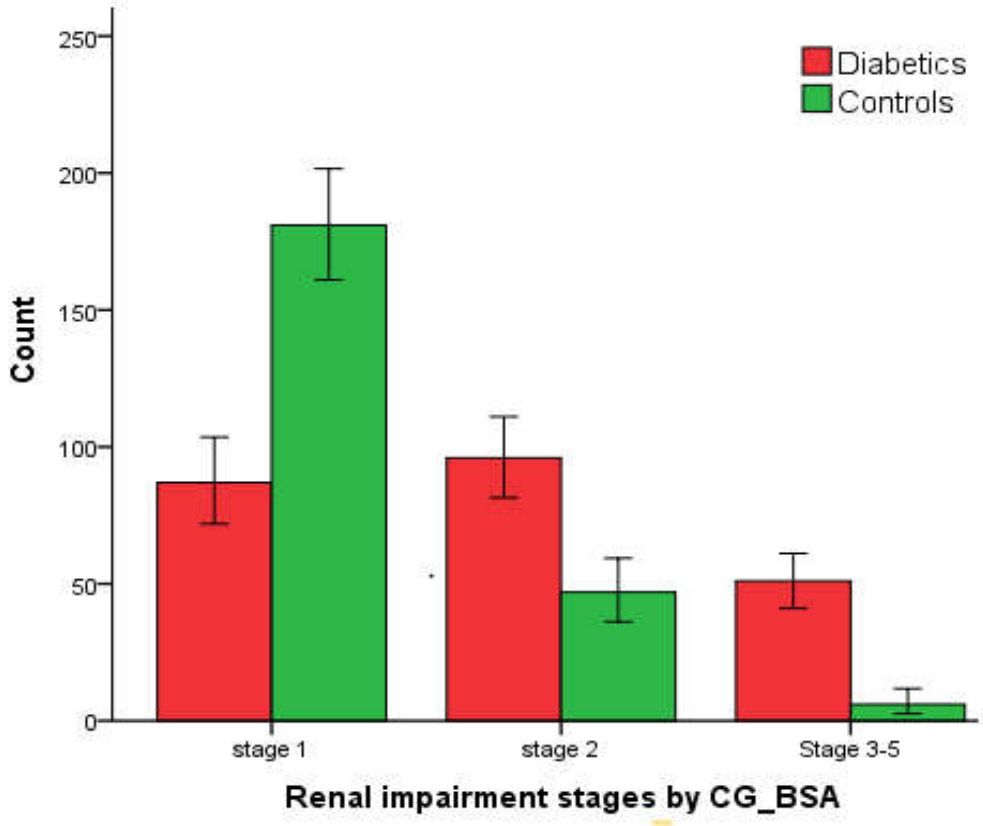

Figure 2.Distribution of study participants based on renal impairment stages calculated by CG – BSA equation at JUMC, South West Ethiopia, from January 9 to March 22, 2017**.**

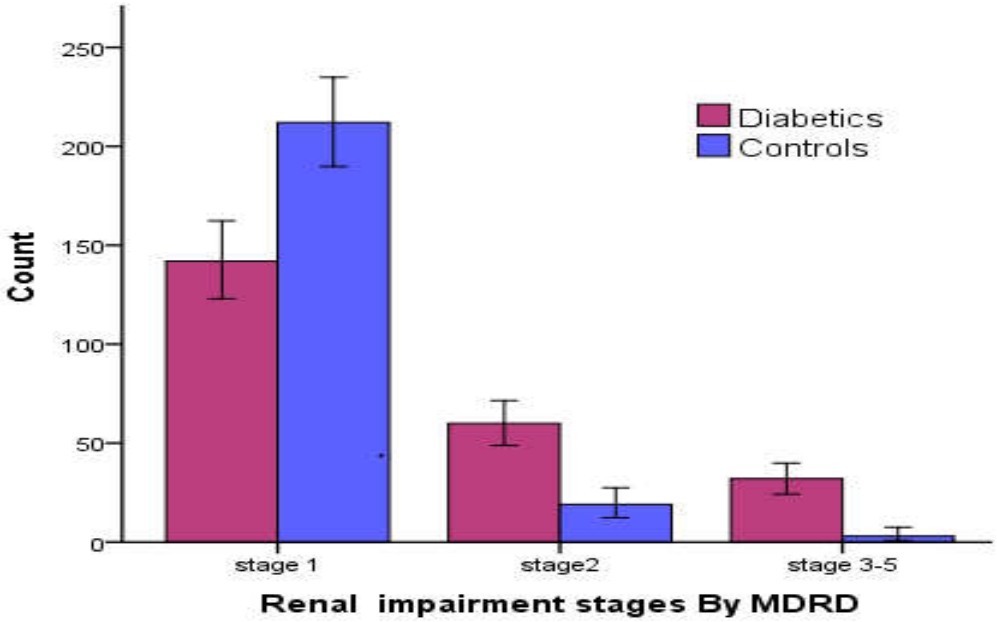

Figure 3. Distribution of study participants based on renal impairment stages calculated by MDRD equation at JUMC, South West Ethiopia, from January 9 to March 22, 2017.

In this study, the association of sociodemographic, life style and clinical factors with renal impairment in people with diabetes were assessed. By age group, the prevalence of renal impairment was significantly higher among diabetes participants ≥50 years old than <50years old: 15.4% versus 6.4% (p =0.001). The prevalence of renal impairment also significantly higher in type2 diabetes compared to type 1 diabetes: 17.5% versus 4.3 %(p=0.002), duration of diabetes above10 years compared to 10 years and below: 11.5% versus 10.3% (p<0.001), oral hypoglycemic agent users (P<0.001), in hypertensive than non-hypertensive patients: 12.0% versus 9.8 %(p<0.001). However, the prevalence renal impairment was not significantly associated with sex (p=0.167), marital status (p=0.166), place of residence (p=0.824), educational level (p=0.764), current smoking (p=1.000), previous smoking (p=0.931), alcohol consumption (p=0.785), traditional medicines using habit (p=0.218) and regular analgesics users (p=0.27) (Table-7).

Table 7. Socio-demographic and clinical characteristics versus reduced renal function by CG-BSA equation in diabetic individuals at JUMC, South-West Ethiopia, from January 9 to March 22, 2017.

Variables	**Normal renal function (eGFR≥60ml/min/1.73m^2)**	**Renal impairment (eGFR<60ml/min/1.73m^2)**	**Total**	***P- value***
	N (%)	**N (%)**	**N (%)**	
Total	n=183	n=51	234	
Sex				
Male	106(45.3)	24(10.3)	130(55.6)	0.167
female	77(32.9)	27(11.5)	104(44.4)	
Age(years)				
<50	102(43.6)	15(6.4)	117(50.0)	0.001
≥50	81(34.6)	36(15.4)	117(50.0)	
Residence				
Urban	125(53.4)	34(14.5)	159(67.9)	0.824
Rural	58(24.8)	17(7.3)	75(32.1)	

Marital status				
Unmarried	32(13.7)	4(1.7)	36(15.4)	0.166
Married	128(54.7)	44(18.8)	172(73.5)	
Divorced	11(4.7)	1(0.4)	12(5.1)	
Widowed	12(5.1)	2(0.9)	14(6.0)	
Education				
<High school	110(47.0)	31(13.2)	141(60.3)	0.931
≥High school	73(31.2)	20(8.5)	93(39.7)	
Occupation				
Unemployed*	100(42.7)	32(13.7)	132(56.4)	0.302
Employed**	83(35.5)	19(8.1)	102(43.6)	
Current smokers				
Yes	2(0.9)	0(0.0%)	2(0.9)	1.000
No	181(77.4)	51(21.8)	232(99.1)	
Previous smokers				
Yes	21(9.0)	6(2.6)	27(11.5)	0.954
No	162(69.2)	45(19.2)	207(88.5)	
Alcohol consumption				
yes	16(6.8)	5(2.1)	21(9.0)	0.785
No	167(71.4)	46(19.7)	213(91.0)	
Traditional medicine				
Yes	11(4.7)	6(2.6)	17(7.3)	0.218
No	172(73.5)	45(19.2)	217(92.7)	
Analgesics users				
Yes	15(6.4)	7(3.0)	22(9.4)	0.276
No	178(71.8)	44(18.8)	212(90.6)	
Type of diabetes				
Type1	80(34.2)	10(4.3)	90(38.5)	0.002

Type2	103(44.0)	41(17.5)	144(61.5)	
Duration of diabetes (years)				
≤10	146(62.4)	24(10.3)	170(72.6)	<0.001
>10	37(15.8)	27(11.5)	64(27.4)	
Diabetic medications				
oral	79(33.8)	28(12.0)	107(45.7)	<0.001
insulin	86(36.8)	10(4.3)	96(41.0)	
Oral/insulin	18(7.7)	13(5.6)	31(13.2)	
Known hypertension				
Yes	25(10.7)	28(12.0)	53(22.6)	<0.001
No	158(67.5)	23(9.8)	181(77.4)	

*= jobless, house makers, farmers and students, ** Government/private employers, Merchants and pensioners

5.2 Factors associated with renal impairment calculated by CG- BSA in diabetic participants

On multivariate analysis, being female sex (OR 3.35,95%CI,1.34-8.22,P=0.008), being aged ≥50 years,(OR 3.12,95%CI,1.26-7.70,P=0.014), having diabetes duration >10 year (OR 2.42,95%CI,1.03-5.72,P=0.043),having history of hypertension (OR 8.84,95%CI,3.12-25.04,P<0.001),having high diastolic pressure (OR 4.48,95%CI,1.70-17.66,p=0.004) and having high fasting blood glucose(OR 8.44,95%CI,2.08-34.32,p=0.003) were independently associate with renal impairment in people with diabetes. However, being unmarried(OR 0.74,95%CI, 0.16-3.51,P=0.439),having type2 diabetes(OR 0.22,95%CI,0.04-1.22,P=0.069) and being treated with insulin injection(OR 0.26,95%CI,0.05-1.30,p=0.214) were not associated with renal impairment as shown (Table -8).

Table 8. Factors associated with renal impairment by standardized CG equation in diabetic individuals at JUMC, South-West Ethiopia, from January 9 to March 22, 2017.

Variables	Category	COR95%CI)	p-value	AOR(95%CI)	P-value
Sex	Male	1*	0.047	1*	0.008
	Female	1.55(0.83-2.89)		3.35(1.34-8.22)	
Age(years)	<50	1*	0.001	1*	0.014
	≥50	3.02(1.55-5.90)		3.12(1.26-7.70)	
Marital status	Married	1*	0.157	1*	0.439
	Unmarried	0.36(0.12-1.09)		0.74(0.16-3.51)	
	Divorced	0.26(0.03-2.11)		0.13(0.01-1.85)	
	Widowed	0.49(0.10-2.25)		0.51(0.09-2.94)	
BMI(kg/m^2)	>25	1*	0.202	1*	0.858
	≥25	1.50(0.80-2.8)		0.93(0.40-2.16)	
Type of diabetes	Type1DM	1*	0.004	1*	0.069
	Type2DM	3.05(1.44-6.45)		0.22(0.04-1.12)	
Duration of diabetes (years)	≤10	1*	0.000	1*	0.043
	>10	4.44(2.30-8.57)		2.42(1.03-5.72)	
Diabetic medication	Oral	1*	0.001	1*	0.214
	Insulin	0.33(0.15-0.72)		0.26(0.05-1.30)	
	Oral & insulin	2.04(0.86-4.69)		1.23(0.39-3.88)	
History of hypertension	No	1*	0.000	1*	<0.001
	Yes	5.62(2.81-11.23)		8.84(3.12-25.04)	
SBP(mmHg)	<140	1*	0.021	1*	0.571
	≥140	2.19(1.12-4.28)		0.72(0.23-2.26)	
DBP(mmHg)	<90	1*	0.000	1*	0.004
	≥90	4.72(2.41-9.23)		5.48(1.70-17.66)	
FBS(mg/dl)	<130	1*	0.001	1*	0.003
	≥130	7.24(2.16-24.22)		8.44(2.08-34.32)	

1* = Reference category, COR = Crud Odds Ratio, AOR = Adjusted Odds Ratio

5.6 Comparisons of quantitative variables and laboratory tests between diabetes and controls

Significance median differences were noted in age (p<0.001), BMI (p<0.001) and systolic blood pressure (p<0.001) and diastolic blood pressures (p<0.001) between diabetic and apparently healthy controls (Table-9).

