Sudan University of Science and Technology
College of Graduate Studies

Assessment of Albumin to Creatinine Ratio and Glomerular Filtration Rate in Type 2 Diabetic Patients in Khartoum state

تقويم نسبة الألبيومين إلى الكرياتنين ومعدل الترشيح الكبيبي لدى مرضى السكري من النوع الثاني في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Science (Clinical Chemistry)

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بسم الله الرحمن الرحيم

قال تعالى :

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

(سورة العلق: الآيات 1-5)

Dedication

Iam extremely grateful to my parents " Basheer and Egbal " for their love , prayers , caring and scarifices for educating and preparing me for my future, Iam very much thankful to my husband "Azhari" for his love, understanding , prayers and continuing support to complete this research work , also I express my thanks to my sisters "Rana and Reem" and brothers "Tilal and Modawi" and everyone help me to complete this research successfully.

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Abstract

Background :diabetic nephropathy is the most common cause of end stage renal disease, the aim of this study was to assess the levels of albumin to creatinine ratio and glomerular filtration rate as an early indicators of diabetic nephropathy .

Materials and methods :this comparative cross- sectional study was carried out in prof. Mahdy center for diabetes and zenam specialist hospital in Khartoum state from September 2019 to October 2020, fifty Sudanese long standing diabetic patients (29 males and 21 females) in addition to fifty Sudanese newly diagnosed diabetic patients 19)males and 31 females) were enrolled , measurements of albumin , creatinine and albumin creatinine ratio were done by using Cobas (C311) fully automated analyzer , collected data were analyzed by a computer system using statistical package for Social sciences (SPSS) program .

Results :there was insignificant difference between the mean of GFR and ACR of newly diagnosed and long standing diabetic patients p-value = 0.398 ,0.491 respectively, the study also showed significant increase in the mean of urine albumin and ACR among insulin medication when compare with metformin in long standing diabetic patients (p-value =0.018 0.031 ,respectively), the present study also showed that 28% of type 2 diabetic patients had microalbuminuria and 7% had macroalbuminuria (40% of long standing diabetic patients had MA while only 16% of newly diagnosed patients had MA (

,there was insignificant defference between the mean of age, BMI , urine albumine, urine creatinine, serum creatinine, ACR, and GFR of males and females in long standing diabetic patients (*p*-value = 0.414 , 0.325 , 0.348, 0.403 ,0.413 ,0.636 0.592 respectively) , the result also revealed a positive correlation between ACR and diabetes mellitus duration and negative correlation between GFR and the duration of the

disease(r=0.65 , r= -0.208 , *p*=0.054 , *p*=0.047 respectively) The present study also revealed a weak negative correlation between the age of diabetic patients and the e-GFR (r= -0.484 , *p*= 0.000)

Conclusion : forty percent of long- standing diabetic patients had microalbuminuria while only 16% of newly diagnosed diabetic patients had microalbuminuria. Insulin treated diabetic patients had increased urine albumin, the e-GFR is decreased and the level Of ACR is increased as the duration of diabetes increases .

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المستخلص

الخلفيّة: اعتلال الكلية السكري هو السبب الأكثر شيوعاً لمرض الكلى في مراحله الأخيرة ، الهدف من هذه الدراسة هو تقييم مستويات الألبيومين إلى نسبة الكرياتنين ومعدل الترشيح الكبيبي بإعتبارهما المؤشرين المبكريين لإعتلال الكلية السكري.

المواد والأساليب: أجريت هذه الدراسة المقطعية في مركز بروف مهدي للسكري ومستشفى زينام التخصصي في ولاية الخرطوم من سبتمبر 2019 إلى اكتوبر 2020 ، تضمنت هذه الدراسه خمسين سودانياً مصاباً بداء السكري منذ فترة طويلة 29 ذكر و 21 انثى بالإضافة إلى خمسين سودانياً تم تشخيصهم حديثاً بالسكري 31 ذكر و19 انثى ، تم قياس مستويات الألبيومين والكرياتنين وحساب نسبة الألبيومين إلى الكرياتنين بواسطه جهاز(Cobas C311)، وتم تحليل البيانات المجمعه بواسطه نظام كمبيوتر باستخدام برنامج الحزمه الإحصائية للعلوم الاجتماعية SPSS .

النتائج: لم يكن هناك فرق معنوي بين متوسط معدل الترشيح الكبيبي و نسبة الألبيومين الي الكرياتنين لدى مرضى السكري المشخصون حديثاً والمرضى المصابون بالسكري منذ فتره طويله 0.398 , p-value = 0.491 على التوالي، كما أظهرت الدراسة زيادة معنوية في معدل ألبومين البول و نسبة الألبيومين الي الكرياتنين بين أدوية الأنسولين عند مقارنتها بالميتفورمين في مرضى السكري لفترات طويلة (0.031 , p-value =0.018 على التوالي) وأظهرت الدراسة الحالية أيضا أن 28 ٪ من مرضى السكري من النوع2 يعانون من البيلة الألبومينية الزهيدة و 7٪ يعانون من البيلة الألبومينية الكبيرة (40 ٪ من مرضى السكري لفترات طويلة يعانون من البيلة الألبيومينيه الزهيدة بينما 16 ٪ فقط من المصابين حديثا يعانون من البيلة الألبيومينيه الزهيدة) لم يكن هناك اختلاف معنوي بُيِّن متوسط العمر ، مؤشر كتلة الجسم ، زلال البول ، كُرٌ ياتنين البول ، كرياتينين الدم ،نسبة الألبيومين الي الكرياتنين و معدل الترشيح الكبيبي للذكور والإناث لدى مرضى السكري لفترات طويله، p-value = 0.636, 0.413, 0.403 , 0.592, 0.348 , 0.325, 0.414 على التوالي)، كان هناك ارتباط إجًابي بين نسبة الألبيومين الي الكرياتنين ومدة مرض السكري وعلاقه سلبيه بين معدل الترشيح الكبيبي ومدة المرض، كشفت الدراسة الحالية عن ارتباط سلبي ضعيف بين عمر مرضى السكري ونسبة الترشيح الكبيبي.

الخلاصة : 40 ٪ من مرضى السكري لفترات طويلة يعانون من البيلة الألبيومينيه الزهيدة بينما 16 ٪ فقط من المصابين حديثا يعانون من البيلة الألبيومينيه الزهيدة ،مرضى السكري الذين يستخدمون الأنسولين لديهم معدلات ألبيومين بول ونسبة الألبيومين الي الكرياتنين أعلى من الذين يستخدمون المتفورمين ، وفي مرض السكري نسبة الألبيومين إلى الكرياتنين تذيد ومعدل الترشيح الكبيبي يقل كلما زادت فترة المرض.

