

1. Introduction and literature review

1.1. Introduction

Diabetes mellitus is systemic metabolic disorder characterized by hyperglycemia resulting deficiency in insulin secretion, insulin action or both. There are two type of diabetes: Type I diabetes mellitus and Type II diabetes mellitus. Type I diabetes mellitus is result of pancreatic islet β -cell destruction, leading to decrease in, and eventually cessation of, insulin secretion .and type II diabetes mellitus is result from insulin resistance.

Generally, the injurious effects of hyperglycemia are separated into macro vascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro vascular complications (diabetic nephropathy, neuropathy, and retinopathy) (Marshall, 2008).

The earliest detectable change in the course of diabetic nephropathy is a thickening in the glomerulus. As diabetic nephropathy progresses, increasing numbers of glomeruli are destroyed by nodular glomerulosclerosis. Now the amounts of albumin being excreted in the urine increases, and may be detected by ordinary urinalysis techniques. At this stage, a kidney biopsy clearly shows diabetic nephropathy. Assessment of diabetic nephropathy: The most tests used to assess renal function are those that assess either the GFR or the integrity of the glomerular filtration barrier, and estimate of the GFR can be made by measuring the urinary excretion of substance that is completely filtered from the blood by the glomeruli and is not secreted, reabsorbed or metabolized by the renal tubule (clearance), experimentally inulin (plant polysaccharide) and creatinine are used. The accurate measurement of

creatinine clearance is difficult since it is necessary to obtain complete and accurately timed sample of urine (Marshall, 2008).

Plasma creatinine concentration is the most reliable simple biochemical test of glomerular function. The plasma creatinine concentration is inversely related to GFR. A normal plasma creatinine does not necessarily imply normal renal function. Estimated GFR: An alternative to formal measurement of creatinine clearance is to calculate and estimate of clearance from the serum creatinine concentration; this is done by a variety of formulas such as the Cockcroft-Gault formula. (Marshall, 2008).

HbA1c include haemoglobinA1c (HbA1c), HbA1 (which comprises HbA1a, HbA1b, and HbA1c), and total glycated hemoglobin (which comprises HbA1 plus other glycated haemoglobin species). Both the Diabetes Mellitus Complications and Control Trial (DCCT) and the United Kingdom Prospective Diabetes Study measured HbA1c. Thus, most clinical outcome data that relate glycemic control to the complications of diabetes are based on HbA1c measurement. The American Diabetes Association (ADA) recommends that HbA1c should be measured at least.

1.2. Literature review

1.2.1. Diabetes mellitus

Diabetes mellitus is group of metabolic disease characterized by hyperglycemia result from defects in insulin secretion or insulin action or both the chronic hyperglycemia of diabetes is associated with long-term damage ,dysfunction and failure of various organs especially the eye , kidneys , heart and blood vessels (Genuth *et al*, 2003).

The National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: type 1, insulin-dependent diabetes mellitus (IDDM); and type 2, non-insulin-dependent diabetes mellitus (NIDDM). Established in 1995, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the sponsorship of the American Diabetes Association, was given the task of updating the 1979 classification system. The proposed changes included eliminating the older terms of IDDM and NIDDM. The categories of type 1 and type 2 were retained, with the adoption of Arabic numerals instead of Roman numerals (Michael *et al*, 2010).

1.2.1.1. Types of diabetes mellitus

Diabetes mellitus is classified into two types; Type 1 diabetes mellitus which is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet Type 1 constitutes only 10% to 20% of all cases of diabetes and commonly occurs in childhood and adolescence. This disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with a genetic predisposition and causes the immune destruction of the cells of the pancreas and, therefore, a decreased production of insulin. Characteristics of type 1 diabetes mellitus

include abrupt onset, insulin dependence, and ketosis tendency. This diabetic type is genetically related.

One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia: islet cell auto antibodies, insulin autoantibodies, glutamic acid decarboxylase auto antibodies, and tyrosine phosphatase IA-2 and IA-2B auto antibodies destruction and a tendency to ketoacidosis (Michael *et al*, 2010).

Type 2 diabetes mellitus is characterized by decline in insulin action due to the resistance of tissue cells to the action of insulin. The problem is intensified by the inability of the beta cells of the pancreas to produce enough insulin to counteract the resistance. Thus, type 2 diabetes is a disorder of both insulin resistance and relative deficiency of insulin. Insulin resistance syndrome, also known as metabolic syndrome and syndrome X, affects the metabolism of many nutrients, including glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol. Individuals who are diagnosed with metabolic syndrome may show abdominal obesity and high blood pressure. Such individuals are at increased risk for cardiovascular disease. The etiology of type 2 diabetes is complex and multifaceted. There is evidence to show that there is an association of obesity with the development of type 2 diabetes. Other factors, such as family history of type 2 diabetes and lack of physical activity, have also been associated with the disorder. Previous diagnosis of gestational diabetes is a risk factor for type 2 diabetes mellitus, as are increasing age, hypertension, and dyslipidemia. Increased risk for developing the disease is also associated with membership in certain racial and ethnic groups, such as African-Americans, Hispanic-Americans, Native Americans, Asian Americans, and Pacific Islanders (Arneson , 2007).