The median of fasting blood sugar of diabetic and control study participants was 175 mg/dl and 84 mg/dl respectively (p <0.001) (Table-9).

Also, the median serum creatinine (0.93mg/dl) and serum urea (24mg/dl) were significantly higher in Diabetic group as compared to control groups (0.82mg/dl) and 19mg/dl) (P<0.001, for both) respectively (Table-9).

On the other hand, the median creatinine clearance in diabetic and control study participants was 83ml/min/1.73m^2) and 102 ml/min/1.73m^2 respectively, (p<0.001) (Table-8).).

Table 9. Comparisions quantitative variables and laboratory tests between diabetes and controls at JUMC, South-West Ethiopia, from January 9 to March 22, 2017.

Variables	Total (n=468)	Diabetes (n=234)	Controls (n=234)	P-Value
	Median(IQR)	Median(IQR)	Median(IQR)	
Age(years)	42(30-51)	50(40-60)	32(27-44)	<0.001
BMI(kg/m^2)	22(20-25)	23(21-26)	22(20-24)	<0.001
Systolic BP(mmHg)	120(110-130)	120(120-136)	120(110-125)	<0.001
Diastolic BP(mmHg)	70(70-80)	80(80-85)	75(70-80)	<0.001
FBS(mg/dl)	102(82-175)	175(127-269)	84(77-92)	<0.001
Creatinine(mg/dl)	0.87(0.75-0.99)	0.93(0.78-1.16)	0.82(0.73-0.94)	<0.001
Urea(mg/dl)	21(16-26)	24(18-28)	19(15-24)	<0.001
eGFR(ml/min/1.73m^2)	91(73-111)	83(64-99)	102(90-118)	<0.001

5.7 Correlation analysis

According to Spearman coefficient correlation in diabetic study participants, there was statistically positive correlation between serum creatinine with age (r_s= 0.219, p=0.001), BMI (r_s =0.162, p=0.013), FBS (r_s=0.518, p<0.001), SBP (r_s =0.150, p=0.022) and DBP (r_s=0.248, p<0.001). In addition, there was significant positive correlation between serum urea with age(r_s=0.218, p=0.001),FBS(r_s=0.269, p<0.001), and DBP((r_s=0.129, p=0.049) while there was no statistically significant correlation between serum urea with BMI (r_s=-0.002,p=0.980),SBP(r_s= 0.082,p=0.213).On the other hand, there was statistically significant negative correlation between estimated GFR with age(r_s=-0.526,p<0.001),FBS(r_s=-0.397,p<0.001),DBP(r_s=-0.265,p<0.001),SBP(r_s=-0.199,p=0.002).However,there was weak negative correlation with BMI (r_s = -0.089) but not significant(p=0.174).

Concerning to apparently healthy controls, there was significant positive correlation between serum urea with fasting blood sugar (r_s=0.166, p=0.011). There was significant negative correlation between estimated creatinine clearance with age (r_s= -0.223, p=0.001), and with BMI (r_s= -0.170,p=0.009).However, no statistically significant correlation was observed between creatinine with age(r_s=-0.027,p=0.680),BMI(r_s=0.007,p=0.916),SBP(r_s=0.070,p=0.2840), DBP(r_s=-0.015,p=0.822) and FBS(r_s=-0.048,p=0.470).There was no statistical significant correlation between serum urea with age(r_s=0.097,p=0.138),BMI(r_s=-0.112,p=0.087), DBP(r_s=0.004,p=0.954) and SBP(r_s=0.077,p=0.240). In addition to this, there was statistically significant positive correlation between estimated GFR with BMI (r_s=238, p<0.001) and FBS(r=0.130, p=0.047), whereas negatively correlated with age (r_s=-358, p<0.001) (Table-10).

Table 10. Spearman's correlation coefficient between age, FBS and clinical characteristics with renal function parameters of the study participants at JUMC, South West thiopia,from Junuary 9 to march 22,2017.

Variables	Diabetes			Controls		
	Creatinine	Urea	eGFR(CG-BSA)	Creatinine	Urea	eGFR(CG-BSA)
	$r_s(p)$	$r_s(p)$	$r_s(p)$	$r_s(p)$	$r_s(p)$	$r_s(p)$
Age	0.219*(0.001)	0.218*(0.001)	-0.526*(<0.001)	-0.027(0.680)	0.097(0.138)	-0.358*(<0.001)
BMI	0.162*(0.013)	-0.002(0.980)	-0.089(0.174)	0.007(0.916)	-0.112(0.087)	0.238*(<0.001)
SBP	0.150*(0.022)	0.082(0.213)	-0.199*(0.002)	0.070(0.284)	0.077(0.240)	-0.019 (0.771)
DBP	0.248*(0.000)	0.129*(0.049)	-0.265*(<0.001)	-0.015(0.822)	0.004(0.954)	0.077(0.239)
FBS	0.518*(0.000)	0.269*(0.000)	-0.397(<0.001)	0.048(0.467)	0.166*(0.01)	0.130*(0.047)

*Correlation is significant at the 0.05 level (2 –sided), P-value <0.05 is considered statistically significant; r_s is Spearman's correlation coefficient and P is the P-value, CG- Cockcroft &Gault

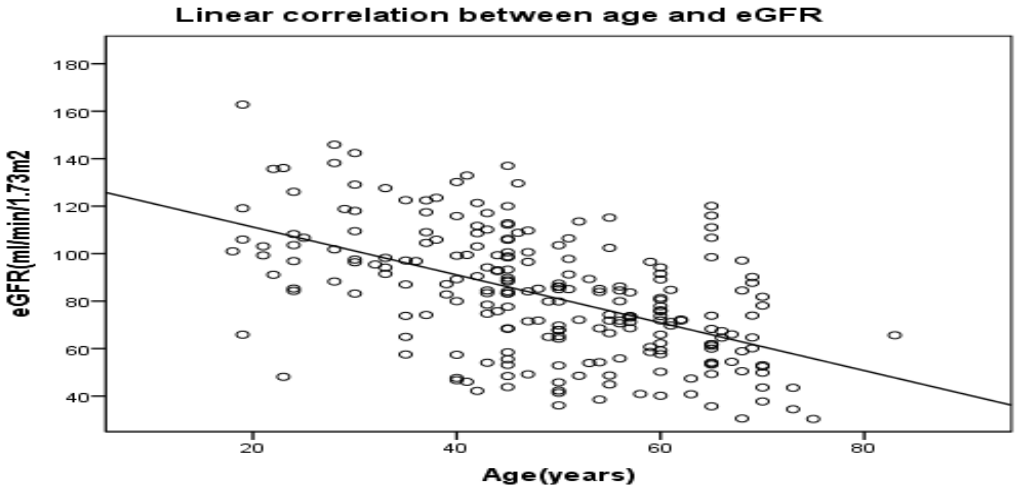

Figure 4.A scatter plot shows the relationship between age (years) and eGFR (ml/min/1.73m^2) in diabetes at JUMC, South West thiopia,from Junuary 9 to march 22,2017.

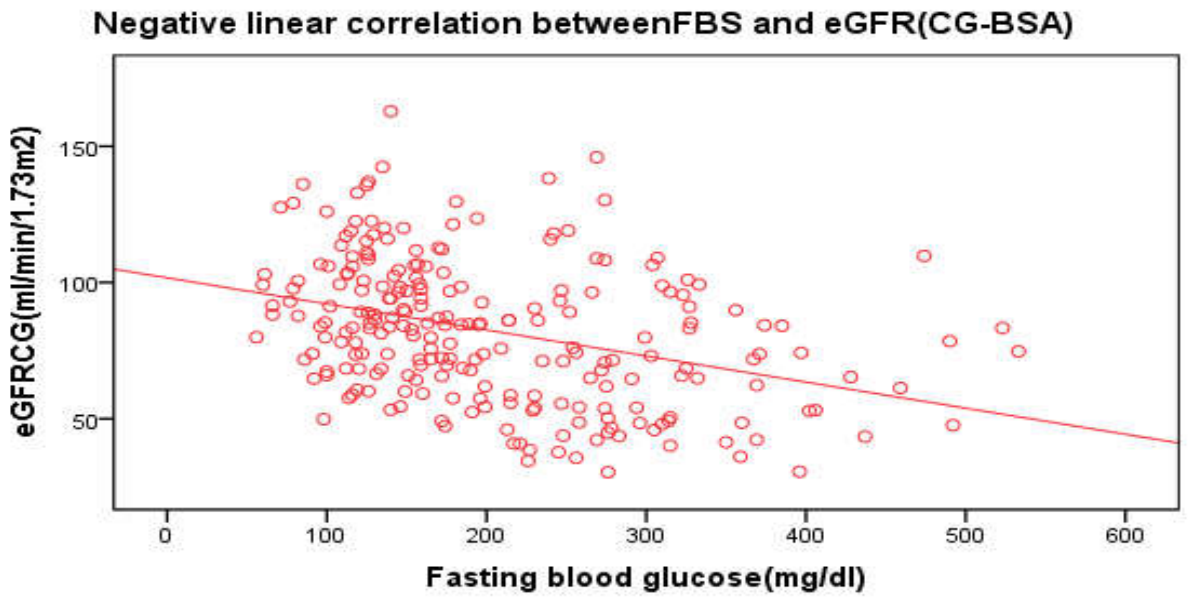

Figure 5.A scatter plot shows the correlation between eGFR and fasting blood glucose values of diabetic patients at JUMC, South West thiopia,from Junuary 9 to march 22,2017.

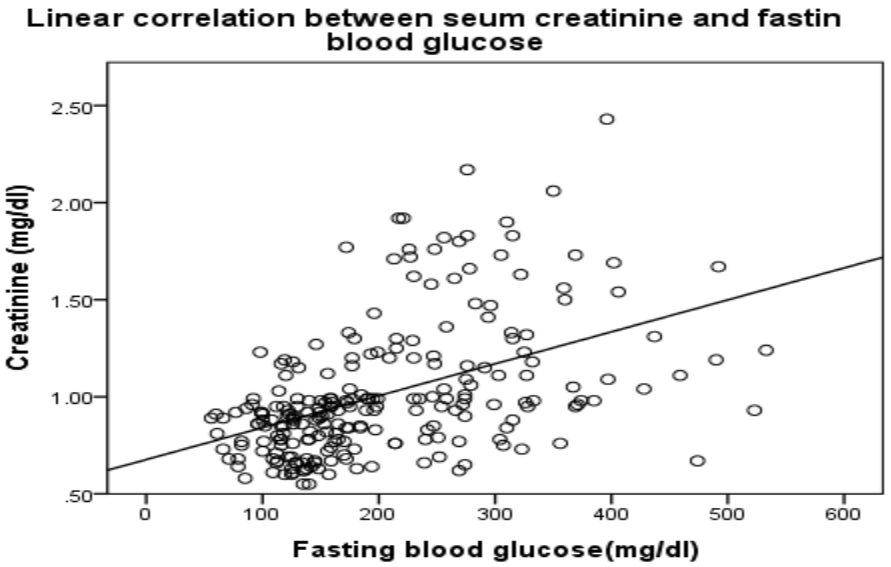

Figure 6.A scatter plot shows the correlation between serum creatinine and fasting blood glucose values of diabetic patients at JUMC, South West thiopia,from Junuary 9 to march 22,2017.