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ACR albumin to creatinine ratio

ADA American diabetes association

BCG bromocresol green

BMI body mass index

BP blood pressure

CKD chronic kidney disease

CAD coronary artery disease

DKA diabetic ketoacidosis
DM diabetes mellitus
DN diabetic nephropathy

DR diabetic retinopathy

ECF extracellular fluid

ESRD end stage renal disease

GAD glutamic acid decarboxylase

GFR glomerular filtration rate

HDL high density lipoprotein

IA_2 insulinoma associated_2 autoantibody

IAA insulin autoantibody
MI myocardial infarction
NEFA non esterified fatty acid

NPDA non proliferative diabetic retinopathy

PDR proliferative diabetic retinopathy

SPSS statistical package for social sciences

T1D type 1 diabetes / T2D type 2 diabetes

Chapter one

1 Introduction , Rationale and Objectives

1.1 Introduction:

Complications of diabetes mellitus include microvascular problems such as nephropathy , neuropathy and retinopathy , increased heart disease is also found in diabetic patients (Bishop et al.,2010) .

Classic diabetic nephropathy is chronic condition developing over many years, Characterized by gradually increasing urinary albumin excretion (UAE) and blood pressure (BP) , Declining glomerular filtration rate (GFR) is relatively late event, as nephropathy progresses , the risk of other chronic complications of diabetes increases, screening for diabetic nephropathy should be performed annually by measuring urine albumin:creatinine ratio and estimated GFR (Richard et al.,2010).

Across- sectional hospital based study was carried out in Elmusbah Medical Center, from November 2008 to March 2009, fifty eight of type 2 diabetic patients studied. Urinary albumin concentration was measured by immunoturbodimetric assay. Microalbuminuria was diagnosed in 26 (44%) patients. It is concluded that the prevalence of microalbuminuria was 8.66% from total populations at risk (N=300), The risk factors associated with microalbuminuria were found to be age and duration of diabetes (Rahamtalla et al., 2012) .

A retrospective chart review study was conducted at department of clinical chemistry, king Abdulaziz medical city in Riyadh , saudi Arabia , during August to December 2014 .The study include 100 male and female patient diagnosed with type 2 DM Result : Increase in mean level of plasma creatinine (138umol/L), urine microalbuminuria (240 mg/L) and ACR (82) was observed among type 2 DM patients, moderate positive correlation was observed between microalbuminuria and urine albumin creatinine ratio

(r=0.509 p=0.0006) and between urine albumin creatinine ratio and plasma creatinine (r=0.553 p= 0.017), it was reported that type 2 DM patient who at risk of developing renal impairment must be regularly monitored for microalbuminuria and urine albumin creatinine ratio level (Karar et al., 2014) .

1.2 Rationale :

It is estimated that more than 346 million people worldwide have diabetes mellitus, by year 2030 , it is predicted that diabetes will become the seventh leading cause of death in the world , Development of chronic kidney disease (CKD) in patients with diabetes adds significantly to the morbidity and mortality and significantly increases health care cost , Even before the development of end stage renal disease (ESRD) (Roshan and Stanton., 2013).

The overall prevalence of chronic kidney disease (CKD) in the sudan was estimated to range from 7.7% to 11% of the population , with 13.3% of CKD patients being diabetics, diabetic nephropathy was reported as the third most common cause of patients being on dialysis therapy in sudan accounting for 10.4% of all cases (Shigidi et al., 2013).

For all these reasons and in order to prevent or delay diabetes complications we conduct this research and we hypothesize that ACR will be increased , e-GFR will be decreased in diabetic patients.

1.3 Objectives :

Divided into general and specific objectives

1.3.1 General objective :

To assess the levels of albumin to creatinine ratio and glomerular filtration rate as an early indicators of diabetic nephropathy in diabetic patients .

1.3.2 Specific objectives :

1- To estimate microalbuminuria and creatinine in long -standing diabetic patients compared to newly diagnosed diabetic patients .

2 -To calculate albumin to creatinine ratio (ACR) in diabetic patients.

3 -To estimate plasma creatinine and to calculate glomerular filtration rate in diabetic patients .

4 -To correlate between duration of diabetes , albumin to creatinine ratio and glomerular filtration rate (GFR) in diabetic patients .

5- To compare between insulin and metformin as medications of diabetes .

Chapter two

2 Literature Review

2.1 Diabetes mellitus:

Diabetes mellitus is a metabolic disorder of multiple etiologies, it is characterized by chronic hyperglycemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both (Ricahard et al., 2010).

2.1.1 Classification of diabetes mellitus:

Clinically, patients with diabetes mellitus may be classified as having primary or secondary diabetes mellitus (Gad Allah, 2009).

2.1.1.1 Primary diabetes mellitus :

This may be caused by a factor in the blood which antagonize or inhibits the action of insulin, or by production of abnormal form of insulin, or by inability of the pancreas to produce sufficient insulin from the earliest stage of the disease, it is divided into type 1 and type 2 (Gad Allah, 2009).

Type 1 Diabetes Mellitus:

It is due to decreased insulin production , circulating insulin level is very low. these patients are dependent on insulin injections. Onset is usually below 30 years of age, most commonly during adolescence.

They are morepone to develop ketosis . An autoimmune basis is attributed to most of these cases. Circulating antibodies against insulin is seen in 50% cases, and antibodies against islet cell cytoplasmic proteins are seen in 80% cases.

Type 1 diabetes mellitus is an autoimmune disease in which pathologic, autoreactive T cells of the immune system attack the insulin-secreting pancreatic islets of Langerhans. Cytotoxic T cells, which bear the CD8 protein on their

membrane, kill islets, thereby leading to lifelong dependence on insulin for affected patients. Often, a period of poorly controlled blood glucose levels inevitably result in early illness and early death.

Insulin and the 65-kD isoform of glutamic acid decarboxylase (GAD) are major autoantigens in patients with type 1 diabetes.

GAD is a naturally occurring protein found in the brain and in insulin-secreting islets of the pancreas. It is a self protein that functions as an autoantigen in patients with type 1 diabetes.

The selfproteins can be subjected to attack not only by autoreactive T cells but also by autoantibodies to GAD. Avariety of other autoantibodies, such as the insulinoma-associated–2 autoantibody (IA-2), insulin autoantibody (IAA), and islet- specific glucose-6-phosphatase–related protein (IGRP), are also seen (Vasudevan et al., 2011).

signs and symptoms include polydipsia , polyphagia , polyuria , rapid weight loss, hyperventilation , mental confusion and possible loss of consciousness (bishop et al., 2010).