Gestational diabetes (GDM) is any degree of glucose intolerance with onset or first recognition during pregnancy. Causes of gestational diabetes include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. However, this disease is associated with increased prenatal complications and an increased risk for development of diabetes in later years. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome, hypocalcemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of a mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant's oversupply of glucose is abruptly terminated, causing severe hypoglycemia (Michael *et al*, 2010).

Other types of diabetes mellitus

The fourth form of diabetes is termed other specific causes of diabetes. This form of hyperglycemia may be the secondary result of non-insulin-related events. Blood glucose levels are increased in endocrine disorders, such as Cushing's syndrome; in exocrine disorders, such as cystic fibrosis; and as a response to specific drugs, such as protease inhibitors and glucocorticoids. Other causes of this form of diabetes are the result of genetic defects that affect pancreatic beta cells or the action of insulin. The disorders of diabetes differ in their presentation as well as their etiology. Approximately 10% of diabetics are of the type 1 variety. The type 1 disease state usually occurs as acute illness, while type 2 diabetes progresses slowly over time (Arneson , 2007).

Type 1 glucose blood levels are usually more severe than type 2. Type 1 diabetics are more likely to develop ketoacidosis than are type 2 diabetes mellitus. Due to the etiology of disease, type 1 diabetes mellitus are insulin dependent, while most type 2 diabetes mellitus are not. Type 1

diabetes mellitus are younger (<18 years old when diagnosed) and thinner; type 2 diabetes mellitus are usually older (>40 years old when diagnosed) and more likely to be obese. However, these characteristics of presentation are not uniform to all type 1 and type 2 diabetes mellitus. Type 1 diabetes may be diagnosed after the age of 18 years. Type 2 diabetes mellitus may develop in obese children. Type 2 diabetes mellitus may need insulin if glycemia cannot be controlled by other measure (Arneson, 2007).

1.2.2.2. Complications of diabetes mellitus

Diabetes mellitus is a group of chronic diseases characterized by hyperglycemia. Modern medical care uses a vast array of lifestyle and pharmaceutical interventions aimed at preventing and controlling hyperglycemia. In addition to ensuring the adequate delivery of glucose to the tissues of the body, treatment of diabetes attempts to decrease the likelihood that the tissues of the body are harmed by hyperglycemia (Fowler, 2008).

The importance of protecting the body from hyperglycemia cannot be overstated; the direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in both type 1 and type 2 diabetes mellitus. Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). It is important for physicians to understand the relationship between diabetes and vascular disease because the prevalence of diabetes mellitus continues to increase in the United States, and the clinical armamentarium for primary and secondary prevention of these complications is also expanding (Fowler, 2008).

1.2.2.3 Acute complication of diabetes mellitus

Hypoglycemia: Probably most common cause of coma seen in diabetes mellitus patients

Diabetic ketoacidosis: diabetic ketoacidosis may be precipitated by infection, acute myocardial infarction or vomiting. The clinical consequences of diabetic ketoacidosis are due to:- hyperglycemia causing plasma hyperosmolarity.

Hyperosmolal non-ketotic coma: the term Hyperosmolal or 'precoma' is usually confined to condition in which there is marked hyperglycemia but not detectable ketoacidosis (Marshall ,2008).

Lactic acidosis: lactic acidosis can cause a high anion gap metabolic acidosis and coma. It may be due to the use of metformin in certain situations, such as high doses in very elderly, those with renal, liver, or cardiac failure or dehydrated patients (Marshall, 2008).

Long term complication of diabetes: Long term complication of diabetes mellitus fall into two groups: Micro vascular that is nephropathy, neuropathy and retinopathy. And macro vascular disease related atherosclerosis. This occurs in both types of diabetics (Marshall, 2008).

Diabetic retinopathy Diabetic retinopathy may be the most common micro vascular complication of diabetes. .The risk of developing diabetic retinopathy or other micro vascular complications of diabetes depends on both the duration and these verities of hyperglycemia. Development of diabetic retinopathy in patients with type 2 diabetes mellitus was found to be related to both severity of hyperglycemia and presence of hypertension, and most patients with type1diabetes develop evidence of retinopathy within20 years of diagnosis. Retinopathy may begin to

develop as early as 7 years before the diagnosis of diabetes mellitus in patients with type 2 diabetes mellitus (Fowler, 2008).

Diabetes mellitus causes micro and macro-vascular changes in the body and this includes diabetic nephropathy. It does this through hyperglycemia which leads to hyperfiltration and hence increased glomerular filtration rate. Later as the disease progresses the patient might progress into end stage renal disease.

It is therefore imperative that diabetic patients' renal function is assessed and monitored in order to avoid the risk of progression to end stage renal disease (Allen Meemeet *al*, 2009).

Diabetic nephropathy is the most common cause of end stage kidney disease (ESKD) in the United States and worldwide. Most diabetic patients with ESKD have type 2 diabetes mellitus. Since only a minority of type 2 diabetes mellitus patients develop kidney disease, predisposing factors for development of the disease are operative. In addition, once clinical kidney disease is evident, the rate of decline of glomerular filtration rate (GFR) is highly variable, ranging from 2 to 20 ml min⁻¹ yr⁻¹ the reasons for these differences in the rate of disease progression are multifactorial,

Including both non-modifiable and modifiable factors Blood pressure control is known to be important in preventing adverse cardiovascular and renal outcomes in diabetic patients with hypertension however it is not clear whether or not blood pressure is an important predictor of GFR decline in diabetes mellitus patients (David J Leehey, 2005).