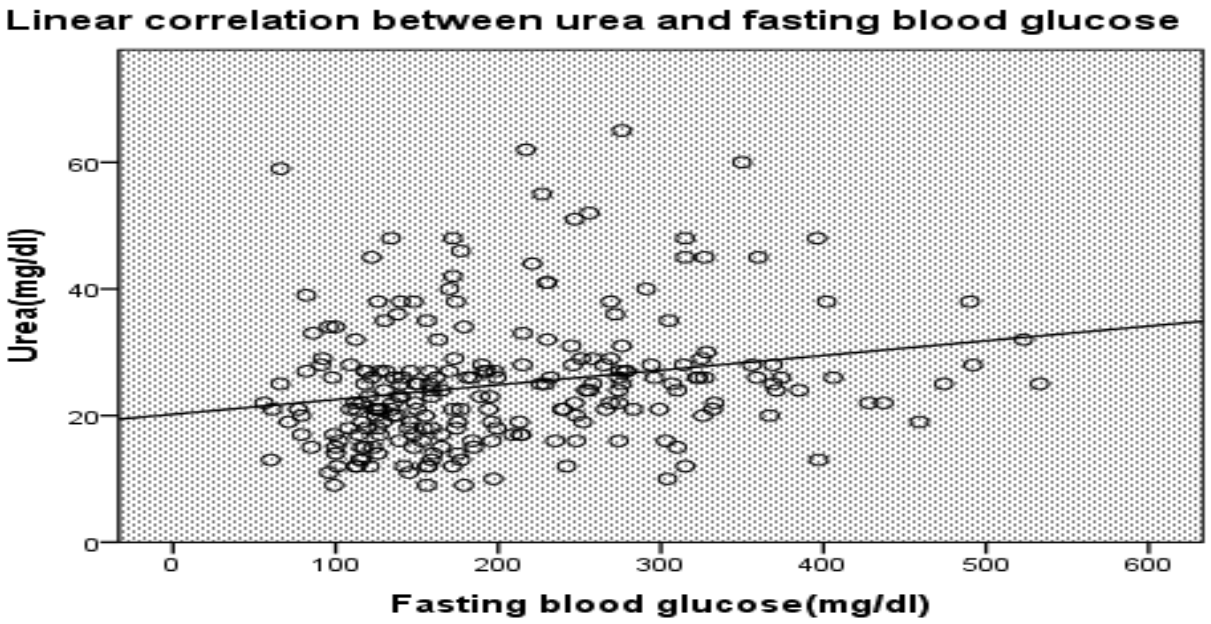

Figure 7.A scatter plot shows linear correlation between fasting blood glucose and serum urea values of diabetic patients at JUMC, South West thiopia,from Junuary 9 to march 22,2017.

CHAPTER SIX: - DISCUSSION

The overall prevalence of renal impairment in diabetic participants in our study based on glomerular filtration rate using the CG-BSA method was 21.8%%.The finding is comparable with a previous study reported from Southern Ethiopia which showed (23.8%)(79). This result is also comparable with other study findings conducted in Korea (24.6%)(63),China (25.8%)(66), Bangladesh (16%)(67), Iran (23.7%)(70) and Ghana (27%)(76), but lower than that reported from United Kingdom(27.5%)(60),The Netherlands (28%)(61), Singapore (51.8%)(64), Nepal (32%)(65), Chile (34%)(71), Oman (42.5%)(69) and Zimbabwe (42.9%)(72),while higher than other studies reported from Canada (15.5%)(59), Turkey (8.77%)(62), Senegal (4.9%)(73), Tanzania (11.0%)(74), Kenya (13.4%)(75) and Eritrea (4%)(78). In the above mentioned studies, the variations in the prevalence of renal impairment can be attributed to disparities in several factors such as; study design, sample size, source of study population, sample selection, race, age and sex structure of the study population, definition of renal impairment, as well as the methods of measurement of creatinine, diabetic duration, and diabetic treatment, eGFR prediction equation etc.

In our study, the prevalence of renal impairment calculated by CG-BSA in apparently healthy subjects was 3.8%.This finding is comparable with other studies done in Turkey (5.58%)(62), Senegal(4.9%)(73), Mwanza city, Tanzania (5.1%)(74), and a Sub-Saharan Africans a genetic epidemiology study (4.8%)(75).However, it is lower than other studies reported from Nepal (18%)(65) and Sudan (11%)(77).These variations might be due to sample size, inclusion criteria, age difference, study design and methods.

As for risk factors, this study found a significant association between older age and renal impairment in people with diabetes. This study is consistent with many other studies(59–61,63,64,66,70,74,79)**.** Age-related decline in the glomerular filtration rate is common and highly prevalent in the elderly(103).In elderly individuals, reduced renal function is accompanied by the decreased in renal blood flow, decrease in kidney mass, particularly from the renal cortex, the glomerular hemodynamic changes and an increase in glomerular basement membrane (GBM) permeability(104).

In our study, female sex is an independent risk factor for renal impairment in diabetic patients. This findings were consistent with other studies (61–64). However ,our study is inconsistent with other previous established studies(63,64,66,69).There are several possible reasons for these contradictory observations, including differences in the patient populations examined, relatively small sample sizes, uncontrolled study designs, the type of diabetes and methods of analysis and differ in eGFR estimating equation.

In our study, duration of disease in diabetes was significantly associated with reduced renal function. This is consistent with results of other previous studies(59,60,69,79) that long duration of diabetes was the independently associated with renal impairment .

In our study, those diabetic subjects on insulin treatment in bivariate analysis was significant, risk factors for reduced renal function while in multivariate analysis being on insulin treatment was not the risk factor for reduced renal function .The present study showed a conflicting result with the previous studies(71,106). The possible explanation for this conflicting finding in the present study may be most Type1 diabetes had short duration of disease so that insulin treatment may not having impact on renal function.

In our study, being hypertensive was significantly associated with diabetic renal impairment. Similar findings were reported in several other studies(62,63,66,75) that hypertension in diabetes may precede the patients towards kidney weakening with all of its symptoms.

On the other hand, this study revealed that high diastolic blood pressure was an independent risk factor for renal impairment in diabetic respondents. The significant association between renal impairment and high diastolic blood pressure was established by other previous studies(63,73,74,76,87).

Additionally, in our study, poor blood glucose control was statistically associated with renal impairment in people with diabetes. This finding is consistent with other previous studies (68,69,79), that high blood glucose is the strong with renal dysfunction in people with diabetes.

On the other hand, in this present study, BMI was not significantly associated renal impairment. This is inconsistent with several other established studies(63,66,68,70,79). This variation in our case might be due to weight loss associated with very poor glycemic control and/or, because of

their inadequate usage and low response to antihyperglycemic treatment, lack of the weight gain often related to glucose lowering drugs.

In this present study, age, BMI, SBP and DBP were significantly higher in diabetes respondents compared to apparently healthy individuals. This findings is comparable with other study(68,81). However, other previous study reported that there was no significant difference in Systolic and diastolic blood pressure between two groups(80). The possible explanation for this might be sample size, inclusion and exclusion criteria and the disease status.

In our study, fasting blood sugar, serum creatinine and urea levels were significantly higher in people with diabetes compared to apparently healthy controls ($p<0.001$). These findings are in tune with other previous studies(80–83,85,88–90).However, the median values of serum creatinine (0.93mg/dl) and urea (24mg/dl) were still lower than the upper normal reference value. This indicated that serum creatinine and urea levels are increased significantly above the normal reference range when 50-60 % of the normal renal function has been lost(17).

The present study indicated that serum creatinine and urea levels were significantly correlated with fasting blood sugar levels. This finding is comparable with other previous studies (90,110,111) that hyperglycemia is one of the major causes of progressive renal damage that leads to renal insufficiency, consequently the nitrogenous waste products like urea and creatinine levels increase abnormally.

In our study, eGFR calculated by CG-BSA was significantly decreased in diabetic patients as compared to control groups. This findings were comparable with other previous studies(81,86,87).However, inconsistent with other previous study showed that there were no statistically significant differences in decreased eGFR between patients with and without diabetes(61). This variation might be due to study design, inclusion and exclusion criteria, age and sex structure of the study population, body mass index, and dilutional effect and differ in eGFR estimation equation. The measurement of the calculated creatinine clearance in our study is considered as less than the lower normal reference value (83ml/min/1.73m^2).The present study indicated that, eGFR is employed for the early identification, diagnosis and appropriate management of reduced renal function.

Limitations

This study has some limitations: Firstly, it does not evaluate a cause–effect relationship between variables and renal impairment in diabetes and apparently healthy individuals because of cross-sectional nature of the study design. Secondly, healthy control study population consisted of mainly middle-aged subjects, meaning that we cannot generalize our findings to other aged healthy control population. Thirdly, the prevalence of renal impairment might have been slightly overestimated and included patients with short term reversible causes of renal impairment using single serum creatinine measurements. Fourthly, despite the Cockcroft–Gault formula is appropriate for estimation of GFR for our population, it has shown to be less accurate to give a firm conclusion of renal function impairment in patients with diabetes and can lead to misclassification of some patients and over estimation of reduced kidney function.

CHAPTER SEVEN:-CONCLUSION AND RECOMMENDATION

7.1 Conclusion

- This study identified prevalence of renal impairment (19.2% among diabetes and 4.3% among apparently healthy controls by Cockcroft Gault equations at Jimma University Medical Center.
- Older age, female sex, duration of diabetes, high fasting blood glucose, hypertension and high diastolic blood pressure are independent contributing risk factors for renal impairment in people with diabetes.
- In the present study, the levels of urea and creatinine were significantly increased for diabetic patients compared to the apparently healthy controls.
- Serum creatinine and urea have significant positive correlation with age, fasting blood sugar and DBP.
- Estimated creatinine clearance has significant negative correlation with age, BMI, FBS and DBP.

7.2 Recommendation

- Monitoring of renal function is important, as impairment is symptomless until at an advanced stage along with proper blood glucose control for peoples living with diabetes.
- Incorporating eGFR into screening for renal impairment in diabetes would enable clinicians to identify individuals with renal impairment early and enabling early effective treatment to delay the progression of CKD.
- If this finding is confirmed in further studies, people with diabetes should be closely monitored

REFERENCES

1. World Health Organization. Global Report on Diabetes. Geneva ,Switzerland .WHO. 2016.

2. Guyton AC, Hall JE. Text Book of Medical physiology. 13th ed. Philadelphia: Elsevier, Inc.; 2016. 994 p.

3. Powers AC. Diabetes mellitus: Diagnosis, Classification, and Pathophysiology. In: Kasper DL, Hauser SL, Jameson JL, Fauci AS, Longo DL, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 19th ed. New York: McGraw-Hill Education; 2015. p. 2399–435.

4. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic Crises in Adult Patients with Diabetes. Diabetes Care. 2009;32(7):1335–43.

5. Al-Awqati Q, Barasch J. Structure and Function of the Kidneys. In: Goldman L, Schafer AI, editors. Goldman's Cecil Medicine. 24th ed. New York: Saunders Elsevier; 2012. p. 117.

6. Drawz P, Rahman M. Chronic Kidney Disease. Ann Intern Med. 2015;162(11):1–5.

7. Satirapoj B. Review on Pathophysiology and Treatment of Diabetic Kidney Disease. J Med Assoc Thai. 2010;93(Suppl.6):228–41.

8. Luis-Rodríguez D, Luis-rodriguez D, Martinez-castelao A, Gorriz JL, Alvaro F De, Navarro-gonzalez JF. Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J Diabetes. 2012;3(1):7.

9. Badal SS, Danesh FR. New Insights into Molecular Mechanisms of Diabetic Kidney Disease. Am J Kidney Dis. 2014;63(202):1–36.

10. Walker S, Beckett G, Rae P, Ashby P. Renal disease. In: Clinical Biochemistry Lecture Notes. 9th ed. John Wiley & Sons, Ltd; 2013. p. 43–59.

11. Delaney MP, Price CP, Lamb EJ. Kidney Disease. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed.

Saunders Elsevier; 2012. p. 1523–601.

12. Paronl R, Arcellonl C, Fermo I, Bonini PA. Determination of Creatinine in Serum and Urine by a Rapid Liquid-Chromatographic Method. ClinChem. 1990;36(6):830–6.