Type 2 Diabetes mellitus:

Type 2 diabetes, previously referred to as "noninsulin- dependent diabetes "or" adult-onset diabetes, accounts for 90–95% of all diabetes. This form encompasses individuals who have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance. At least initially, and often throughout their lifetime , these individuals may not need insulin treatment to survive. There are various causes of type 2 diabetes , Although the specific etiologies are not known , autoimmune destruction of B-cells does not occur

(ADA ,2017).

most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region (Bishop et al., 2010).

Type 2 diabetes is associated with central obesity which increases the risk of type 2 diabetes 80-100 fold, hypertension, hypertriglyceridaemia, decreased high density lipoproteins (HDL)cholesterol, disturbed haemostatic variables and modest increases in a number of pro-inflammatory markers (Kumar and Clark, 2012).

Pathophysiology of type 2 diabetes:

There is no evidence of an autoimmune etiology, rather polymorphism in genes associated with beta-cell function and insulin secretion confer the greatest genetic risk, Type 2 diabetes is characterized by decrease response of peripheral tissue to insulin and beta-cell dysfunction (inadequate insulin secretion in the setting of hyperglycemia) (Mitchell et al., 2012).

Insulin resistance :

Insulin resistance can be defined as the imnability of insulin to produce its usual biologic actions at circulating concentrations that are effective in normal subjects. Insulin resistance in the context of glucose metabolism leads to impaired suppression of endogenous glucose production − under basal conditions as well as after eating (when the physiologic rise in insulin in response to glucose entry from the gut normally shuts down glucose production by the liver) − and to reduced peripheral uptake of glucose. Resistance to the ability of insulin to suppress very low density lipoprotein (VLDL) cholesterol production increases circulating serum triglycerides, while resistance in adipose tissue increases the flux of non - esterified fatty acid (NEFA) both to the liver and skeletal muscle and impairs the action of insulin on glucose metabolism in these tissues (Richard et al., 2010).

2.1.1.2 Secondary diabetes Mellitus :

This occurs as a consequence of other diseases, either pancreatic or endocrine. With pancreatic diabetes, the secretion of insulin is reduced due to pancreatitis, haemochromatosis or resection of the pancreas. In diabetes secondary to other endocrine disorders , ineffective insulin caused by abnormal secretion of hormones with diabetogenic activity. Several drugs adversely affect glucose tolerance and a number of genetic disorders (Gad Allah, 2009).

2.1.2 Clinical Presentation of Diabetes Mellitus：

When the blood glucose level exceeds the renal threshold, glucose is excreted in urine (glocosuria). Due to osmotic effect, more water accompanies the glucose (polyuria). To compensate for this loss of water, thirst center is activated, and more water is taken (polydypsia). To compensate the loss of glucose and protein, patient will take more food (polyphagia). The loss and ineffective utilization of glucose leads to break down of fat and protein, this would lead to loss of weight. Important differential diagnosis for weight loss is diabetes mellitus, tuberculosis, hyperthyroidism, cancer and acquired immune deficiency syndrome (AIDS). Often the presenting complaint of the patient may be chronic recurrent infections such as boils, abscesses, etc. Any person with recurrent infections should be investigated for diabetes. When glucose level in extracellular fluid is increased, bacteria get good nutrition for multiplication. At the same time, macrophage function of the host is inefficient due to lack of efficient utilization of glucose (Vasudevan et al., 2011).

2.1.3 Complications of diabetes mellitus :

Complications of diabetes mellitus can be acute or long-term complications

2.1.3.1 Acute complications :

Both diabetic ketoacidosis (DKA) and hyperosmolar non ketotic hyperglycemia (HH) are caused by a lack of insulin leading to unrestricted flux of stored lipid, carbohydrate and amino acid nutrients into the blood .these conditions are both acute and life-threatening (Richard et al., 2010).

I-Diabetic Keto Acidosis:

Ketosis is more common in type 1 diabetes mellitus. Normally the blood level of ketone bodies is less than 1 mg/dl and only traces are excreted in urine (not detectable by usual tests). But when the rate of synthesis exceeds the ability of extra hepatic tissues to utilize them, there will be accumulation of ketone bodies in blood. This leads to ketonemia, excretion in urine (ketonuria)and smell of acetone in breath. All these three together constitute the condition known as ketosis (Vasudevan et al., 2011).

The breath may have a classic „"fruity odor"" due to excretion of acetone in expired breath, and the patient is hypotensive due to metabolic acidosis which causes electrolyte imbalance (Glew and Rosenthal, 2007).

II- Hyperosmolar Nonketotic Coma :

It can result due to elevation of glucose to very high levels (900 mg/dl or more). This would increase the osmolality of extracellular fluid (ECF). Osmotic diuresis leads to water and electrolyte depletion.The coma results from dehydration of cerebral cells due to hypertonicity of ECF (Vasudevan et al., 2011).

Glucose concentrations exceed 300 to 500 mg/dL (17-28 mmol/L) and severe dehydration is present. The severe dehydration contributes to the inability to excrete

glucose in the urine. Mortality is high with this condition. ketons are not observed because the severe hyperosmolar state inhibits the ability of glucagon to stimulate lipolysis (Bishop et al., 2010).

III-Hypoglycemia :

Define as low level of blood glucose that occurs when there is imbalance between insulin, food intake, and physical exertion. The most common cause of low blood sugar is excessive use of insulin or other glucose-lowering medications to lower the blood glucose level in diabetic patients in the presence of a delayed or absent meal (Gad Allah, 2009).

Hyperglycemia causes harm; but hypoglycemia is fatal. A fall in plasma glucose less than 50 mg/dl is life-threatening. Causes of hypoglycemia are :

1 - over dose of insulin" This is the most common cause. The differentiation of hypoglycemic coma from hyperglycemic coma (ketosis) is important ,since treatment is exactly opposite. The diagnosis is mainly based on blood glucose estimation.

2 - Post-prandial hypoglycemia " 2-3 hours after a meal, transient hypoglycemia is seen in some persons, This is due to over-secretion of insulin

3 - Insulinoma " Insulin secreting tumors are rare " Von Gierke‟s disease (Vasudevan et.,2011).

2.1.3.2 Long-term complications:

Complications that take long time to appear, Which divided into macrovascular and microvascular complications.