1.2.2. Glycatedhaemoglobin

Glycatedhaemoglobin: Glycosylated hemoglobin is the term used to describe the formation of a hemoglobin compound produced when glucose (a reducing sugar) reacts with the aminogroup of haemoglobin (a protein).

Glycation is the nonenzymatic addition of sugar to amino groups of proteins. While virtually any protein in the body can be glycated (David B *et al.*, 2007).

These include haemoglobinA1c (HbA1c), HbA1 (which comprises HbA1a, HbA1b, and HbA1c), and total glycatedhaemoglobin (which comprises HbA1 plus other glycatedhaemoglobin species). Both the diabetes mellitus Complications and Control Trial (DCCT) and the United Kingdom Prospective Diabetes Study measured HbA1c. Thus, most clinical outcome data that relate glycemic control to the complications of diabetes are based on HbA1c measurement. The American Diabetes Association (ADA) recommends that HbA1c should be measured at least

Twice a year in persons with diabetes (David B *et al.*, 2007).

For convenience and ease of obtaining a sample, glycated hemoglobin is measured in the blood obtained from a patient.

Blood glucose concentrations exhibit wide diurnal fluctuations due to food ingestion, exercise, and other factors. In contrast, the concentration of glycated hemoglobin remains relatively stable with time. This is due to the life span of red blood cells, which is usually ~120 days. In individuals with a normal erythrocyte life span, glycated hemoglobin is directly proportional to the blood glucose concentration over the preceding 8 to 12 week (David B *et al.*, 2007).

A high cardiovascular morbidity and mortality has been associated with albuminuria and/or reduced kidney function, regardless of age. Thus, screening of HbA1c concentrations in the general population could be crucial to identify different HbA1c phenotypes associated with CKD and CVD, even in the absence of diabetes (DHernandez .D *et al.*2013).

1.2.3. Serum creatinine

Creatinine is formed from creatine and creatine phosphate in muscle and is excreted into the plasma at a constant rate related to muscle mass. Plasma creatinine is inversely related to glomerular filtration rate (GFR) and, although an imperfect measure, Creatine is synthesized primarily in the liver from arginine, glycine, and methionine. It is then transported to other tissues, such as muscle, where it is converted to creatine phosphate, which serves as a high-energy source. Creatine phosphate loses phosphoric acid and creatine loses water to form the cyclic compound, creatinine, which diffuses into the plasma and is excreted in the urine (Marshall, 2008).

The daily production of creatinine is about 2% of total body creatine, which remain constant if muscle mass is not significantly changed. In normal patients at steady state, the rate of creatinine production equals its excretion. Therefore, creatinine concentration in the serum creatinine vary little from day to day in patients with healthy kidneys. Although there is an inverse relationship between serum creatinine and kidney function, serum creatinine should not be the sole basis for the evaluation of renal function. There are several issues to consider when evaluating a patient's serum creatinine. Some of factors that affect serum creatinine concentration are muscle mass, sex, age, race, drugs, method of laboratory analysis and low protein diets. Additionally acute changes in patient's GFR may not be initially manifested as an increase in serum

creatinine concentration since it takes time for new steady state concentrations of serum creatinine to be achieved. the time required to reach 95% of steady state in patient with 50%,25%,and 10% of normal kidney function is about 1,2and 4days respectively (Mary ,2009).

The normal level of creatinine in the blood is approximately 0.6 to 1.2 milligrams (mg) per decilitre (dl) in adult males and 0.5 to 1.1 milligrams per deciliter in adult females. Muscular young or middle-aged adults may have more creatinine in their blood than the norm for the general population. Elderly persons, on the other hand, may have less creatinine in their blood than the normal. Infants have normal levels of about 0.2 or more, depending on their muscle development. A person with only one kidney may have a normal level of about 1.8 or 1.9. Creatinine levels that reach 2.0 or more in babies and 10.0 or more in adults may indicate the need for a dialysis machine to remove wastes from the blood. Certain drugs can sometimes cause abnormally elevated creatinine (Bazari H, 2007).

1.2.4. Creatinine clearance, a measure of the amount of creatinine eliminated from the blood by the kidneys, and GFR are used to gauge renal function. The GFR is the Creatinine clearance is usually reported in units of mL/minute Creatinine clearance overestimates GFR because a small amount of creatinine is reabsorbed by the renal tubules and up to 10% of urine creatinine is secreted by the tubules. However, Creatinine clearance provides a reasonable approximation of GFR.

The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because the active secretion of creatinine will account for a larger fraction of the total creatinine cleared. Ketoacids, cimetidine and trimethoprim reduce creatinine tubular

secretion and therefore increase the accuracy of the GFR estimate, particularly in severe renal dysfunction. (In the absence of secretion, creatinine behaves like inulin (Delanghe, J 1989).

Abnormal results (lower-than-normal creatinine clearance) may indicate: Acute tubular necrosis, Bladder outlet obstruction, Congestive heart failure, Dehydration, Glomerulonephritis, Renal ischemia (blood deficiency, Renal outflow obstruction (usually must affect both kidneys to reduce the creatinine clearance), Shock, Acute renal failure and Chronic renal failure and an End-stage renal disease (Bazari, 2007).