13. Oh MS. Evaluation of Renal Function, Water, Electrolytes, and Acid-Base. In: McPherson RA, Pincus MR, editors. Henry's Clinical Diagnosis and Management by Laboratory Methods. 22nd ed. philadelphia: Saunders Elsevier; 2011. p. 169–92.

14. McMahon GM, Waikar SS. Biomarkers in Nephrology. Am J Kidney Dis. 2013;62(1):165–78.

15. Frank EL. Nonprotein Nitrogen Compounds. In: Bishop ML, Fody EP, Schoeff LE, editors. Clinical chemistry principles, techniques, and correlations. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 246–61.

16. Van Leeuwen AM, Poelhuis-Leth DJ. Davis's Comprehensive Handbook of Laboratory and Diagnostic Tests with Nursing Implications. 3rd ed. Philadelphia: F. A. Davis Company; 2009. 1177 p.

17. Traynor J, Mactier R, Geddes CC, Fox JG. How to Measure Renal Function in Clinical Practice. BMJ. 2006;333:733–7.

18. Schrier RW. Blood Urea Nitrogen and Serum Creatinine not Married in Heart Failure. Circ Hear Fail. 2008;1:2–5.

19. Dasgupta A, Wahed A. Clinical Chemistry, Immunology and Laboratory Quality Control:A Comprehensive Review for Board Preparation, Certification and Clinical Practice. Texas: Elsevier Inc.; 2014. 197-212 p.

20. Amin N, Mahmood RT, Asad MJ, Zafar M, Raja AM. Evaluating Urea and Creatinine Levels in Chronic Renal Failure Pre and Post Dialysis: A Prospective Study. J Cardiovasc Dis. 2014;2(2).

21. Rhoades RA, Bell DR. Renal Physiology and Body Fluids: Kidney Function. In: Medical Physiology Principles for Clinical Medicine. 4th ed. Philadelphia: Lippincott Williams &

Wilkins; 2013. p. 399–426.

22. Stevens LA, Coresh J, Greene T, Levey AS. Assessing Kidney Function – Measured and Estimated Glomerular Filtration Rate. N Engl J Med. 2006;354(23):2473–83.

23. Levey AS, Inker LA, Coresh J. GFR Estimation: From Physiology to Public Health. Am J Kidney Dis. 2014;63(5):820–34.

24. Stevens LA, Levey AS. Measured GFR as a Confirmatory Test for Estimated GFR. J Am Soc Nephrol. 2009;20:2305–13.

25. Acker BA c. V, C.M.Koomen G, G.Koopman M, Waart DR d., Arisz L. Creatinine Clearance during Cimetidine Administration for Measurement of Glomerular Filtration Rate. Lancet. 1992;340:1326–9.

26. National Kidney Foundation. K/DOQI ™ Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis. 2007;49(2):S1–S180(Supp2).

27. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Inter,Suppl. 2013;3(1):1–150.

28. International Diabetes Federation. IDF Diabetes Atlas. 6th ed. 2013.

29. Bargman JM, Skorecki K. Chronic Kidney Disease. In: Harrison's Online (Harrison's Principles of Internal Medicine, 18th ed) [Internet]. McGraw-Hill Medical. [cited 2017 Dec20].Availablefrom:http://accessmedicine.mhmedical.com/content.aspx?bookid=331&sectionid=40727069

30. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B et al. Chronic Kidney Disease: Global Dimension and Perspectives. Lancet. 2013;379:165–80.

31. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. Am J Kidney Dis. 2002;39:S1–S266(Supp1).

32. Mohammad R, Hassan M, Wahab A. Renal function Impairment in Insulin Dependent and Non Insulin Dependent Diabetic Patients. J Drug Deliv Ther. 2015;5(1):49–56.

33. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic Kidney Disease: Worldwide Difference of Prevalence and Risk Factors. J Nephropharmacol. 2016;5(1):49–56.

34. Chan S, Chan H, Baboolal K. A Review of Diabetic Kidney Disease. J Fam Med. 2017;4(2):1–5.

35. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, De Boer IH, Goldstein-Fuchs J, et al. Diabetic Kidney Disease: A Report from an ADA Consensus Conference. Diabetes Care. 2014;37(10):2864–83.

36. American Diabetes Association. Microvascular Complications and Foot Care. Diabetes Care. 2015;38((Suppl.1)):S58–66.

37. Ajiboye O, Sega JB. National trends in the treatment of diabetic nephropathy in the United States. J Clin Pharm Ther. 2017;42:311–7.

38. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal Trends in the Prevalence of Diabetic Kidney Disease in the United States,. JAMA. 2011;305(24):2532–9.

39. National Diabetes Statistics Report. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services. 2017.

40. Perico N, Remuzzi G. Chronic Kidney Disease in Sub-Saharan Africa: A Public Health Priority. Lancet Glob Heal. Perico et al. Open Access article distributed under the terms of CC BY-NC-ND; 2014;2(3):e124–5.

41. Stanifer JW, Turner EL, Egger JR, Thielman N, Karia F, Maro V, et al. Knowledge, Attitudes, and Practices Associated with Chronic Kidney Disease in Northern Tanzania: A Community-Based Study. PLoS One. 2016;11(6):1–14.

42. Okoye OCA, Oviasu E, Ojogwu L. Prevalence of Chronic Kidney Disease and its Risk Factors amongst Adults in a Rural Population in Edo State, Nigeria. J US-China Med Sci. 2011;8(8):471–81.

43. Janmohamed MN, Kalluvya SE, Mueller A, Kabangila R, Smart LR, Downs JA, et al. Prevalence of Chronic Kidney Disease in Diabetic Adult Out-Patients in Tanzania. BMC Nephrol. BMC Nephrology; 2013;14(183).

44. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, Naicker S, et al. The Epidemiology of Chronic Kidney Disease in Sub-Saharan Africa: A Systematic Review and Meta-analysis. Lancet Glob Heal. 2014;2(3):e174–81.

45. Naicker S. Integrated Management: Chronic Kidney Disease, Diabetes Mellitus, Hypertension. African J Nephrol. 2013;16(1):6–13.

46. Noubiap JJN, Naidoo J, Kengne AP, Jean APK. Diabetic nephropathy in Africa: A systematic review. World J Diabetes. 2015;6(5):759–73.

47. Gill G, Gebrekidan A, English P, Wile D, Tesfaye S. Diabetic Complications and Glycaemic Control in Remote North Africa. Q J Med. 2008;101:793–8.

48. Phillips L, Allen N, Phillips B, Abera A, Diro E, Riley S, et al. Acute Kidney Injury Risk Factor Recognition in Three Teaching Hospitals in Ethiopia. South African Med J. 2013;103(6):413–8.

49. Worku D, Hamza L, Woldemichael K. Patterns of Diabetic Complications at Jimma University Specialized Hospital, SouthWest Ethiopia. Ethiop J Health Sci. 2010;20(1):33–9.

50. Tefera G. Determinants of Proteinuria among Type 2 Diabetic Patients at Shakiso Health Center , Southern Ethiopia : A Retrospective Study. Adv Diabetes Metab. 2014;2(3):48–54.

51. Tesfaye S, Gill G. Chronic diabetic complications in Africa. African J diabetes Med. 2011;19(1):4–7.

52. International Diabetes Federation. IDF Diabetes Atlas. 7th ed. Brussels, Belgium: International Diabetes Federation. 2015.

53. Peer N, Kengne AP, Motala AA, Mbanya JC. IDF Diabetes Atlas. Diabetes in the Africa Region: An Update. Diabetes Res Clin Pract. Elsevier Ireland Ltd; 2014;103:197–205.

54. Toth-Manikowski S, Atta MG. Diabetic Kidney Disease: Pathophysiology and Therapeutic Targets. J Diabetes Res. 2015;2015:1–16.

55. Rajput R, Kumar KP, Seshadri K, Agarwal P, Talwalkar P, Kotak B, et al. Prevalence of Chronic Kidney Disease (CKD) in Type 2 Diabetes Mellitus Patients: START-India Study. J Diabetes Metab. 2017;8(2):722.

56. Mora-Fernández C, Domínguez-Pimentel V, de Fuentes MM, Górriz JL, Martínez-Castelao A, Navarro-González JF. Diabetic Kidney Disease: From Physiology to Therapeutics. J Physiol. 2014;592(18):3997–4012.

57. Filho RP, Abensur H, Betonico CCR, Machado AD, Parente EB, Queiroz M, et al. Interactions between Kidney Disease and Diabetes: Dangerous Liaisons. Diabetol Metab Syndr. 2016;8(1):50.

58. Phillips AO. Diabetic nephropathy. Medicine (Baltimore). Elsevier Ltd; 2011;39(8):470–4.

59. Dyck RF, Hayward MN, Harris SB. Prevalence, Determinants and Co-morbidities of Chronic Kidney Disease among First Nations Adults with Diabetes: Results from the CIRCLE Study. BMC Nephrol. 2012;13(57):1–10.

60. Middleton RJ, Foley RN, Hegarty J, Cheung CM, Mcelduff P, Gibson JM, et al. The Unrecognized Prevalence of Chronic Kidney Disease in Diabetes. Nephrol Dial Transpl. 2006;21:88–92.

61. Van Der Meer V, Wielders HPM, Grootendorst DC, De Kanter JS, Sijpkens YWJ, Assendelft WJJ, et al. Chronic Kidney Disease in Patients with Diabetes Mellitus Type 2 or Hypertension in General Practice. Br J Gen Pract. 2010;60(581):884–90.

62. Sahin I, Yildirim B, Cetin I, Etikan I, Ozturk B, Ozyurt H, et al. Prevalence of Chronic Kidney Disease in the Black Sea Region, Turkey, and Investigation of the Related Factors with Chronic Kidney Disease. Ren Fail. 2009;31:920–7.

63. Kang YU, Bae EH, Ma SK, Kim SW. Determinants and Burden of Chronic Kidney Disease in a High-Risk Population in Korea : Results from a Cross-Sectional Study. Korean J Intern Med. 2016;31:920–9.

64. Shankar A, Klein R, Klein BEK. The Association among Smoking, Heavy Drinking, and Chronic Kidney Disease. Am J Epidemiol. 2006;164(3):263–71.

65. Cravedi P, Sharma SK, Bravo RF, Islam N, Tchokhonelidze I, Ghimire M, et al. Preventing Renal and Cardiovascular Risk by Renal Function Assessment: Insights from a Cross-Sectional Study in Low-Income Countries and the USA. BMJ. 2012;2(e001357):1–16.

66. Zhou Y, Echouffo-Tcheugui JB, Gu J, Ruan X, Zhao G, Xu W, et al. Prevalence of Chronic Kidney Disease across Levels of Glycemia among Adults in Pudong New Area, Shanghai, China. BMC Nephrol. 2013;14(253):1–10.

67. Huda MN, Alam KS, Harun -Ur-Rashid. Prevalence of Chronic Kidney Disease and Its Association with Risk Factors in Disadvantageous Population. Int J Nephrol. 2012;2012(267329):1–7.

68. Wijesuriya MA, De-abrew WK, Weerathunga A, Perera A, Vasantharajah L. Association of Chronic Complications of Type 2 Diabetes with the Biochemical and Physical Estimations in Subjects Attending Single Visit Screening for Complications. 2012;3(1):3.

69. Alrawahi AH, Rizvi SGA, Al-riami D, Al-anqoodi Z. Prevalence and Risk Factors of Diabetic Nephropathy in Omani Type 2 Diabetics in Al-Dakhiliyah Region. Oman Med J. 2012;27(3):212–6.

70. Sepanlou SG, Barahimi H, Najafi I, Kamangar F, Poustchi H, Shakeri R, et al. Prevalence and Determinants of Chronic Kidney Disease in Northeast of Iran: Results of the Golestan Cohort Study. PLoS One. 2017;12(5):1–14.

71. Villarroel P, Parra X, Ardiles L. Frequency of Chronic Kidney Disease among Ambulatory Patients with Type2 Diabetes. Rev Med Chile. 2012;140:287–94.