Macrovascular Complications in Diabetes:

Diabetes accounts for 75 – 90% of the excess coronary artery disease (CAD) risk and enhances the effects of other cardiovascular risk factors. Death from stroke and myocardial infarction (MI) are the leading causes of mortality in T1DM and T2DM (Nathan et al., 2005)

Microvascular complications in diabetes:

Microvascular complications are caused by prolonged sxposure to hyperglycemia (Richard et al., 2010).

I-Diabetic neuropathy:

Is a common disorder and is defined as signs and symptoms of peripheral nerve dysfunction in a patient with DM in whom other causes of peripheral nerve dysfunction have been excluded (Sadikot et al., 2004).

Diabetic neuropathy has been defined as a demonstrable disorder, either clinically evident or subclinical, that occurs in the setting of diabetes without other causes for peripheral neuropathy. It includes manifestations in the somatic and/or autonomic parts of the peripheral nervous system (Richard et al., 2010).

II- Diabetic retinopathy (DR) :

Most common cause of new cases of blindness among adults aged 20-74 years

Type 1 patients are more susceptible because in type 2 other causes of blindness are common because of age progression. Characterized by vascular closture and growth of new blood vessels (Fong et al., 2003).

proliferative diabetic retinopathy (PDR) occurred in 67% of persons with type 1 diabetes mellitus (T1DM) for 35 or more years so that two - thirds of people with T1DM would need laser treatment for PDR during their lifetime (Klein et al., 1984)

The prevalence of blindness is influenced by duration of diabetes, blood glucose and blood pressure control, and by the presence or absence of screening and preventive laser treatment (Richard et al., 2010).

Clinical Types of Diabetic Retinopathy:

1- Proliferative: in which there is angiogenesis.

2-Non-Proliferative Diabetic Retinopathy(NPDR)

The lesions in the retina at this stage are within the retina and include micro-aneurysms, small "dot and blot" haemorrhages, "splinter" haemorrhages, intraretinal microvascular abnormalities (IRMA)and (cotton wool) spots. The presence of these lesions in various degrees determines whether the NPDR is mild, moderate, severe and verysevere (Viswanath and McGavin, 2003).

III- Diabetic nephropathy (DN):

Classic diabetic nephropathy is a chronic condition developing over many years, characterized by gradually increasing urinary albumin excretion (UAE) and blood

pressure (BP) , Declining glomerular filtration rate (GFR) is a relatively late event. Kidney disease is staged on the basis of both the level of UAE and GFR. In diabetic nephropathy, UAE increases gradually from normal, through microalbuminuria to proteinuria (Richard et al., 2010).

Risk factors for diabetic nephropathy:

Glycemic control, BP and blood lipid levels, obesity and smoking , all those are modifiable risk factors.

Non-modifiable risk factors include long duration of diabetes , advanced age ,female sex and retinopathy.

New risk factors for diabetic nephropathy include oxidative stress, subclinical inflammation ,genetic background and ethnicity (Tziomalos and Athyros, 2015).

The cause of DN is not well understood, but it is thought that high blood sugar, advanced glycation product formation and cytokines may be involved (Batuman,

2017).

Diagnosis of diabetic nephropathy:

Increased UAE is most common feature of diabetic nephropathy , measuring albuminurea is an essential component in the diagnosis of diabetic nephropathy.

According to guidelines issued by (ADA) urinary albumin should be measured at least yearly in patients with T1D duration 5 years or more and in all patients with T2D, UAE should be evaluatwd by measuring the urinary albumin/creatinine ratio in a spot urine sample, since it is accurate and more convenient than the measurement of UAE in 24-h urine collection. UAE varies over time and therefore 2 or more urine samples collected within a period of 3-6 months should show increased albumin excretion before making a diagnosis of nephropathy.

UAE should not be measured in patients with febrile infections. The patients should avoid exercise before testing , and glucose level and BP should be controlled before evaluating UAE.

A substantial proportion of patients with T1D and T2D has decreased GFR .

Urinary proteome analysis also appears to be a promising new modality for early diagnosis of diabetic nephropathy . it detects a variety of non-albumin urinary proteins . however the high cost and limited availability of this method are important barriers for its wider implementation. (Tziomalos and Athyros, 2015)

Staging system for Diabetic Nephropathy :

Stage 1 : Glomerular Hyperfiltration , the earliest observation in development of nephropathy is an increase of up to 50% in the glomerular filtration arte(GFR), Stage 2 : thickening of the glomerular capillary basement membrane (BM) is found histologically, Stage 3 : Development of microalbuminuria (20-200 mcg/min or 30300mg/24h , not detectable by routine urine dipsticks) , stage 4 : Overt diabetic nephropathy and macroalbuminuria (>200mcg/min or 300mg/24h , that is detectable by routine dipsticks, Stage 5 : End-stage renal disease (ESRD) (usually 25-30 years after diagnosis) with glomerular closure and resultant decrease in proteinuria (Roshan and Stanton , 2013).

Good blood glucose and BP control are key to prevention of nephropathy (Richard et al., 2010).

2.2 Long standing diabetes mellitus and nephropathy:

Diabetic duration is one of the strongest risk factors for nephropathy (Richard et al., 2010).

Urinary excretion of albumin (microalbuminuria) and lowered GFR are the early markers of nephropathy, The importance of screening for microalbuminuria and lowered GFR in hypertensive and diabetic patients lies in the detection of preclinical kidney disease, and identification of individuals at increased risk of progressive renal disease, cardiovascular events, and mortality , the American Diabetes Association (ADA) recommended that people with diabetes should do an annual microalbuminuria urine test and measurement blood creatinine at least once a year (ADA 2017).

2.2.1 Creatine (Cr) and Creatinine (Crn):

creatine is synthesized mainly in the liver from arginine ,glycine , mthionine . it is then transported to other tissues such as muscle ,where it converted to phosphocreatine which serves as a high energy source . creatine phosphate loss phosphoric acid and creatin loss water to form creatinine ,which is released into the plasma . creatinine is excreted into the circulation at relatively constant rate that has been shown to be proportional to the individual's muscle mass , it is removed from circulation by glomerular filtration and excreted in the urine, plasma levels of creatinine are related to the relative muscle mass , the rate of creatine turnover and renal function (Bishop et al., 2010).

The reference values for creatinine are 0.6_1.2 mg/dl for male and 0.5_ 1.1 mg/dl for female (Bishop et al., 2010)

Plasma creatinine concentration is observed to be significantly higher in diabetic patients compared to non-diabetic control subjects, and used to predict and monitor kidney failure caused by diabetes (Almamory, 2014).