The Cockcroft-Gault formula

Although now less widely used and not validated for use screening for chronic kidney diseases. The Cockcroft-Gault formula, which is applicable over wider range of GFR also provides an estimate of the creatinine clearance and hence GFR. This formula takes in to account body weight in addition to sex, age and serum creatinine concentration:

Creatinine clearances (ml/min) =

$$\frac{(140 - \text{age in years}) \times (\text{weight in KG})}{72 \times \text{Serum creatinine in } \mu\text{mol/L}} \times 0.85 \text{ for females}$$

Serum creatinine in $\mu\text{mol/L} \times 0.81$

OR:

$$\frac{(140 - \text{age in years}) \times (\text{weight in KG})}{72 \times \text{Serum creatinine in mg/dl}} \times 0.85 \text{ for females}$$

72 x Serum creatinine in mg/dl

Cockcroft-Gault still has an interest in screening the decline in renal function in subjects with normal serum creatinine who are at risk, such as diabetic patients.

This formula is more accurate than MDRD In subjects with a normal or increased GFR (Marshall 2008).

1.5. Rationale

Until recently, type 2 diabetes mellitus was typically regarded as a disease of middle-aged and elderly. However, current data indicates that its prevalence among children and adolescents is increasing particularly among certain ethnic groups .This increase has been linked to the rising problem of obesity and physical inactivity. The other risk factors are a family history of type 2 diabetes mellitus.

Certain areas of the world have been mapped as type 2 diabetes mellitus red zone including Sudan, where the prevalence of type 2 diabetes mellitus has increased from 8.6% in Khartoum State in 1996, to as high as 19% in a recent house-hold survey . However, there are no published data on the prevalence of this problem among children and adolescents (Osman , 2013).

Diabetic nephropathy is the most common cause of end stage kidney disease (ESKD) in the United States and worldwide. Most diabetic patients with ESKD have type 2 diabetes mellitus.

The study aimed to evaluate HbA1c level in type 2 diabetic patients and also to assess the renal functions (which may impair as a result of complication of diabetes mellitus) through evaluation of creatinine clearance.

1.6. Objectives

General Objective:

To study the levels of creatinine clearance and HbA1c in type 2 diabetic patients.

Specific objectives:

1. To estimate serum creatinine and HBA₁C levels in type 2 diabetes mellitus and healthy individuals samples as control group
2. To determine creatinine clearance level by using formula in diabetics and control groups.
3. To correlate between levels of creatinine clearance and HbA1c in type 2 diabetes mellitus, duration of diseases and type of treatment.

2. Materials and Methods

2.1. Materials

2.1.1. Study design: This is a case control study.

2.1.2. Study area: The study was conducted in ALshaheedAlzepair center in Wad Madani city during March to June 2015 .

2.1.3. Study population: 50 patients with diabetes Mellitus Type 2 and 50 apparently healthy individuals to serve as control group were enrolled in this study.

Inclusion Criteria: Type 2 diabetic patients were included.

Exclusion criteria: non diabetics type 1 diabetics and type 2 diabetic patients with kidney diseases, anemia, hypertension and heart diseases were excluded.

2.1.4. Samples: About 5ml of venous blood were collected from each patient at the Random state, Divided to 2.5ml in EDTA containers for HbA1c and 2.5ml in plane containers for serum creatinine .The samples were collected under aseptic conditions . For serum creatinine, after clotting centrifuged for 3 minutes at 3000 RPM to obtain serum, and analyzed.

2.1.5 Ethical consideration: Patients who voluntarily accepted to participate in the study were included.

2.1.6.Equipments:

- Spectrophotormeter, model Model=BTS.302Serial NO. =801560278
- I chroma TM reader

- Centrifuge
- E DTA containers and Sterile plane containers
- Disposable syringes
- 70% alcohol
- Tourniquets
- Cotton
- Micropipettes (automatic pipettes)
- Graduated pipettes

2.1.7. Data analysis: Data was analyzed by using the SPSS computer program

2.2. Methodology

2.2.1 Estimation of serum creatinine concentration using Jaffe-kinetic method (Appendix II)

Principle Jaffe-kinetic method

In Jaffe-kinetic method, creatinine react with picric acid in alkaline solution to form red orange chromogens.

Calculation:

Concentration of test =

$$\frac{(\text{Optical density of test} \times \text{concentration of STD})}{\text{Optical density of STD}}$$

Optical density of STD

Reference values:

0.6 to 1.2 mg/dl in male

0.5 to 1.1 mg/dl in female

2.2.2 Calculation of creatinin clearance by The Cockcroft-Gault formula:

The cock-coft-gault formula, which is applicable over wider range of GFR also provides an estimate of the creatinine clearance and hence GFR. This formula takes in to account body weight in addition to sex, age and serum creatinine concentration.

$$= \frac{(140 - \text{age in years}) \times (\text{weight in KG})}{72 \times \text{Serum creatinine in mg/dl}} \quad (\times 0.85 \text{ for females})$$

72 x Serum creatinine in mg/dl

This formula is more accurate than MDRD In subjects with a normal or increased GFR .