72. Machingura PI, Chikwasha V, Okwanga PN, Gomo E. Prevalence of and Factors Associated with Nephropathy in Diabetic Patients Attending an Outpatient Clinic in Harare, Zimbabwe. Am J Trop Med Hyg. 2017;96(2):477–82.

73. Seck SM, Doupa D, Gueye L, Abdou Dia C. Prevalence of Chronic Kidney Disease and Associated Factors in Senegalese Populations: A Community-Based Study in Saint-Louis. Nephro Urol Mon. 2014;6(5):e19085.

74. Peck R, Baisley K, Kavishe B, Were J, Mghamba J, Smeeth L, et al. Decreased Renal Function and Associated Factors in Cities, Towns and Rural Areas of Tanzania: A Community-Based Population Survey. Trop Med Int Heal. 2016;21(3):393–404.

75. Adebamowo SN, Adeyemo AA, Tekola-Ayele F, Doumatey AP, Bentley AR, Chen G, et al. Impact of Type 2 Diabetes on Impaired Kidney Function in Sub-Saharan African Populations. Front Endocrinol (Lausanne). 2016;7(50):1–6.

76. Ephraim RK, Biekpe S, Sakyi SA, Adoba P, Agbodjakey H, Antoh EO. Prevalence of Chronic Kidney Disease among the High Risk Population in South-Western Ghana; A Cross Sectional Study. Can J Kidney Heal Dis. Canadian Journal of Kidney Health and Disease; 2015;2(40):1–7.

77. Abu-Aisha H, Elhassan EA, Khamis AH, Abu-Elmaali A. Chronic Kidney Disease in Police Forces Households in Khartoum, Sudan: Pilot Report. Arab J Nephrol Transplant. 2009;2(2):21–6.

78. Ahamed SM, Zeratsion R, Elfaki I, Modawe G. Assessment of Renal Function of Eritrean Diabetic Patients Using the GFR derived from the Serum levels Creatinine, Cystatin C or Creatinine- Cystatin C. Sch J Appl Med Sci. 2015;3(3A):1064–8.

79. Fiseha T, Kassim M, Yemane T. Prevalence of Chronic Kidney Disease and Associated Risk Factors among Diabetic Patients in Southern Ethiopia. Am J Heal Res. 2014;2(4):216–21.

80. Matys U, Bachorzewska-Gajewska H, Malyszko J, Dobrzycki S. Assessment of Kidney Function in Diabetic Patients. Is there a Role for New Biomarkers NGAL, Cystatin C and KIM-1? Adv Med Sci. 2013;58(2):353–61.

81. Ahn JH, Yu JH, Ko S, Kwon H, Kim DJ, Kim JH, et al. Prevalence and Determinants of Diabetic Nephropathy in Korea: Korea National Health and Nutrition Examination Survey Study Population. Diabetes Metab J. 2014;38:109–19.

82. Jose MJ, Varkey V, Chandni R, Zubaida PA, Maliekkal J. The Role of Smoking as a Modifiable Risk Factor in Diabetic Nephropathy. J Assoc Physicians India. 2016;64:34–8.

83. Singh P, Khan S, Mittal RK. Renal Function Test on the Basis of Serum Creatinine and Urea in Type-2 Diabetics and Nondiabetics. Bali Med J. 2014;3(1):11–4.

84. Shrestha S, Gyawali P, Shrestha R, Poudel B, Sigdel M, Regmi P, et al. Serum Urea and Creatinine in Diabetic and non-diabetic Subjects. J Nepal Assoc Med Lab Sci. 2008;9(1):11–2.

85. Alam J, Mallik SC, Mokarrama MN, Hoque M, Hasan M, Islam S, et al. Comparative Analysis of Biochemical and Hematological Parameters in Diabetic and Non-Diabetic Adults. Adv Med Sci An Int J. 2015;2(1):1–9.

86. Fontela PC, Winkelmann ER, Ott JN, Uggeri DP. Estimated Glomerular Filtration Rate in Patients with Type 2 Diabetes Mellitus. Rev Assoc Med Bras. 2014;60(6):531–7.

87. Salazar MR, Carbajal HA, Marillet AG, Gallo DM, Valli ML, Novello M, et al. Glomerular Filtration Rate, Cardiovascular Risk Factors and Insulin Resistance. Med (Buenos Aries). 2009;69(5):541–6.

88. Idonije BO, Festus O, Oluba OM. Plasma Glucose, Creatinine and Urea Levels in Type-2 Diabetic Patients Attending a Nigerian Teaching Hospital. Res J Med Sci. 2011;5(1):1–3.

89. Amartey NAA, Nsiah K, Mensah FO. Plasma Levels of Uric acid, Urea and Creatinine in Diabetics who visit the Clinical Analysis Laboratory (CAn-Lab) at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. J Clin Diagnostic Res. 2015;9(2):BC05-BC09.

90. Elfaki EM, Mohamed M, Ali A, Abdul-raheem EM. Assessment of Plasma Levels of Urea Nitrogen, Creatinine and Albumin among Sudanese Patients with Type 2 Diabetes Mellitus. Int J Heal Sci Res. 2013;3(11):1–7.

91. Jimma University Specialized Hospital(JUSH). https://www.ju.edu.et/jimma-university-specialized-hospital-jush.

92. Mhlanga CM, Mogale MA, Adu A, Shai LJ. Serum AGEs in Black South African Patients with Type 2 Diabetes. J Endocrinol Metab Diabetes South Africa. Taylor & Francis; 2016;21(3):56–62.

93. Frese EM, Fick A, Sadowsky HS. Blood Pressure Measurement Guidelines for Physical Therapists. Cardiopulm Phys Ther J. 2011;22(2):5–12.

94. WHO. A Global Brief on Hypertension. Silent Killer, Global Public Health Crisis. Geneva, Switzerland.WHO. 2013.

95. WHO. Training Course on Child Growth Assessment. Geneva,Switzerland.WHO. 2008.

96. WHO. Obesity.Preventing and Managing the Global Epidemic. Report of a WHO Consultation. WHO Technical Report Series 894.Geneva, Switzerland. 2000.

97. WHO. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. Geneva, Switzerland.WHO. 2008.

98. American Diabetes Association. Standards of Medical Care in Diabetes. Diabetes Care. 2015;38(Suppl. 1):S1–2.

99. Cockcroft DW, Gault MH. Prediction of Creatinine Clearance from Serum Creatinine. Nephron. 1976;16:31–41.

100. Mosteller RD. Simplified Calculation of Body-Surface Area. N Engl J Med. 1987;317:1098.

101. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A More Accurate Method to Estimate Glomerular Filtration Rate from Serum Creatinine: A New Prediction Equation. Ann Intern Med. 1999;130(6):461–70.

102. Schwandt A, Denkinger M, Fasching P, Pfeifer M, Wagner C, Weiland J, et al. Comparison of MDRD, CKD-EPI, and Cockcroft-Gault Equation in Relation to Measured Glomerular Filtration Rate among a Large Cohort with Diabetes. J Diabetes Complications. Elsevier Inc.; 2017;31(9):1376–83.

103. Duque GC, Passos MT, Nishida SK, Sabino ARP ,Kirsztajn GM. Assessment of Glomerular Filtration Rate in Older Adults in Brazil. J Nephrol Urol. 2017;1(1):1–4.

104. Weinstein JR, Anderson S. The Aging Kidney: Physiological Changes. Adv Chronic Kidney Dis. 2010;17(4):302–7.

105. Rodriguez-Poncelas A, Garre-Olmo J, Franch-Nadal J, Diez-Espino J, Mundet-Tuduri X, Barrot-De la Puente J, et al. Prevalence of Chronic Kidney Disease in Patients with Type2 Diabetes in Spain: PERCEDIME2 Study. BMC Nephrol. 2013;14(46):1–8.

106. Coll-de-Tuero G, Mata-Cases M, Rodriguez-Poncelas A, Pepió JMA, Roura P, Benito B, et al. Chronic Kidney Disease in the type2 Diabetic Patients: Prevalence and Associated Variables in a Random Sample of 2642 Patients of a Mediterranean Area. BMC Nephrol. 2012;13(87):1–9.

107. Su S-L, Lin C, Kao S, Wu C-C, Lu K-C, Lai C-H, et al. Risk factors and their Interaction on Chronic Kidney Disease: A Multi-Centre Case Control Study in Taiwan. BMC Nephrol. BMC Nephrology; 2015;16(83):1–10.

108. Ferguson TS, Tulloch-Reid MK, Younger-Coleman NO, Wright-Pascoe RA, Boyne MS, Soyibo AK, et al. Prevalence of Chronic Kidney Disease among Patients attending a Specialist Diabetes Clinic in Jamaica. West Indian Med J. 2015;64(3):201–8.

109. Inoue Y, Howard AG, Thompson AL, Mendez MA, Herring AH, Gordon-Larsen P. The Association between Urbanization and Reduced Renal Function: Findings from the China Health and Nutrition Survey. BMC Nephrol. BMC Nephrology; 2017;18(1):1–10.

110. Bamanikar SA, Bamanikar AA, Arora A. Study of Serum Urea and Creatinine in Diabetic and Non- Diabetic Patients in a Tertiary Teaching Hospital. J Med Res. 2016;2(1):12–5.

111. Deepa K, Goud MB, Devi OS, Devaki R, Nayal B, Prabhu A, et al. Serum Urea,

Creatinine in Relation to Fasting Plasma Glucose Levels in Type 2 Diabetic Patients. Int J Pharm Biol Sci. 2011;1(3):279–83.

ANNEXES

Annex I: Information sheets

Information sheet (English version)

Title of the research project: Renal impairment and associated factors among diabetic patients at Jimma University Medical Center, 2017.

Study design: Hospital based comparative cross-sectional study.

Name of researcher: Mohammed Adem

Name of the Organization: Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Name of the sponsor Organization: Jimma University

Introduction: This information sheet is prepared for the aim of explaining the research project that you are asked to join by the group of research team.

This information sheet what provided or read to you describes about the research. When the data collector reads the information sheet, we will expect attentive listening and you can ask questions at any time.

This research team includes one researcher, one clinical nurse as data collector, one laboratory technologist for laboratory test analysis and two advisors from Jimma University, School of medical laboratory Sciences.

Aim of the study: The aim of this research project is to determine renal impairment and associated factors among diabetic patients and apparently healthy individuals at Jimma University Medical Center. This study may have a great importance to assess renal impairment on both diabetic and apparently healthy subjects. In addition, it is used for a base line data for other consecutive studies to be done in our country.

Procedure: If you agree to take part in the study, one of the investigators or a nurse will give you verbal and/or written information about the study and you will be given the consent form to

sign. You are kindly requested to give us the correct information about yourself and the necessary measurements are performed by the assigned nurse. If you are fit for the study 5 ml of blood samples will also be collected for laboratory examination of fasting blood glucose, creatinine and urea.

Risk and discomfort: Participating in this project will not cause more discomfort than is required you could go through for routine examination. But there could be minor pain and change in color of your skin following the blood drawing and which would disappear in short duration. If there comes any discomfort, we shall offer you necessary medical treatment freely. The amount of blood taken from each volunteer throughout the study period is 5ml which will not affect your health.

Benefits: If you are participating in this research project, there may not be direct benefit to you but your participation is likely to help us an important input to find the prevalence of renal impairment and associated factors on renal function parameters among DM patients which will be important to assess the extent of and to increase patient quality of life. And if the medical examination reveals any abnormalities that need immediate treatment, your doctor will be notified about the result.

Incentives and payment for participating in the study: You will not be provided with any direct incentives for your participation in this study. But the cost for your laboratory tests will be covered by the project.

Confidentiality: All information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a computer and it will not be revealed to anyone except your physician and the principal investigator.

Right to refused or withdraw: You have full right to withdraw from participating in this study at any time before and after consent without explaining the reason and not respond to some or all the questions. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact Address

If you have any question or concern, you can contact Mohammed Adem at any time using the following address:

Mohammed Adem, Jimma University, Institute Health, School of Medical Laboratory Science

Tel: +2519-11-00-54-15

Email:ma389870@gmail.com

Jimma, Ethiopia

Information sheet (Afan Oromo version)

Mata duree qorannoo: Wal'aanamtoota dhibee sukkaaraa irratti qorannoo kalee gochuudhaan rakkina dhibeen sukkaaraa irratti fidu hospitaala Univarsiitii jimmaa keessatti ilaaluu ta'a.