2.2.2 Albumin and microalbuminuria:

the name is derived from the white precipitate formed when egg is boiled (Latin, albus =white). Albumin constitutes the major part of plasma proteins ,It has one polypeptide chain with 585 aminoacids, It has a molecular weight of 69,000 D. It is elliptical in shape, It is synthesized by hepatocytes; therefore estimation of albumin is a liver function test, half-life of albumin is about 20 days. Liver produces about 12 g of albumin per day, representing about 25% of total hepatic protein synthesis (Vasudevan et al., 2011).

In most patients the first sign of diabetic nephropathy is increased urinary albumin excretion , microalbuminuria is defined as persistent albuminuria in two out of three urine collections of 30 to 300 mg/24 h, or an albumin–creatinine ratio (ACR) of 30 to 300 mg/g creatinine, cannot be detected by routine urine strip (Bishop et al., 2010).

Patients who develop severely increased albuminuria >300 mg albumin/g creatinine in a spot urine sample (also called macroalbuminuria or clinical albuminuria) are at particularly high risk for developing a decline in renal function (Tziomalos and Athyros, 2015) .

In Africa, it is estimated that within 5 – 10 years following diagnosis of , 32 – 57% of people with diabetes will have developed microalbuminuria (Majaliwa et al., 2007).

2.2.3 Glomerular filtration rate (GFR):

Measurement of glomerular filtration rate (GFR) provides the most useful general index for the assessment of the severity of renal damage. A decrease in the renal function is due to the loss of functional nephrons, rather than a decrease in the function of individual nephron.

GFR is the product of filtration rate in single nephrons and the number of nephrons in both kidneys. Substantial kidney damage occurs before GFR is decreased.GFR is also affected by age, sex, body size ,protein intake and pregnancy. Normal GFR for youngadults is 120-130 ml/minute/1.73 m2. GFR is constant in a normal individual, but may vary even with normal kidney function. A decline with age is significant and more than 25% of people older than70 years may have a GFR less than 60 ml/minute. This may be due to decline with age or any systemic disease that may be coexisting.GFR cannot be measured directly, it is estimated from the clearance of a filtration marker (Vasudevan et al., 2011).

Grading of chronic kidney disease (GFR ml/mt/1.73 m2) : grade1 :Minimal damage (GFR>90) , grade 2 : Mild damage with slightly low GFR (GFR 60-89) grade 3 :Moderately low GFR (GFR 30-59) ,grade 4: Severely low GFR (GFR 1529) ,grade 5: Kidney failure (GFR<15) (Vasudevan et al., 2011).

Chapter there

3 Materials and methods

3.1 Materials

3.1.1 Study design:

This is a comparative cross- sectional study.

3.1.2 Study area:

The study was done in Khartoum state , in prof. Mahdy center for diabetes and zanam specialist hospital .

3.1.3 Study period:

This study was carried out during the period from september 2019 to october 2020.

3.1.4 Ethical consideration:

The study was approved by the scientific Committee of Clinical Chemistry Department at College of Medical Laboratory science of the Sudan University of Science and Technology , an informed consent was obtained from each participant in the study (appendix I) , demographic and clinical data was collected from records and by questionnaire (appendix II) .

3.1.5 Study population:

Study population divided into two groups, first group include 50 diabetic patients (29 males aged 40 -73 years and 21 females aged 41-85 years) with long standing diabetes, second group include 50 newly cases type 2 diabetes (19 males aged 28-70 years and 31 females aged 40-81 years)

3.1.6 Inclusion criteria

Sudanese patients with type 2 diabetes mellitus, at least have diabetes mellitus for 10 years were included as group A, and Sudanese patients with type 2 diabetes (diabetes duration 1-5 years) were included as group B .

3.1.7 Exclusion criteria:

Those who have diabetes mellitus for 6 _ 9 years , high blood pressure, renal impairments, and liver diseases.

3.2 Methods:

3.2.1 Sampling:

Blood and urine samples were used in this study. After the use of local antiseptic for skin (70% ethanol), 2.5 ml of venous blood was collected from each volunteer in this study, using a disposable plastic syringe, the blood was collected and then poured in lithium heparin container, mixed gently and centrifuged at 3000 rpm for 5 min to obtain the plasma. After informed consent, a random urine sample was collected in a disposable clean and dry urine container from each volunteer in this study.

3.2.2 Estimation of albumin:

Measurement of albumin was done by using Cobas (C311) fully automated analyzer.

Colorimetric assay. At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex. The color intensity of the blue-green color is directly proportional

to the albumin concentration in the sample and is measured photometrically (appendix III).

3.2.3 Estimation of creatinine:

Measurement of creatinine and calculation of albumin creatinine ratio was done by using Cobas (C311) fully automated analyzer.

This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase, the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration in the reaction mixture (appendix IV)

In order to calculate GFR thr following formula was used :

$$\text{e-GFR} = \frac{(140 - \text{Age in years})(\text{weight in kg})}{72 * \text{plasma creatinine (mg / dl)}} * (0.85 \text{ for females})$$

3.2.4 Calculation of body mass index (BMI):

BMI was calculated from the formula:

BMI= weight in kilograms/square of height in meters. (Gad Allah, 2009).

The patient is categorized as under-weight if BMI is less than 18.5 kg/m2, normal weight if BMI is 18.5 to 25 kg/m2, over-weight if BMI is 25 to 30kg/m2, or obese if BMI is over 30 kg/m2. (WHO expert consultation, 2004).

3.3 Quality control :

Pathological and normal control sera were used to assure the accuracy and validity of results , internal quality control scheme and procedure were established for corrective action if controls do not recover within the acceptable tolerance, the applicable government regulations and local guide lines for quality control were followed.

3.4 Data analysis:

Collected data were analyzed by a computer system using statistical package for social sciences (SPSS) program version 14, the means and standard deviations of the ACR and GFR were obtained for both groups , the t-test was used for comparison , pearson's correlation test was used to find association between the duration of diabetes mellitus (in years) and the level of ACR (in mg/g) and GFR (in ml/min) , also correlation was assessed between ACR and GFR.

Chapter four

4 Result

(Table 4.1) shows insignificant difference between the mean of urine creatinine, serum creatinine , urine albumin, GFR and ACR of newly diagnosed and long standing–diabetic.patients. **(Table 4.2)** shows insignificant difference between the mean of age, BMI , urine albumine, urine creatinine, serum creatinine, ACR, and GFR of males (n=29) and females (n=21) in long - standing diabetic patients .

(Table (4.3) shows percentage of microalbuminuria in newly diagnosed and long – standing diabetic patients

(Table 4.4) shows significant increase in the mean of urine albumin and ACR among insulin medication when compare with metformin in long - standing diabetic patients .