Reference value:

Male 97 to 137 ml/min

Female 88 to 128 ml/min

2.2.3 Estimation of HbA1c method: (Appendix II)**Principle and procedure of method:**

HbA_{1c} is based in the fluoresce immunoassay technology, (Sandwich immune – detection method). Plasma was added to the mixture of hemolytic buffer and detection buffer, which result in hemolysis of red blood cell.

The sample mixture was loaded and migration on the matrix of the test cartridge, the higher concentration of the Hb A_{1c} produced a higher fluorescence, as signal interpreted and the result – displayed on Ichroma^M Reader in unit of % (NDSP), mmol/mol (IFCC) and mg / dl

Reference range is NGSP (%) : 4.5 – 6.5 %

IFCC (mmol / mol) : 26 – 78 mmol / mol.

2.2.4. Quality control

2.2.4.1. Quality control of jaffe method

We used a standard to calculate the results to obtain accurate results independent of the system or instrument used.

We used normal and abnormal known values control in each run to insure quality control, if the values were found outside of defined range we checked the instruments, reagent and procedure

2.2.4.2 Quality control of HbA_{1c} method

we used commercially available controls

(Boditeen med Inc, I chromtmHb A_{1c} control) to confirm the expected Q.C result, validity of assay and to assure the accuracy of result, we insured cartridge is ready to used with patient specimens.

3. Results

A hundred samples were collected (50 from type 2 diabetes mellitus patients and 50 from apparently healthy individual) to evaluate the level of creatinin, creatinin clearance and HbA1c. Analysis was done by jaffe kinetic method for creatinine, creatinine clearance was calculate by Cockcroft –Gault formula and HbA1c analyzed by I chroma instrument. SPSS computer program was used for data analysis and the results were as follow:

Table 3.1 shows comparison between means of levels of creatinine clearance, serum creatinine and HbA1c in test and control groups.

Crcl significant decreased, Hba1c significant increased, Sc significant increased in test groups

Table 3.2 shows the mean of HbA1c & creatinine clearance in diabetes mellitus patients according to drugs. insignificant relationship between HbA1c & creatinine clearance to the drugs.

Table 3.3 shows a classification of diabetes mellitus patients to controlled and uncontrolled groups according to the HbA1c.

Figure 3.1 a scatter plot show a significant negative correlation. (p. value 0.000. $r = -0.361$) between creatinine clearance and age among test group.

Figure 3.2 a scatter plot shows a significant positive correlation (p. value 0.004. $r = 0.403$) between Duration of diabetes millets and HbA1c among test group.

Figure 3.3 a scatter plot shows a significant negative correlation (p. value 0.000. $r = -0.612$) between creatinine clearance and HbA1c among test group.

Figure 3.4 shows correlation between creatinine clearance and duration among test group, p . Value 0.000. $r = -0.612$

Table 3.1: Comparison between means of creatinine clearance, serum creatinine and HbA1c in test and control groups.

Variable	Mean \pm SD	<i>P.</i> value
Creatinine clearance ml/min		
Test N =50	85.9 \pm 27.8	
Control N = 50	138.7 \pm 30.5	0.000
HbA1c %		
Test N= 50	10. 16 \pm 2.6	
Control N =50	05.37 \pm 0.6	0.000
Serum Creatinine mg/dl		
Test N=50	0.97 \pm 0.36	
Control N =50	0.57 \pm 0.11	0.000

T. test was used for analysis

P. value ≤ 0.05 is considered to be significant

Table 3.2: The mean of HbA1c and creatinine clearance in diabetes mellitus patients according to drugs.

	No	Mean \pm SD creatinine clearance in ml/minute	<i>p.</i> <i>value</i>	Mean \pm SD <i>HBA1C</i>	<i>p.</i> <i>value</i>
Tab group	50	88.4 \pm 27.2	0.181	10.2 \pm 2.6	0.506
Insulin group	50	72.6 \pm 28.5		9.5 \pm 2.9	

t. test was used for analysis

p. value ≤ 0.05 is considered to be significant

Table3.3: Controlled versus uncontroleddiabetes mellitus patients according to the HbA_{1c}

	Frequency	<i>Percent</i>
controlled	10	10%
Uncontrolled	40	40%

Reference range is ; 4.5-6.5%.