Qorataa:-Obbo Mohammed Adem

Maqaa Dhaabataa: Yuuniversittii Jimmatti Instituyittii Faayyaa Fakaltii Fayyaa saayinsii,Mana Barumsaa Meedikalii Laaboratorii .

Dhaabbata rawii baasii: Yuuneversitii Jimmaa

Seensa: Ibsii fi woliigalteen gucichaa ammaisin akka irratti hirmaatan Kan ibsuudha.Qorannoo kana irratti osoo hinmurteessin dura ragaa kanakanneen funaanan yeroo dubbisan hubannoo dhaandhaggeefetan rawwachun gaffiiyoo qabaatan hagaafatn rawatan qorannoo kana erga eegalanii booda gaaffiiyoo qabaatan gaafachu ni danda u.

Kaayyoo qorannoo: Qorannoo dhibee kalee gochuudhaan dhibee fi wantoota dhibicha waliin wal qabatan beeksisuu ta'a. Dabalataanis qorronnowwan biro biyyattii keessatti dalagamaniif galtee ta,uuf gargaara

Akkaataa adeemsa hoji: Qorannoo kana kessatti kan woliigalteen hubachuun waliigallu raga funaanuun guca kennuu irratti mallattoon mirkanessuun dirqama ta a.Kanaan booda qorannoo hubachuun gaafatama.Namatti himuun hinbarbaachisu. Ofumaa hordoffii yaalaa qorannoo dhiigaatiin irradeebi ee waan agarsiisuuf degarsani godhu.Qorannoo kanatti kan hirmaatan dhukubsattoota sukkara fi hordoffi namoota dhuunfaan fidan gafaadha.

Rakkoo lee mudachu danda an: Qorannoo fayyaa kana irratti kan hirmaatan rakkoo fayyaa irratti hinfida jedhee hinyaadu garuu yeroo talaallii fudhatan qaama talaallii itti fudhatan irratti miiri dhukubbii mudachu danda a.Haata u malee yeroo muraasa booda badu danda a. Qorannoo gegeefamu irratti hundaa uun rakkoon yoo mudate talaalliin tola kennama.

Bw aa qorannoo: Qorannoo kanarratti hirmaachuudhaan faayidaa kallattii argachuu dhiisuu dandeessu. Haata'u malee waa'ee dhibee kalee dursanii beekuu fi wantoota dhibicha gara sadarkaa cimaatti jijjiiran beekuuf faayidaa qaba. Dabalataanis rakkoowwan dhibee kana irratti

dhufuu danda'an ittisuuf fayyaa dhukkubsataa fooyyessuuf fayidaa guddaa qaba. Firii qorannichaa ilaaludhaan rakkina jiru ogeessota fayyaaf bekisisna.

Kutaa wol aansaa: Kutaa kanatti fayyadamuun gargaarsas ta e baasi homaa hinqabu.Garuu baasii kan kan raawatu dhaabbata qorannoo kana gegeessu dha.

Icitii eeguu irratti: Qorannoo kana irraa ragaan funaanamu icitiin isaa eegamuu qaba. Ragaan (qorannoo) funaanamu kun keessan ta uun isaa maqaan hin ibsamu. Qorannichi faayila qorataa olaanaa fi godhamee furtuu icitiin kompiyutara irratti erga olkaa ameen booda namni akka argu hinta u.

Mirga mormii: Qorannoo kana irratti hirmaachuu yoo hinbarbaadne mirga guutuu qabu.Hirmaachuu dhiisuu fi gaaffi gaafatame deebisuu fi deebisuu dhiisuuf mirga qabu.Sababii qoranicha irratti hin hirmaanneef hospitaalichi yaalii inni sukkaaraa irratti kennu irratti gafuun ykn kufaatiin kamiyyuu hin jiru.

Teessoo: Gaaffii ykn yaada yoo qabaattan yeroo barbaada meetti Mohaammad adamiin quunnamuu nidandessu.

Mohaammad Adam:-Yuuniversitti Jimmaa, Institutii Fayyaa, kutaa barnoota qorannoo laabiraatorii.

Telephona: +2519-11-00-54-15

e-mail:-ma389870@gmail.com

Jimmaa, Itiyoopiyaa

Information sheet (Amharic version)

-

-

-

-

-

-

-

5

-

-

-

-

-

-

-

- +2519-11-00-54-15

- ma389870@gmail.com

-

Annex II: Consent forms

Consent form (English version)

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 conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal is explained to me in the appropriate language I understand. I_____ do here by give consent to Dr. /Mr. /Mrs. /Miss _____to include me in the proposed research.

Unique number of the participant _____

Participant (signature) _____date_____

Name of the data collector_____

Data collector (signature) _____date_____

Consent form (Afan Oromo version)

Yaada ragaa armaanolitti barreefame dubbisee kayyoo qorannoo fi faayidaa isaa hubadheera yeroo kamitti yyuu qoranocharraa rakkoo fi mormiin aka hin jirre hubadheera. Kana keessatti gartuu qorannoo malee fedhii guutuun hirmaachuuf ilaalcha narra eergamu bahuuf galata galchuukoo mallattoonan mirkanessa.

Lakkoofsa dhoksaa nama itti hirmaate_____

Mallattoo hirmaataa_____guyyaa_____

Maqaa nama ragaa funaanee_____

Mallattoo nama ragaa funaanee_____guyyaa_____

Consent form (Amharic version)

--- ---

--- ---

Annex III. Questionnaires

A. Questionnaires for diabetes mellitus participants

Card No_____

Code No_____

Part I. Socio-demographic characteristics

S/N	Questions	Choices	Remark
101	Sex	1. Male 2. Female	
102	Age	Age in years_____	
103	Place of Residence	1. Urban 2. Rural	
104	Marital Status	1. Unmarried 2. Married 3. Divorced 4. Widowed/Widower	
105	Educational Level	1. No formal education 2. Primary School 3. Secondary School 4. College/University	
106	Ethnicity	1. Oromo 2. Amhara 3. Kefa 4. Gurage 5. Tigrie 6. Others, specify_____	
107	Religion	1. Muslim 2. Orthodox 3. Protestant 4. Others	
108	Monthly income(ETB)	_____Birr	
109	Occupational status	1. Unemployed 2. Government/Private employed 3. Farmer 4. pensioner	

		5. House maker	

Part **II.** Smoking habit and Alcohol consumption

110	Do you smoke cigarette currently?	1. Yes, for how long? _____ 2. No	
111	Have you been a smoker previously?	1. Yes, for how long? _____ 2. No	
112	Do you consume alcohol?	1. Yes 2. No	
113	If yes, how much drinks per week?		

Part III .Use of traditional medicine

114	Do you have history of using traditional medicine for a long period time?	1. Yes 2. No 3. I don't know	
115	Do you still use traditional medicine?	1. Yes 2. No	

Part IV. History of modern medicines

116	Do you have history of using analgesics for a long period of time?	1. Yes 2. No	
117	If yes, justify it		
118	Do you have using modern medicine now?	1. Yes 2. No	
119	If yes, justify it		

Part V. Questions for chronic disease conditions

120	Do you have family members who have Chronic Kidney Disease?	1. Yes 2. No 3. I don't now	
121	Do you have history of known Hypertension?	1. Yes 2. No	
122	Do you have history of heart problem?	1. Yes 2. No 3. I don't know	
123	Do you have any history of chronic diseases?	1. Yes	

		2. No 3. I don't know	
124	If yeas, please justify it.		

Part VI. Clinical data

125	Type of diabetes	1. Type 1 DM 2. Type 2 DM	
126	Duration of Diabetes	1. <5 years 2. 5-10 years 3. 11-20years 4. >20 years	
127	Types of hypoglycemic medications	1. Oral(tablate) 2. Injection(Insulin) 3. Oral and injection	

Part VII. Anthropometric Measurements

128	Height(m)		
129	Weight(Kg)		
130	BMI(Kg/m^2)		

Part VIII. Blood Pressure measurement

131	Systolic Blood Pressure(mmHg)		
132	Diastolic Blood Pressure(mmHg)		

Thank you!

B. Questionnaire for healthy individuals (controls)

Code No_____

Part I. Socio-demographic characteristics

S/N	Questions	Choices	Remark
301	Sex	1. Male 2. Female	
302	Age		
303	Place of residence	1. Urban 2. Rural	
304	Marital status	1. Unmarried 2. Married 3. Divorced 4. Widowed/widower	
305	Educational level	1. No formal education 2. Primary school 3. Secondary school 4. College/University	
306	Ethnicity	1. Oromo 2. Amhara 3. Kefa 4. Gurage 5. Tigrie 6. Other, specify_____	
307	Religion	1. Muslim 2. Orthodox 3. Protestant 4. Others	
308	Monthly income(ETB)	_____Birr	
309	Occupational Status	1. Unemployed 2. Government/private employed 3. Farmer 4. pensioner 5. House maker	

Part II. Smoking habit and Alcohol consumption

310	Do you smoke cigarette currently?	1. Yes, For how long_____	

		2. No	
311	Have you been a smoker previously?	1. Yes ,for how long_____ 2. No	
312	Do you consume alcohol?	1. Yes 2. No	
313	If yes, how much drinks per week?		

Part III. Use of traditional medicine

314	Do you have history of using traditional medicine for a long period time?	1. Yes 2. No 3. I don't know	
315	Do you still use traditional medicine?	1. Yes 2. No	

Part IV. Questions for modern medicine

316	Do you have history of using analgesics for a long period of time?	1. Yes 2. No	
317	If yes, justify it		
318	Do you have using modern medicine now?	1. Yes 2. No	
319	If yes, justify it		

Part V. Questions for diabetes mellitus and other chronic disease conditions

320	Do you have Polyphagia	1. Yes 2. No	
321	Do you have thirst?	1. Yes 2. No	
322	Do you have urgency for micturation?	1. Yes 2. No	
323	Do you have history of hypertension previously?	1. Yes 2. No	
324	Do you have family members who have Chronic Kidney Disease?	1. Yes 2. No 3. I don't now	
325	Do you have known Hypertension?	1. Yes 2. No	

326	Do you have known heart problem?	1. Yes 2. No	
327	Do you have any history of chronic diseases?	1. Yes 2. No 3. I don't know	

Part VI. Anthropometric measurements

328	Height(m)		
329	Weight(Kg)		
330	BMI(Kg/m^2)		

Part VI. Blood Pressure measurements

331	Systolic blood pressure(mmHg)		
332	Diastolic blood pressure(mmHg)		

Thank you!