(Tabble 4.5) shows significant increase in the mean of urine albumin among (ACR >30mg/mmol category) when compare with (3-30mg/mmol, <3mg/mmol categories) while There was insignificant difference of Age, BMI, urine creatinine, serum creatinine and GFR among ACR categories in long- standing diabetic patient .

(Table 4.6) shows insignificant difference between the mean of Age, BMI, Urine Albumin, Urine creatinine, Serum creatinine, ACR and GFR in newly diagnosed diabetic patients**.**

(Table 4.7) shows insignificant difference between urine creatinine, serum creatinine, urine albumin, GFR and ACR with medications in newly diagnosed diabetic patients.

(Table 4.8) shows significant increase in the mean of urine albumin among (ACR >30mg/mmol category) when compared with (3-30mg/mmol, <3mg/mmol categories) in newly diagnosed diabetic patient. While there was insignificant difference of Age,

BMI, urine creatinine, serum creatinine and GFR amomg ACR categories in newly diagnosed diabetic patients.

(Table 4.9) shows positive correlation between ACR and age, duration of diabetes melitus, negative correlation between GFR and age, duration of diabetes in long - standing diabetic patients**.**

(Table 4.10) shows Negative correlation between GFR and age ,BMI. Also Negative correlation between ACR and duration in newly diagnosed diabetic patients**.**

Table (4.1): comparison between newly and long- standing diabetic patients

Variables	Newly diagnosis N=50	Long stand D.M N=50	P. value
Urine albumin	3.7 ± 7	6.7 ± 10.2	0.090
Urine creatinine	74.4 ± 56.4	111 ± 73	0.210
Serum creatinine	0.53 ± 0.47	0.62 ± 0.52	0.434
ACR	5.8 ± 14	7.6 ± 11.6	0.491
GFR	187 ± 92	173 ± 72	0.398

Table (4.2): Comparison of gender in long- standing diabetic patients

	Male N=29	Female N=21	p. value
Age	57.3 ± 8.5	59.6 ± 11.2	0.414
BMI	24.7± 4.6	25.9 ± 3.8	0.325
Urine albumin	5.4 ± 8.6	8.4 ± 12.2	0.348
Urine creatinine	103 ± 58.6	122 ± 89.7	0.403
Serum creatinine	0.56 ± 0.17	0.7 ± 0.78	0.413
ACR	7 ± 7.5	8.6 ± 14.1	0.636
GFR	186 ± 64.9	179 ± 81.7	0.592

T- test was used for comparision.

p-value < 0.05 is considered significant.

-Table (4.3) percentage of microalbuminuria in newly diagnosed and long – standing diabetic patients

Duration of disease	Percentage of microalbuminuria
Diabetic patients	28%
Newly diagnosed diabetic patients	16%
Long- standing diabetic patients	40%

Table (4.4): comparison of medication in long- standing diabetic patients

	Insulin N=20	Metformin N=30	P. value

Urine albumin	11.3 ± 12.4	3.6 ± 7.2	0.018
Urine creatinine	131 ± 78	97 ± 67.4	0.108
Serum creatinine	0.67 ± 0.57	0.58 ± 0.49	0.592
ACR	12.5 ± 15.1	4.3 ± 7	0.031
GFR	155 ± 61.8	185 ± 76.6	0.157

T- test was used for comparision.

p-value < 0.05 is considered significant.

Table **(4.5): categorization of ACR in long- standing- diabetic patients**

Variables	<3mg/m mol N=27	3--30mg/m mol N=20	>30mg/m mol N=3	P. value
Age	57 ± 8	60 ± 11.2	60 ± 14.2	0.420
BMI	25.7 ± 4	24.4 ± 4.1	27.7 ± 8.6	0.304
Urine albumin	1.2 ± 1.3	10.4 ± 10.2	31.2 ± 10	0.000
Urine creatinine	108 ± 68	118 ± 85	86.5 ± 21.5	0.760
Serum creatinine	0.57 ± 0.51	0.69 ± 0.57	0.53 ± 0.25	0.748
GFR	193 ± 76.4	146 ± 50	171 ± 118	0.079

ANOVA test was used for comparision.

p-value < 0.05 is considered significant.

Table (4.6): Comparison of gender in newly diagnosed diabetic patients

	Male N=19	Female N=31	p. value

Age	51.7 ± 11.5	54.7 ± 11.8	0.397
BMI	27.3 ± 5.4	38.5 ± 42.3	0.260
Urine albumin	5.2 ± 9.4	2.7 ± 5.1	0.287
Urine creatinine	81 ± 40.3	102 ± 63.7	0.205
Serum creatinine	0.6 ± 0.15	0.5 ± 0.59	0.472
ACR	7.8 ± 18	4.6 ± 11.2	0.440
GFR	175 ± 69	194 ± 104	0.478

T- test was used for comparision.

p-value < 0.05 is considered significant.

Table (4.7): medication among newly diagnosed diabetic patients

	Insulin N=14	Metformin N=32	Without Medication N=4	P. value
Urine albumin	4.4 ± 6.5	3.6 ± 7.7	1.4 ± 1.3	0.760
Urine creatinine	98.1 ± 84.1	91.6 ± 43	104 ± 44.5	0.887
Serum creatinine	0.65 ± 0.83	0.49 ± 0.22	0.53 ± 0.15	0.581
ACR	11.8 ± 21.9	3.8 ± 9.4	1.3 ± 0.75	0.164
GFR	182 ± 86.6	192 ± 100	167 ± 25	0.861

T- test was used for comparision.

p-value < 0.05 is considered significant.

Table (4.8): categorization of ACR in newly diagnosed diabetic patients

Variables	<3mg/m mol N=38	3--30mg/m mol N=8	>30mg/m mol N=4	P. value

Age	54 ± 11.4	54 ± 12.4	51 ± 15.9	0.887
BMI	36.3 ± 38.5	26.1 ± 2	30.4 ± 4.9	0.626
Urine albumin	1.7 ± 5.1	5.9 ± 3.5	18.2 ± 10.7	0.000
Urine creatinine	91.7 ± 44	125 ± 100	59 ± 15.9	0.141
Serum creatinine	0.47 ± 0.21	0.68 ± 1.1	0.5 ± 0.22	0.102
GFR	195 ± 97.8	153 ± 71	179 ± 72	0.510

ANOVA test was used for comparision.

p-value < 0.05 is considered significant.

Table (4.9): correlation between ACR ,GFR and Age, BMI and Duration in long- standing diabetic patients

	ACR	GFR
Age	r=0.256 p=0.073	r= - 0.484 P=0.000
BMI	r=0.048 P=0.739	r=0.028 P=0.847
Duration	r=0.65 P=0.054	r= -0.208 P=0.047

Pearson's correlation test was used for correlation.