7% uncontrolled.>

7% controlled.<

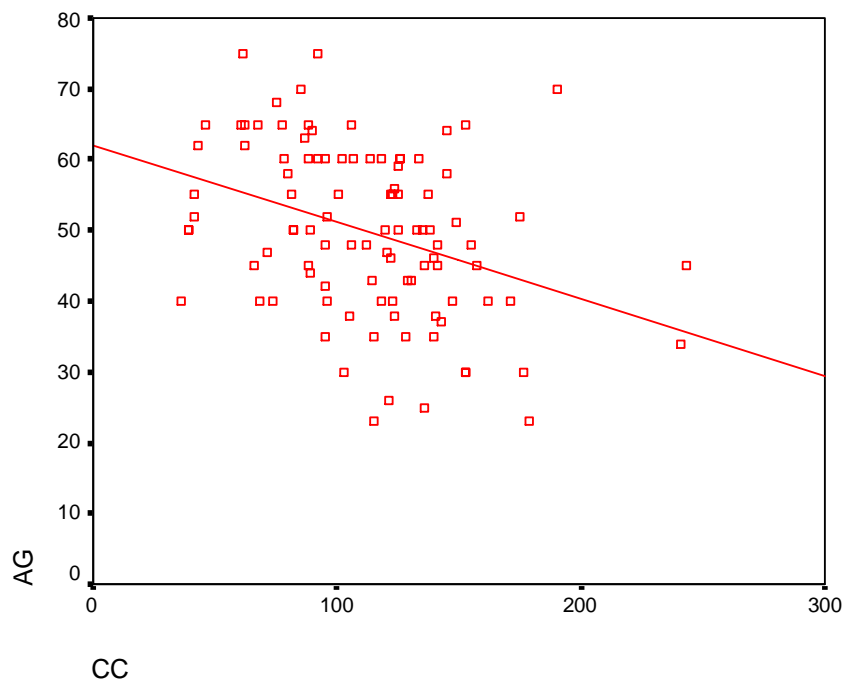


Figure3.1: Correlation between creatinine clearance and age among test group.

p . value 0.000. $r = -0.361$

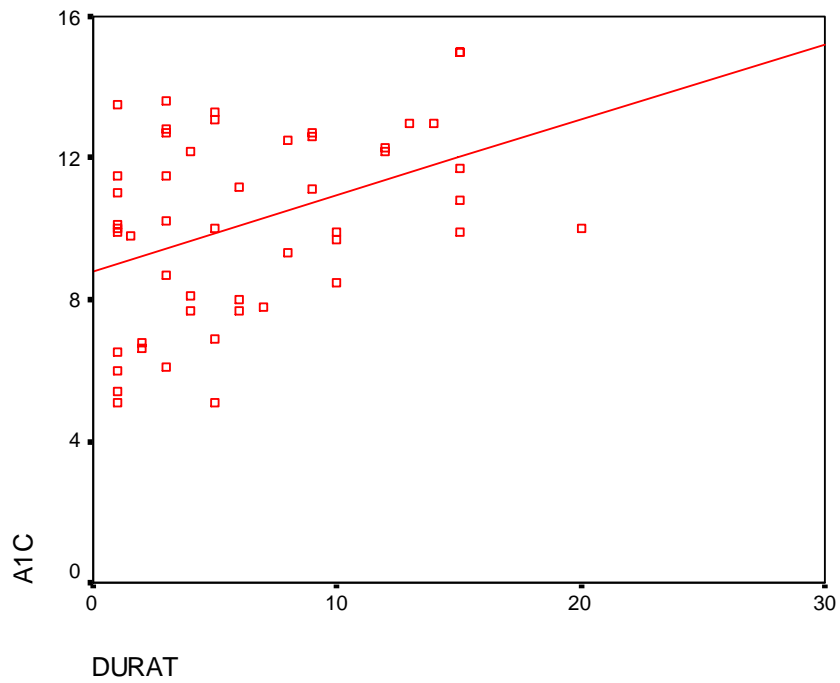


Figure3.2: Correlation between Duration of diabetes millets and HbA1c among test group.

p . Value 0.004. $r = 0.403$

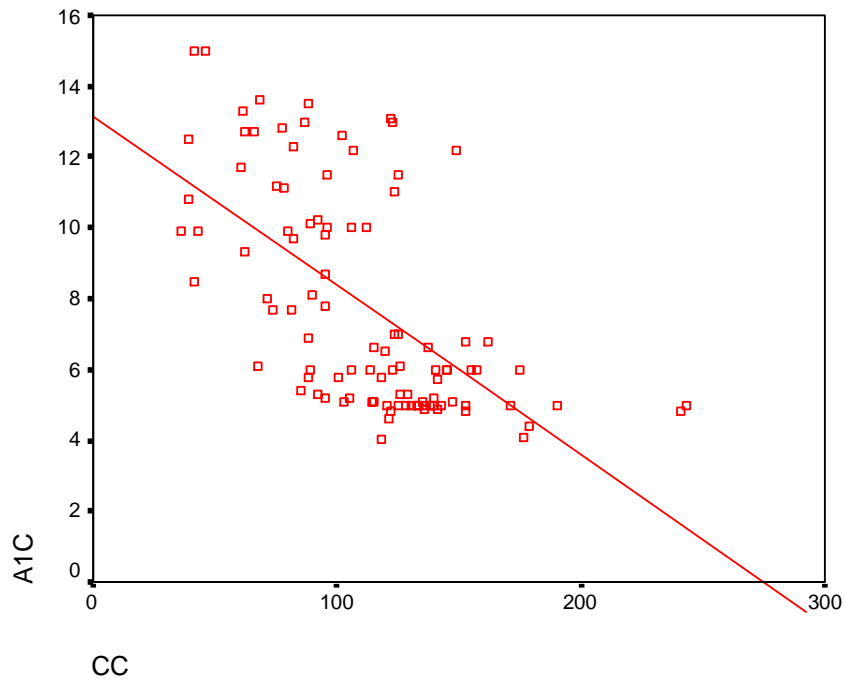


Figure3.3: Correlation between creatinineclearance and HbA1c among test group.