A. Gaaffilee dhukkubsattoota shukaaraaf

Lakk.Karrdii_____

Lakk.kodii_____

Kutaa 1. Odeeffannoo haala hawaas-dinagdee

Lakk	Gaaffii	Filannoo	Yaada
101	Saala	1. Dhiira 2. Dhalaa	
102	Umurii Waggaan		
103	Bakka Jireenyaa	1. Magaalaa 2. Baadiyyaa	
104	Haala Gaa'ilaa	1. Baaffee 2. Heerumte(kan fudhe) 3. Kan hiike/te 4. Kan irraa due'e/duute	
105	Sadarkaa barnootaa	1. Kan hin baranne 2. Sadarkaa 1ffaa 3. Sadarkaa 2ffaa 4. Kolleejjiirsi/Yuuniversiitii	
106	Qomoo	1. Oromoo 2. Amaaraa 3. Kafaa 4. Guraagee 5. Tigiree 6. Kan biraa, ibsi_____	
107	Amantii	1. Musliima 2. Ortodoksii 3. Pirootestaantii 4. Kan biraa	
108	Galii Ji'aa(Birriidhaan)	Birrii_____	
109	Haala Ogummaa	1. Hojii dhabaa 2. Hojjetaa mootummaa/dhuunfaa	

		3. Qonnaan Bulaa 4. Sooromaa 5. Hojii mana kessa	

Kutaa **2.** Amala Xuuxuufi alkoolii dhuguu

110	Yeroo ammaa tamboo ni xuuxxaa?	1. Eeyee, yeroo hammamiif? _____ 2. Miti	
111	Duris ni xuuxxa turtee?	1. Eeyee, yeroo hammamiif? ____ 2. Miti	
112	Dhugati alkooli ni dhugdu?	1. Eeyee 2. Miti	
113	Deebiiin keessaan eeyee yoo ta'e torbaniti hammam dhugdu?		

kutaa 3 .Qoricha aadaa fayyadamuu

114	Kanaan dura qoricha aadaa yeroo dheeraaf fayyadamtanitu?	1. Eeyee 2. Miti 3. Hin beeku	
115	Amayyuu qoricha aadaa ni fayyadamtaa?	1. Eeyee 2. Miti	

Kutaa 4. Odeeffanno qoricha ammayyaa fayyadamuu

116	Yeroo dheerafi qoricha dhibee tasgabeessuu fayyadamtanii beektu?	1. Eeyee 2. Miti	
117	Eeyee yoo ta'e adda baasii ibsi		
118	Amma qoricha ammayyaa fayyadamaa jirtaa?	1. Eeyee 2. Miti	
119	Odeeffanno qoricha ammayyaa fayyadamuu		

Kutaa 5. Gaaffilee dhukkubbii yeroo dheeraa kan hin daddarbinee

120	Miseensa maatii kee kessa namni dhukkuba kale qabu jiraa?	1. Eeyee 2. Miti 3. Hin beeku	
121	Dhiibbaa dhiigaa ni qabdaa?	1. Eeyee 2. Miti	

122	Rakkoo onnee ni qabdaa?	1. Eeyee 2. Miti 3. Hin beeku	
123	Dhukkuba yeroo dheeraaf namarra turu kamiinuu qabda turtee?	1. Eeyee 2. Miti 3. Hin beeku	
124	Eeyee yoo ta'e adda baasi		

Kutaa 6. Odeeffannoo kiliinikaalaa

125	Gosa dhukkuba shukkaaraa	1. Gosa 1ffaa 2. Gosa 2ffaa	
126	Turtii dhukkuba shukkaaraa	1. Waggaa 5 gadi 2. Waggaa 5-10 3. Waggaa 11-20 4. Waggaa 20 oli	
127	Gosoota qorichaa sukkaaraa ga-bu'eef kennamu	1. Afaaniin(qoricha liqimsaa) 2. Lilma(Insuliinii) 3. Liqimsaa fi lilmoo	

Kutaa 7. Odeeffannoo dhaab-qaamaa

128	Hojjaa(m)		
129	Ulfaatina(Kg)		
130	BMI(Kg/m^2)		

Kutaa 8. Safara dhiibbaa dhiigaa

131	Dhiibbaa dhiigaa siistoolikii(mmHg)		
132	Dhiibbaa dhiga dyaastoolikii(mmHg)		

Galatoomaa!

B. Gaaffilee namoota fayya-qabeeyyiif qophaa'e(madaaliif/birqabaaf)

Lakk.koodii_____

Kutaa 1. Odeeffannoo haala hawaas-dinagdee

Lakk	Gaaffii	Filannoo	Yaada
301	Saala	1. Dhiira 2. Dhalaa	
302	Umurii	Umurii Waggaan	
303	Bakka Jireenyaa	1. Magaalaa 2. Baadiyyaa	
304	Haala Gaa'ilaa	1. Baaffee 2. Heerumte(kan fudhe) 3. Kan hiike/te 4. Kan irraa due'e/duute	
305	Sadarkaa barnootaa	1. Kan hin baranne 2. Sadarkaa 1ffaa 3. Sadarkaa 2ffaa 4. Kolleejjiirsi/Yuuniversiitii	
306	Qomoo	1. Oromoo 2. Amaaraa 3. Kafaa 4. Guraagee 5. Tigiree 6. Kan biraa, ibsi_____	
307	Amantii	1. Musliima 2. Ortodoksii 3. Pirootestaantii 4. Kan biraa	
308	Galii Ji'aa(Birriidhaan)	Birrii_____	

309	Haala Ogummaa	1. Hojii dhabaa 2. Hojjetaa mootummaa/dhuunfaa 3. Qonnaan Bulaa 4. Sooromaa 5. Hojii mana kessa	

Kutaa **2.** Amala Xuuxuufi alkoolii dhuguu

310	Yeroo ammaa tamboo ni xuuxxaa?	1. Eeyee, yeroo hammamiif? ____ 2. Miti	
311	Duris ni xuuxxa turtee?	1. Eeyee, yeroo hammamiif? ____ 2. Miti	
312	Dhugati alkooli ni dhugdu?	1. Eeyee 2. Miti	
313	Deebiiin keessaan eeyee yoo ta'e torbaniti hammam dhugdu?		

kutaa 3 .Qoricha aadaa fayyadamuu

314	Kanaan dura qoricha aadaa yeroo dheeraaf fayyadamtanitu?	1. Eeyee 2. Miti 3. Hin beeku	
315	Amayyuu qoricha aadaa ni fayyadamtaa?	1. Eeyee 2. Miti	

Kutaa 4. Odeeffanno qoricha ammayyaa fayyadamuu

316	Kanaan dura qoricha aadaa yeroo dheeraaf fayyadamtanitu?	1. Eeyee 2. Miti	
317	Eeyee yoo ta'e adda baasii ibsi		
318	Amma qoricha ammayyaa fayyadamaa jirtaa?	1. Eeyee 2. Miti	
319	Odeeffanno qoricha ammayyaa fayyadamuu		

Kutaa 5. Gaaffilee dhukkubbii shukkaaraa fi yeroo dheeraa kan hin daddarbinee

320	Nyaata baayee nyaattaa?	1. Eeyee 2. Miti	
321	Dheebuu qabdaa?	1. Eeyee 2. Miti	
322	Fincaan yeroo fincooftu sisardaa?	1. Eeyee 2. Miti	

323	Miseensa maatii kee kessa namni dhukkuba kale qabu jiraa?	3. Eeyee 4. Miti 5. Hin beeku	
324	Dhiibbaa dhiigaa ni qabdaa?	1. Eeyee 2. Miti	
325	Rakkoo onnee ni qabdaa?	1. Eeyee 2. Miti 3. Hin beeku	
326	Dhukkuba yeroo dheeraaf namarra turu kamiinuu qabda turtee?	1. Eeyee 2. Miti 3. Hin beeku	

Kutaa 6. Odeeffannoo dhaab-qaamaa

327	Hojjaa(m)		
328	Ulfaatina(Kg)		
329	BMI(Kg/m^2)		

Kutaa 7. Safara dhiibbaa dhiigaa

330	Dhiibbaa dhiigaa siistoolikii(mmHg)		
331	Dhiibbaa dhiga dyaastoolikii(mmHg)		

Galatoomaa!

.

_____

_____

1.

.			
101		1. 2.	
102	/ / ?		
103	?	1. 2.	
104		1. / 2. / 3. / 4. /	
105		1. / 2. 3. 4. /	
106	?	1. 2. 3. 4. 5. 6. _____	
107	?	1. 2. 3. 4.	
108	?	_____	
109	?	1. / 2. /	

		3. 4. 5.	

2.

110	?	1. , _____ 2.	
111	?	1. ____ 2.	
112	?	1. 2.	
113	?		

3.

114	?	1. 2. 3.	
115	?	1. 2.	

4.

116	?	1. 2.	
117			
118	?	1. 2.	
119			

5.

120	?	1. 2. 3.	
121	?	1. 2.	
122	?	1. 2. 3.	

123	?	1. 2. 3.	
124			

6.

125		1. 2.	
126		1. 5 2. 5-10 3. 10-20 4. 20	
127		1. / 2. ( ) 3. ( )	

7.

128	(.)		
129	(.)		
130	(. / ^{2})		

8.

131	(mmHg)		
132	(mmHg)		

!

. -

_____

1.

.			
301		1. 2.	
302	/ / ?		
303	?	1. 2.	
304		1. / 2. / 3. / 4. /	
305		1. / 2. 3. 4. /	
306	?	1. 2. 3. 4. 5. 6. _____	
307	?	1. 2. 3. 4.	
308	?	_____	
309	?	1. / 2. / 3.	

		4. 5.	

2.

310	?	1. , 2.	_____
311	?	1. 2.	____
312	?	1. 2.	
313	?		

3.

314	?	1. 2. 3.	
315	?	1. 2.	

4.

316	?	1. 2.	
317			
318	?	1. 2.	
319			

5.

320	?	1. 2.	
321	?	1. 2.	
322	?	1. 2.	

323	?	1. 2.	
324	?	1. 2. 3.	
325	?	1. 2. 3.	
326	?	1. 2. 3.	

6.

327	(.)		
328	(.)		
329	(. / ^{2})		

7.

330	(mmHg)		
331	(mmHg)		

!

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES, SCHOOL OF MEDICAL LABORATORY SCIENCES

Annex IV: Laboratory request form for study participants

SN	Parameters	Results	Comment
1.	Fasting blood glucose	mg/dl	
2.	Creatinine	mg/dl	
3.	Urea	mg/dl	

Reported by:

Name of lab technologist_____

Date of report_____

Signature_____

Annex X: Laboratory principles and procedures

Mindray BS-200E chemistry analyzer

This instrument has been designed to perform spectroscopic measurement at predetermined wavelengths of analyte concentrations and enzyme activity using various reagents. We can perform any combination of tests up to 36-sample pipetting, incubations, photometric measurements and calculations. Programming and operating the analyzer is simple and made easy by windows software. The software, which is supplied with the analyzer, should be installed on a PC connected to the instrument.

Its sophisticated software allows us to program and permanently store in the memory of your PC almost unlimited number of tests and up to 41 test profiles, calibrators and controls. We can create routine sample request by assigning patients data and test and / or profiles to sample. Once the results have been obtained, you can request reports organized per patient or per test or examine the quality control data.

The analyzer can perform end-point or equilibrium method (one or two reagents, monochromatic or dichromatic), fixed time reaction (namely, first-order kinetic method or initial rate method) and kinetic mode method (namely, zero-order kinetic or continuous-monitoring method). The analyzer provides two calibration methods: linear calibration and nonlinear calibration.

The linear calibration includes one-point linear calibration, two-point linear calibration and multi-point linear calibration. They are mainly used for tests determined by colorimetry.

The nonlinear calibration includes Logit-Log 4P, Logit-Log 5P, Exponential 5P, Polynomial 5P, Parabola and Spline. They are mainly used for tests determined by turbidity.

General working procedures

1. Check the connections among the analyzing unit, operation unit and printer.
2. Ensure a detergent is in position 39 and sufficient distilled water is in position 40 on the reagent disk.
3. Check how much deionized water is left in the tank. If not much, add deionized water to the tank.

4. Check how much wash solution is left in the tank. If not much, add wash solution to the tank.
5. Ensure the waste tank is empty. If it is not empty, empty the waste tank.
6. Place the Power to ON.
7. Press the power button on the monitor of the operation unit
8. Press the power button on the monitor of the operation unit
9. Press the power button on the computer of the operation unit
10. After you have logged on the Windows operating system, double-click the shortcut icon of the operating software on the desktop or select the program of the operating software from [Start] to startup the operating software.
11. After startup, the analyzer will check automatically the operation system and resolution of the screen, close screen saver, check color configuration, initialize database and examine the printer
12. When checking is finished, the following dialog box will pop up to ask you to enter the username and password, and then click *OK***.**
13. Select a serial port from Serial Port in the Startup dialog box, and then click *Start* to initialize the system. After that, operate according to the screen prompt until the main screen of the operating software is displayed.
14. Wait the analyzer's working temperate reaches at 37^{o}c.
15. Select and click the QC request button on group buttons area to request the QC samples
16. Click the QC request button on group buttons area to request the QC samples
17. Then click in "sample request" button in the group button area to prepare work list by entering the sample id or patient's name and to enter which test you want to perform on each sample.
18. Click "Ok" at the middle of the sample request screen.
19. Click the arrow buttons to view sample programming information at the bottom on the left side of the sample disk.
20. After preparing the work list, go to the next step and click "Status" to enter the screen, which is used to display the current status of the sample disk, reagent disk and reaction disk.