Table (4.10): correlation between ACR , GFR and Age, BMI and Duration in Newly diagnosed diabetic patients

	ACR	GFR
Age	r= -0.185 p=0.198	r= -0.433 P=0.002
BMI	r= -0.065 P=0.654	r= - 0.279 P=0.050
Duration	r= -0.333 P=0.018	r= -0.037 P=0.801

Pearson's correlation test was used for correlation.

Chapter five

5 Discussion, conclusion and recommendations

5.1 Discussion :

Diabetic nephropathy is a major factor in the development of chronic kidney disease (CKD) and is recognized as the leading cause of end-stage renal disease (Bennett and Aditya, 2015).

The curretn study results revealed insignificant difference between the mean of urine ceratinie , serum creatinine , urine uluminuria , ACR and GFR of newly diagnosed and long standing diabetic patients these findings disagreed with study done by (Hoy et al., 2001) who found that Albuminuria progresses and GFR is lost over time in individuals in study community, at rates that are strongly dependent on levels of pre-existing albuminuria , also (Pasko et al., 2013) found The microalbuminuric patients in their study had a longer duration of diabetes than the normoalbuminuric group, according to UK Prospective Diabetes Study 74 (UKPDS) for microalbuminuria became statistically significantly increased only at 20 years or more after the diagnosis of type 2 diabetes, in our study the mean of diabetes duratin in long standing diabetic group is 14 years which is not enough to make significant defferance in ACR between the two groups of the study.

The study also showed insignificant difference between the mean of age, BMI ,urine albumine, urine creatinine, serum creatinine, ACR, and GFR of diabetic males and females , these finding disagreed with study done by (Margaret et al., 2012) who found that Compared to women, men had higher mean serum creatinine , lower mean eGFR and a greater prevalence of microalbuminuria , (Clotet et al., 2016) also found that male sex has been associated with higher rates of albuminuria compared with females in the context of type 2 diabetes mellitus, (Savage et al., 1995) found that the male sex significantly correlated with microalbuminuria and progression to

ESRD was lost after menopause ,when women lose female macroalbuminuria , but the protective effect of the female sex with T2DM in the hormones the positive effect disappears so that our study shows insignificant difference between males and females due to increased age of females in this study.

The study also showed significant increase in the mean of urine albumin and ACR among insulin medication when compare with metformin in long standing diabetic patients , these findings agreed with study done by (Chih-Cheng et al., 2011) who found that insulin resistance could significantly predict development of microalbuminuria in type 2 diabetic patients,(Anyanwagu et al., 2019) found that among a large cohort of insulin-treated T2D patients in routine practice, the increased ACR was associaed with the greatest risk of premature death, (Savage rt al., 1995) also found insulin use was significantly correlated with microalbuminuria and macroalbuminuria , insulin resistance is the inability of a known quanity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population which leads to hyperglycaemia that causes alteration in structure and function of podocytes which are adhering to GBM leading to increased basement membrane pore size, all contribute to the albuminuria .

The study showed that 28% of type 2 diabetic patients had microalbuminuria and 7% had macroalbuminuria, subjects with macroalbuminuria had significantly increased urine albumin ،Similar result obtained in Egypt by (Elhefnawy et al., 2019) who found 31.8% of type 2 diabetes had microalbuminuria, and 7.9% had macroalbuminuria, (Farahat et al., 2014) reported that in type 2 diabetics patients, the overall prevalence of MA and macroalbuminuria was 34.2 and 12.8%, respectively, (alsalman et al., 2009) found that In Bahrain, the prevalence of MA and macroalbuminuria among type 2 diabetic patients were 22 and 5.8%, respectively , (Shebl 2008) found that the prevalence of MA was 58.2% , these variations in the prevalence rate of proteinuria can be attributed to the differences in several factors

such as the study design, source of study population, sample selection, race, age, sex structure of the study population, diagnostic criteria, as well as the methods of measurement of proteinuria and urine collection, diabetic duration, diabetic treatment, and presence of hypertension.

The present study showed a weak positive correlation between the duration of the disease and the ACR in diabetic patients , low level of ACR was found in patients with short disease duration, and high level was found in patients with long disease duration, this weak correlation may be attributed to the difference in glycemic control between patients. Our data support a study done in Sudan by (Rahamtalla et al., 2012) , this finding also agreed with (AggArwAl and kumar 2014). who found that ACR was linearly correlated to the duration of diabetes and HbA1c , mechanisms responsible for increased ACR in prolonged hyperglycemic state include endothelial dysfunction and chronic inflammation triggered by oxidative stress, inflammatory cytokines and growth factors

The present study also showed a weak negative correlation between the duration of diabetes and the e-GFR (high level of e-GFR was found in patients with short duration while low level of e-GFR was found in patients with long duration), this weak correlation may also be attributed to the difference in glycemic control between patients, This agreed with study carried out in Saudi Arabia by (Alwakeel et al., 2011) , (Hovid et 2001) demonstrate that the decine in glomeruiar filtration rate (GFR) is highly veriable , ranging from 2 to 20 , with median of 12 ml /min/year , prolonged hyperglycemia cause more advanced diabetic glomerular lesions resulting in dicreased GFR .

The present study also revealed a weak negative correlation between the age of diabetic patients and the e-GFR , similar result obtained by (Rius et al., 1995) who found that an inverse correlation was found between GFR and age, but not with known duration of NIDDM. It was a weak correlation ($r=-0.41$) but statistically

significant (P<0.001), (Taniwaki et al., 1998) found that GFR was significantly correlated with the patients age (r = -0.256, P < 0.05) , The decline of GFR appears to be a part of the normal physiologic process of cellular and organ senescence and is associated with structural changes in the kidneys.

5.2 Conclusion :

Forty percent of long- standing diabetic patients had microalbuminuria while only 16% of newly diagnosed diabetic patients had microalbuminuria.

Insulin treated diabetic patients had increased urine albumin.

the e-GFR is decreased and the level Of ACR is increased as the duration of diabetes increases

.

5.3 Recommendations :

1) Diabetic patients should be monitored regularly by measuring their albumin to creatinine ratio and glomerular filtration rate; to delay or avoid the development of diabetic nephropathy and end stage renal disease .

2) Measuring the real glomerular filtration rate using cystatin c may give better result than estimated glomerular filtration rate since it depends on creatinine it will be affected by muscle mass .

3) Health education programs for diabetics; so as to be aware of diabetic complications, in order to prevent dibetes complications .