p . value 0.000. $r = -0.612$

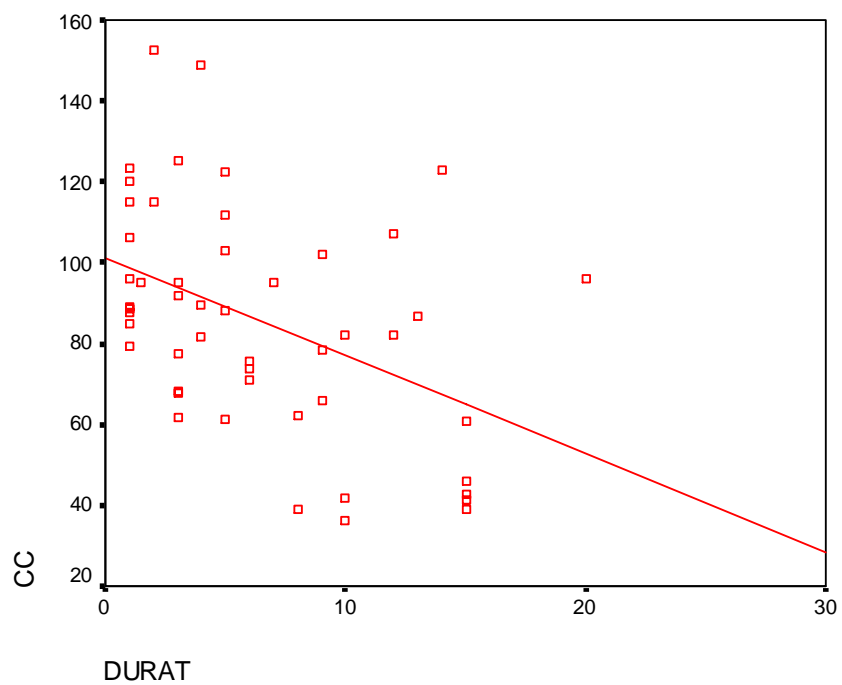


Figure 3.4: Correlation between creatinine clearance and duration among test group.

p . value 0.001. $r = -0.414$

4. Discussion, Conclusion and Recommendation

4.1. Discussion

Diabetes mellitus is a systemic metabolic disorder characterized by hyperglycemia resulting from a deficiency in insulin secretion, insulin action or both. Type II diabetes mellitus results from insulin resistance, generally, the injurious effects of hyperglycemia are separated into macro vascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro vascular complications (diabetic nephropathy, neuropathy, and retinopathy) (Marshall, 2008).

The present study is a case control study aimed to determine the level of creatinine clearance, serum creatinine, and HbA1c in patients with diabetes mellitus type 2. The study includes 100 individuals (50 of them were diabetic patients and 50 of them apparently healthy individuals as control group).

The finding showed that there was a significant decrease of creatinine clearance between case and control groups (mean \pm SD: 85.9 ± 27.8 , 138.7 ± 30.5 respectively), p value = 0.000. This finding was agreed with the study done by Nawar M and his group to show the decrease GFR estimated by Modification of diet in renal disease and Cockcroft –Gault equation show low GFR (≤ 60 ml /min per 1.73m^2) (Shara *et al*., 2009) and Allen Meeme to show the effect of glycaemic control on glomerular filtration rate in Diabetes, showed that decline GFR (mean : 157.4 ± 86.4 ml /min respectively) characterized patient with both type 1 and type 2 diabetes mellitus (p value = 0.001) (Allen Meeme, *et al*., 2009).

Also statistical analysis showed a significant increase of HbA1c in case compared to control group (mean \pm SD: 10.16 ± 2.6 , 5.37 ± 0.6 respectively) at level of p value = 0.000, this finding was agreed with

the study done by Jennifer Nicholas and his team conducted in recent HbA1c values and mortality risk in type 2 diabetes showed a significant increase in HbA1c in cases compared to control group and (mean \pm SD: 9 ± 75 Vs 7.7 ± 75) (Jennifer, *et al* .2013).

The statistical analysis also showed insignificant relationship between HBA1C and type of treatment (mean \pm SD: 10.2 ± 2.6 , 9.5 ± 2.9 .tab,insulin respectively) at level of p.value = 0.506, this was contradict with the results of study done by SH.Talib and his team, they were found a significant relationship between HBA1C and type of treatment (mean \pm SD: 8.18 ± 0.76 VS 7.26 ± 0.47 tab,insulin respectively) (Talib,etal.2014).of diabetic patientswere controlled and 40% wereuncontrolled accordingto HbA1C.

The results of this study showed a significant negative correlation between creatinine clearance and age and this agreed with the study done by Boston in Tutis university , they found decline of creatinine clearance with age by $0.63 \text{ ml/min per } 1.73 \text{ m}^2$ (Boston , 1994).

And the study results showed a significant positive correlation between duration of diabetes millets and HbA1c , and this results agree with study done by Gluseppe , they found increased HbA1c with longer diabetes duration (HbA1c = 6.7 ± 0.2 , duration 10-14 years) (Gluseppe *et al*, 2013).

The statistical analysis also showed that creatinine clearance is a significantly negative correlation with HbA1c levels. This results agreed with results of study done by Gluseppe and his team, they found an increase in HbA1c with decrease of creatinine clearance, $\text{GFR} < 60 \text{ ml/min}$, HbA1c = $7.5 \pm 0.4\%$ (Gluseppe *et al*, 2013).