21. Then close plexi-glass (front glass cover of the instrument), and click on “start” button on the short cut button area of the main screen. It lets you start the session of measurement.

I. GLUCOSE liquicolor

Glucose is the central energy source of the cells in the organism. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation. Glucose regulations become executive the diagnosis and course control of carbohydrate metabolism illness like the diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia and with insulinomas.

Method: Enzymatic colorimetric test with deproteinization

Principle

In the presence of glucose oxidase, the glucose in the sample is oxidized to gluconic acid and hydrogen peroxide. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to generate a red violet quinone imine dye as indicator. The red-violet quinone imine formed is proportional to the amount of glucose present in the sample and can be measured colorimetrically at 500nm.

β -D -Glucose+ O_2+ H_2O $\xrightarrow{\text{glucose oxidase}}$ gluconic acid+ H_2O_2

$2H_2O_2$+4-aminophenazone+phenol $\xrightarrow{\text{peroxidase}}$ quinoneimine+$4H_2O$

Contents, Reagent composition

Monoreagent (RGT): Enzyme reagent

Phosphate buffer, PH 7	0.1mmol/l
4-aminoantipyrine	0.25mmol/l
Phenol	0.75mmol/l
Glucose Oxidase	>15KU/l
Peroxidase	>1.5KU/l

Mutarotase >2.0 KU/l

Stabilizers

Deproteinizing solution (DEPR)

Uranyl acetate 1.6g/l

Sodium Chloride 9g/l

Standard (STD): Glucose 100mg/dl or 5.55mmol/l

Calibrator: Autocal

Controls

- Humatrol N (normal control for auto method system (Prepared by manufacturer)
- Humatrol P (pathologic control for auto method system (Prepared by manufacturer)

Reagent preparation

DPR and RGT are ready for use.STD has to be diluted 1+10 with distilled water.

Storage and stability

The reagents are stable up to the given expiry date when stored at 2…8^{o}c

When opened contamination must be avoided. RGT is stable for 2 weeks at 15…25 ^{o}c

Specimen

Whole blood, Serum and Plasma

The glucose stable for 5 days at 15…25 ^{o}c, if deproteinization and centrifugation of the whole blood is performed promptly after collection. The glucose is stable for 24hours at 2…8^{o}c, if serum or plasma is prepared within 30min. after collection.

Assay:

Wavelength: 500nm

Temperature: 20…25 ^{o}c or 37 ^{o}c

Measurement: Against reagent blank

Procedure

1. Separate the sample
2. Write patient's demographic history and the type of test to be ordered on the work list
3. Put the reagent into the appropriate reagent disk
4. Put the sample tube into the appropriate sample disk
5. Order the machine according to the ordered test

Calculation

$C = 100x\frac{Asample}{ASTD}$ [mg/dl] or

$C= 5.55x\frac{Asample}{ASTD}$ [mmol/l]

Performance characteristics

Linearity: The test is linear up to a glucose concentration of 700mg/dl or 38.85mmol/l. dilute the protein free supernatant 1+1 with Deproteinizing solution, if the glucose concentration of the sample is over this limit and repeat the determination. Multiply the result by 2.

Normal Values

Whole blood (fasting) 70-100mg/dl or 3.9-5.6mmol/l

Serum/Plasma (fasting) 75-115mg/dl or 4.2-6.4mmol/l

Quality control

All control sera with glucose values determined by this method can be used. The manufacturer recommends using its on animal serum based HUMATROL or its on human serum based SERODOS control sera.

Notes

This test is influenced by uric acid, ascorbic acid, glutathione, anticoagulant, bilirubin and creatinine in physiological concentrations.

II. CREATININE liquicolor

Creatinine is a waste product removed by the kidneys mainly by glomerular filtration. The concentration of creatinine in plasma of a healthy individual is fairly constant, independent from water intake, exercise and rate of urine concentration. Therefore increased plasma creatinine values always indicate decreased excretion i.e. impaired kidney function. The creatinine clearance enables a quite good estimation of the glomerular filtration rate which allows better detection of kidney disease and monitoring of renal function.

Method: Photometric Colorimetric test for kinetic measurements: Method without deproteinization

Principle

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Creatinine +picric acid $\longrightarrow$ creatinine-picrate complex

Contents, Reagent composition

R1: Picric acid (PIC) 26mmol/l

R2: Sodium Hydroxide 1.6mol/l

Creatinine Standard (STD): 2mg/dl (176.8µmol/l

Calibrator: Autocal

Controls

- Humatrol N (normal control for auto method system (Prepared by manufacturer)
- Humatrol P (pathologic control for auto method system (Prepared by manufacturer)

Reagent preparation

- Dilute NAOH with distilled water in the ratio 1+4.Store the solution in a plastic bottle
- Mix PIC and diluted NAOH for the working reagent in the ratio 1+1
- The standard is ready for use

Reagent stability

- The reagents/diluted NAOH are stable, even after opening, up to the stated expiry date when stored at 15-25^{o}c
- Contamination must be avoided
- The working reagent, protected from light, is stable for 4 weeks at 15-25^{o}c

Specimen

- Serum, heparinized or EDTA plasma
- Stability:24 hours at 2-8^{o}c
- **Assay:**
- Wavelength: 492 nm
- Temperature: 25 ^{o}C or 37 ^{o}C
- Measurement against air(increasing absorbance)

Procedure

1. Separate the sample
2. Write patient's demographic history and the type of test to be ordered on the work list
3. Put the reagent into the appropriate reagent disk
4. Put the sample tube into the appropriate sample disk
5. Order the machine according to the ordered test

Calculation

$C=2.0x \frac{\Delta A \text{ sample}}{\Delta A \text{ STD}}$ mg/dl

$C=176.8x \frac{\Delta A \text{ sample}}{\Delta A \text{ STD}}$ µmol/l

Conversion of mg/dl into µmol and vice versa: mg/dl X88.402; µmol/lX0.0113=mg/dl

Performance characteristics

Detection limit: 0.12mg/dl

Linearity: 13mg/dl or 1,150µmol/l

Reference values

Serum	mg/dl	µmol/l
Men Women	0.6-1.1 0.5-0.9	53-97 44-80

Result will be reported by mg/dl

Quality control

All control sera with creatinine values determined by this method can be employed. The manufacturer recommends using its on animal serum based HUMATROL or its human serum based SERODOS quality control sera.

Notes

1. The reaction is highly sensitive to temperature. The reaction temperature must be kept constant at 25^{o}c
2. PIC is harmful when inhaled, swallowed or in contact with the skin or mucous membranes wash with plenty of water. In case of sickness, contact a doctor
3. The assay can be affected by the presence of reducing compounds
4. A slight precipitate in the NAOH solution is insignificant

III. UREA liquiUV

The Non Protein Nitrogen (NPN) compound present in highest concentration in the blood is urea. Urea is the major excretory product of protein metabolism which is formed in the liver from the deamination of amino acids and free ammonia generated during protein catabolism. It was also artificially synthesized as of an inorganic forerunner. Urea is an important marker for

evaluation of renal function. An increase in urea level in blood and urine can be caused by renal failure, urinary tract obstruction, dehydration, shock, burns, and gastrointestinal bleeding. Moreover, reduced urea level may be seen in hepatic failure, nephritic syndrome, and cachexia. The measurement of serum / plasma Urea/BUN is indicative of renal damage.

Method: Fully Enzymatic kinetic method

Principle

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia from this reaction combines with 2-oxaloglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD^+. There has been optimized so that the GLDH is the rate limiting enzyme. The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the urea concentration.

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

$$2\text{-oxoglutarate} + 2NH4^+ + 2NADH \xrightarrow{GLDH} \text{L-glutamate} + H_2O + 2NAD^+$$

Contents, Reagent compositions

RGT1:

Tris buffer (PH 7.8)	125mmol/l
ADP0.	88mmol/l
Urease	≥20ku/l
GLDH	≥0.3ku/l
Sodium Azide	0.095%

RGT2:

2-oxoglutarate	25mmol/l
NADH	1.25mmol/l

Sodium Azide 0.095%

Standard (STD)

Urea 80mg/dl or 13.3mmol/l

Sodium azide 0.095%

Calibrator Autocal

Controls

- Humatrol N (normal control for auto method system (Prepared by manufacturer)
- Humatrol P (pathologic control for auto method system (Prepared by manufacturer)

Reagent preparation

- The reagents are ready for use and can directly be applied on automated analyzers (reagent start procedure).
- For sample start procedure working reagent is prepared by mixing 4parts of ENZ with 1part of SUB
- 40ml ENZ+10ml SUB

Reagent stability

- The individual reagents are stable, even after opening, up to the stated expiry date when stored at 2.....8^{o}c.
- Contamination of the reagents must be strictly avoided.
- STD is stable up to the expiry date even after opening
- The working reagent is stable for 5 days at 15…..25^{o}c and for 4weeks at 2…8^{o}c

Specimen

- Serum, plasma, except ammonium heparinate plasma
- Serum or plasma can be stored for up to 3 days at 4^{o}c, for longer periods they should be kept frozen at -20^{o}c.

Assay:

Wavelength: 340nm, Hg 334nm,365nm

Temperature: 25 ^{o}C, 30 ^{o}C or 37 ^{o}C

Measurement: against the reagent blank (RB)

Only one reagent blank per series is required

2-point kinetic

Procedure

1. Separate the sample
2. Write patient's demographic history and the type of test to be ordered on the work list
3. Put the reagent into the appropriate reagent disk
4. Put the sample tube into the appropriate sample disk
5. Order the machine according to the ordered test

Calculations

C $= \frac{\text{Asample}}{\text{A STD}}$ x Factor

C$= \frac{\text{Asample}}{\text{A STD}}$ x80.0 (mg/dl)

C$= \frac{\text{Asample}}{\text{A STD}}$ x13.3 (mmol/l)

Urea (mg/dl) = A sample/ A STD X concentration of standard

Conversion factor for BUN/Urea [mg/dl]

C (BUN) =0.47xC (Urea)

C (Urea) =2.14xx C (BUN)

Performance characteristics

Linearity:

- Serum/Plasma up to 300mg/dl or 50 mmol/l (Urea)

- Samples with a higher urea concentration have to be diluted 1+1 with distilled water, repeat assay and multiply the results by 2

Normal values

Serum (Urea) 10-50 mg/dl or 1.7-8.3 mmol/l

Quality control

All control sera with urea values determined by this method can be employed. The manufacturer recommends using its on animal serum based HUMATROL quality control sera or its human serum based SERODOS.

Notes

- All reagents contain sodium azide (0.095%) as preservative.
- Do not swallow and avoid contact with skin and mucus membranes

Annex XI: Declaration sheet

I, the under signed declared that this thesis is my original work, has not been presented for degree in this or any other university and that all sources of material used for this thesis have been fully acknowledged.

Principal investigator

Name_____

Signature_____

Date_____

Name of institution: Jimma University

Date of submission_____

This thesis has been submitted for examination with my approval as university advisor.

Internal assessor

Name_____

Signature_____

Date_____

Advisors

Name of first advisor_____

Signature_____

Date_____

Name of second advisor_____

Signature_____

Date_____

APPROVAL SHEET OF THESIS

As a member of the board of examiners of the Master of Science thesis open defense examination, I certify that I have read, evaluate the thesis prepared by Mohammed Adem, and

examined the candidates as well. I recommended that the thesis be accepted by fulfilling the thesis requirements for the degree of Master of Science in Clinical Laboratory Science specialty

in Clinical Chemistry.

Principal investigator

Name_____

Signature_____

Date_____

Internal Examiner

Name_____

Date_____

Signature_____