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Appendix I

Informed Consent Form

إستمارة موافقة الشخص المشارك في البحث

أنا الباحث: رزاز بشير مضوي ، كلية الدراسات العليا، جامعة السودان للعلوم والتكنولوجيا، أقوم ببحث وتقويم نسبة الألبيومين إلى الكرياتنين ومعدل الترشيح الكبيبي في مرضى السكري من النوع الثاني بولاية الخرطوم

(Assessment of albumin to creatinine ratio and glomerular filtration rate in type 2 diabetic patiets in Khartoum state)

لقد تم إختيارك لتشارك في هذا البحث ومعك عدد آخر من المشاركين.

نتوقع بمشاركتك أنت والمشاركين الآخرين أن نتحصل على نتائج تفيد فهم طبيعة هذا المرض وقد يعود البحث بفوائد على كل من المشارك نفسه أو المجتمع أو مقدمي الخدمات الصحية.

خلال هذه الدراسة سأقوم بأخذ معلومات عنك وعن المرض وأخذ عينة بول ودم من الوريد حوالي 3ملليتر، ثم إجراء قياس الألبيومين والكرياتنين من العينات.

الإجراء الذي سأقوم به تجاهك ليس به أي مخاطر أو أعراض جانبية على المشارك.

ونحن إذ نأمل فى مشاركتك معنا فى هذا البحث، نؤكد لك على سرية المعلومات والوثائق الخاصة بك، وأنه لن يطلع عليها إلا الباحث المعنى و لجنة أخلاقيات البحوث الصحية القومية أو المحلية.

و سوف نخبرك بنتيجة الفحوصات عن طريق طبيبك المعالج، لن نجمع منك أى عينات أخرى، أما العينات التى يتم جمعها سوف تستعمل لغرض هذه الدراسة فقط. و نود أن نشير كذلك إلى أن المشاركة فى البحث طوعية وأن رفضك للمشاركة فى البحث لا تفقدك الحق فى أى فوائد من البحث، مع التأكيد على أنه لن يتم منحك أي قيمة نقدية مقابل المشاركة فى هذا البحث وأنه بمشاركتك ستكون أحد المتطوعين الذين يشملهم البحث.

<u>فورم إقرار موافقة المشارك في البحث</u>

إقرار المشارك :

لقد إطلعت على المعلومات الحالية والتي تم شرحها لي وأتيح لى طرح الأسئلة عنها كيفما شئت، وقد تلقيت الإجابات الوافية عن كل الأسئلة، وأنا أقر بالموافقة على المشاركة طواعية فى هذه الدراسة وأعلم بحقى فى التوقف عن المشاركة فى أى وقت دون أن يؤثر ذلك على حقوقى فى تلقى العناية الطبية اللازمة فى أى وقت.

رمز المشارك: .....

إسم المشارك: .....

توقيع المشارك: .....

في حال عدم قدرة المشارك على قراءة الإقرار ويحتاج إلى من يشرح أو يترجم له:

..... :(إسم الشارح (المترجم

..... :(عنوان الشارح أو(المترجم

..... :(توقيع الشارح أو(المترجم

..... :توقيع الباحث

..... : التاريخ

AppendIx II

Questionnaire

Assessment of Albumin to Creatinine Ratio and Glomerular Filtration Rate in type 2 Diabetic Patients in Khartoum state

Sex: …….

Age: …… (in years).

Height: ……... (in centimeters).

Weight: …… (in Kg).

Type of medication : ……...

Duration of diabetes: ……. (in years).

Investigations required:

Investigation	Result
Serum creatinine (mg/dL)	
Urine creatinine (mg/dL)	
Urine albumin (mg/L)	
Albumin to creatinine ratio (mg/g)	
Glomerular filtration rate (ml/min)	

GUNDERSEN HEALTH SYSTEM

Standard Operating Procedure

Subject	Albumin – Cobas c501 and c502
Index Number	Lab-4215
Section	Laboratory
Subsection	Chemistry
Category	Departmental
Contact	Amy VanLin
Last Revised	8/16/2019

References

Required document for Laboratory Accreditation by the College of American Pathologists (CAP), Centers for Medicare and Medicaid Services (CMS) and/or COLA.

Applicable To

Employees of the Gundersen Health System and Gundersen Tri-County Hospital laboratories.

Detail

INTENDED USE:
In vitro test for the quantitative determination of albumin in human serum and plasma on Roche/Hitachi cobas c systems.

PRINCIPLE:
Colorimetric assay. At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex. The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

CLINICAL SIGNIFICANCE:
Albumin is a carbohydrate-free protein which constitutes 55-65% of the total plasma proteins. It maintains plasma oncotic pressure and is also involved in the transport and storage of a wide variety of ligands and is a source of endogenous amino acids. Albumin binds and solubilizes various compounds, e.g. bilirubin, calcium, and long-chain fatty acids. Furthermore, albumin is capable of binding toxic heavy metal ions as well as numerous pharmaceuticals, which is the reason why lower albumin concentrations in blood have a significant effect on pharmacokinetics.

Hyperalbuminemia is of little diagnostic significance except in dehydration. Hypoalbuminemia occurs during many illnesses and is caused by several factors: compromised synthesis due either to liver disease or as a consequence of reduced protein intake; elevated catabolism due to tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria as a consequence of nephrotic syndrome; protein loss via the stool (neoplastic disease). In severe cases of hypoalbuminemia, the maximum albumin concentration of plasma is 2.5 g/dL. Due to the low osmotic pressure of the plasma, water permeates through blood capillaries into tissue (edema). The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

SPECIMEN:

GUNDERSEN HEALTH SYSTEM

Standard Operating Procedure

Subject	Creatinine – Cobas c501
Index Number	Lab-4217
Section	Laboratory
Subsection	Chemistry
Category	Departmental
Contact	Amy Vanlin
Last Revised	9/13/2018

References

Required document for Laboratory Accreditation by the College of American Pathologists (CAP), Centers for Medicare and Medicaid Services (CMS) and/or COLA.

Applicable To

Employees of the Gundersen Health System clinical laboratory and Gundersen Tri-County Hospital laboratories.

Detail

INTENDED USE:
In vitro test for the quantitative determination of creatinine concentration in human serum, plasma, and urine on Roche/Hitachi cobas c systems.

PRINCIPLE:
This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase, the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration in the reaction mixture.

CLINICAL SIGNIFICANCE:
Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause. The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of GFR is given by the creatinine clearance test based on creatinine's concentration in urine and serum/plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum/plasma have been made. Among the various approaches suggested, two have found wide recognition: that of