The statistical analysis also showed insignificant relationship between Creatinine clearanse and type of treatment (mean \pm SD: 88.4 \pm 27.2, 72.6 \pm 28.5 respectively) at level of p.value = 0.181.

The statistical analysis also showed significant negative Correlation between Creatinine clearance and duration (p. Value 0.001 r = - 0.414)

4.2. Conclusions

1. The serum creatinine and HbA1c are significantly increased while the creatinine clearance is significantly decreased in diabetes mellitus type 2.
2. There were insignificant relationship between HBA1C, creatinine clearance and type of diabetes mellitus treatment.
3. The creatinine clearance had a significantnegative correlation with age and duration in diabetic patients.
4. The HbA1c had a significant positive correlation with duration of diabetes millets.
5. The creatinine clearance had a significantly negative correlation with HbA1c levels in diabetic patients.

4.3. Recommendations

1. Further studies with larger sample size should be done to get reliable results.
2. A Cohort study needs to be done to follow up Diabetic patient type2 for long time to actually assess their overall renal disease progression while on treatment.

References

Allen Meeme and HanningtonKasoz. (2009), Effect of glycaemic control on glomerular filtration rate in Diabetes, African Health Sciences Volume:9, Issue1, 23-26.

ArnesonW and Brickell.(2007), Clinical Chemistry a Laboratory Perspective,Library of Congress Cataloging-in-Publication Data .

Bazari H.(2007), Approach to the patient with renal disease, 23rd edition, Philadelphia, Pa: Saunders Elsevier.

David B. Sacks, MB and ChB.(.2007), Correlation between Hemoglobin A1c (HbA1c) and Average Blood Glucose, Diabetes Science and Technology Journal Volume: 1, Issue 6,801-803.

David J Leehey, Holly J Kramer, Tarek M Daoud, Maninder P Chatha, and Majd A Isreb. (2005), Progression of kidney disease in type 2 diabetes beyond blood pressure control . BMC Nephrology, 6-8 .

Delanghe J, De Slypere JP, De Buyzere M, Robbrecht J, Wieme R and Vermeulen A. (1989), Normal reference values for creatine, creatinine, and carnitine are lower in vegetarians" Clin. Chem..

Domingo Hernandez, , Ana Espejo-Gil, M Rosa Bernal-Lopez, , Jose Mancera-Romero, Antonio J Baca-Osorio, Francisco J Tinahones, Ana M Armas-Padron, Pedro Ruiz-Esteban, Armando Torres and

Ricardo Gomez-Huelgas. (2013), Association of HbA1c and cardiovascular and renal disease in an adult Mediterranean population, BMC nephropathy.14:151, 1471-2369.

Fowler M J. (2008), ClinicalDiabetes, p 77-81.

Giuseppe Penno,AnnaSolini,Enzo Bonor,Cecilia Fondelli,Emanuela Orsi,Gianpalo Zerbini,Susanna Morano,Franco Cavalot,Olga Lamacchia,LuigiLaviola,GiuseppePugliese and Antonion

Necolucci.(2013),HbA1cVariability as Independent correlate of Nephropathy,in patient With Type2 Diabetes.Diabetes Care,Volume: 36,2301-2310.

Hala AM Osman , Nadia E Isadek and Mohamed A Abdullah. (2013), type 2 diabetes in Sudanese Children and Adolescents , Sudanese Journal of pediatrics vol : 13 issue 13 .17-23.

Jennifer Nicholas, Judith , Charlton andAlex Dergon. (2013) ,Martine recent HBA1C Values and mortality risk in type2 diabetes .open access volume:8 issue 7 ,68008.

Marshall W J, Bangert S K .(2008) , Clinical Chemistry, 6thedition.**British** library cataloging in publication data .Mellitus patientsAfrican Health Sciences Special Issue 1.

Micheal, Bishop, Edward.P,Fody, Larry E and Schoeff.(2010) ,Clinical chemistry : techniques, principles, correlations 6thEdition.

Nawar M , Shara , Helein E, Resnick , lilu , Jiagion Xu , SumaVupputuri ,Barbar V ,Howard and Jason G . Umanns .(2009),decrease GFR estimated by Modification of diet in renal disease and Cockcrft –gault. equation. J Nephrol; 22(3) : 373-380 .

SH talib,vigayKumer,AshishTapadiand AniketInamdar.(2014), Astudy in Insulin and its analogues for control of upnormal glycosylated HBA1C or near controlled type 2 diabetes mellitus patients on oral hypoglycemic agent , in India , journal , Indian academy of clinical medicine , vol 15 (2):106-150.

Sugam Shrestha , prajwal Gyawali , Rojeet Shrestha, Bibek Poudel , Manoj Sgidel , PrashantRegmi , ManoranjanShresthaand Binod kumar yadav.(2008),serum urea and creatinine in diabetic and nondiabetic subjects Journal of Nepal volume: 9 ,Issue1. 11-12.

Appendix I

College of Graduate Studies

Clinical Chemistry

Questionnaire

Name:NO ()

Age:years

Sex: Male ☐ Female ☐

Weight:kg

Duration of diabetes mellitus:years

Other diseases

Drugs used: Tab ☐ insulin ☐

Serum creatinine:mg/dl

Creatinine clearance:
.....ml/minute

HbA_{1c}:%

Date: