بسم الله الرحمن الرحيم

Sudan University for science and technology
College of post graduated studies

Estimations of Serum Creatinine, Urea, and Some Urine parameters for Type2 Diabetic Patients After More than Ten Years Disease Onset

تقويم مستوي الكرياتيتين و البولينا في مصل الدم وفحص بعض معاملات البول للمرضى المصابين بارتفاع السكر النوع الثاني لاكثر من عشرة اعوام بعد حدوث المرض

A thesis is submitted for partial fulfillment of M.Sc. degree in medical laboratory science (clinical chemistry)

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DEDICATION

TO…….

My model role father , moatasim ahmed haj fadol

TO……

My sweet hearted supportive mother Dar-alsalam ahmed ali Whom unless Allah and then Her I will not write this research, I want to said to her you Are Given me the live and hope and arise at dote Acquaintance and Learning.

TO……

ALL my kin especially my aunt son cardiologist:

Dr. ASIM ALMAHI, uncle osman, aunt nyda and friends

TO…….

Whom pave the way to me to incoming to heyday of knowledge

I dedicate this modest hard work

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ABSTRACT

`This study aims assess renal function described as serum creatinine, urea and some urine parameters in type2 diabetics' after more than ten years onset. To detect impact of patient age and the duration on renal function. One hundred people (57 males and 43 females) were divided into Fifty healthy (30 males and 20 females) was the control group. And Fifty (27 males and 23 females), mean age±SD(67.42±8.8),meanfastingbloodglucose±SD(201.52±26,274) mg/dl. At probability less than 0.05 serum creatinine and urea highly significant in Diabetics' compared to there copartner with p.value 0.000 and mean±SD (3.29±2.25 , 1.28±0.35) mg/dl and (63.88±3.38, 21.28±5.48) mg/dl for Diabetics' and non Diabetics' respectively. urine parameters showed elevation and presence of albumin (35)% in Diabetics' and (1)% in non Diabetics', sugar (99)% for Diabetics' and (0)% in non Diabetics', acetone (60)% for Diabetics' and (0)% for non Diabetics', pus (55)% for Diabetics' and (5)% for non Diabetics', Calcium oxalate (66)% for Diabetics' and (1)% for non Diabetics', RBCs (5)% for Diabetics' and (2)% for non Diabetics', Yeast cells(2)% for Diabetics' and (1)% for non Diabetics', Epithelial cells (11)% for Diabetics' and (1)% for non Diabetics'. At correlation coefficient .283. positive correlation between urea and duration. And at correlation coefficient -.015 no correlation between creatinine and duration. conclusion there were Significant increased in serum urea, creatinine, and Albumin, sugar, acetone, pus and Calcium oxalate and positive correlation between duration and urea and no between correlation between duration creatinine in case group. There were not significant changes in RBCs, Yeast cells and epithelial cells. Patients should have regular screening Renal function tests.

الخلاصة

تهدف هذه الدراسة لقياس وظائف الكلي ممثلة في البولينا والكرياتينين والبول عام في مرضي السكري النوع الثاني السودانيين المصابين باكثر من عشر سنوات بالسكري لكشف تأثيرالعمر وعدد سنوات الاصابه بالمرض علي وظائف الكلي.

مائة شخص (57 ذكر و 43 انثي) قسموا الي مجموعتين ,50 فرد اصحاء (30 ذكر و20 انثي) متوسط اعمارهم 44 سنة هم كمقياس. والمجموه الثانيه هم الحالات 50 فرد (27 ذكر و23 انثي) مع متوسط اعمار 67 سنه , متوسط فحص سكري صيام 201 مع اكثر من عشر سنوات اصابه بالسكري.

علي احتماليه اقل من05. 0 في هذه الدراسه اليوريا والكرياتينين فيهما زيادة مؤثرة عاليه في مرضي السكري مقارنة مع قرنائهم الاصحاء مع معدل 000. 0 ومتوسط , 2.25±3.29) mg/dl (63.88±3.38 , 21.28±5.48) mg/dl (1.28±0.35 لمرضي السكري والاصحاء علي التوالي. اما بالنسبه لفحص البول فقد اظهر ارتفاع وظهور كل من الزلال بنسبة %(35)لمرضي السكري و%(1) للاصحاء ,السكر بنسبة%(99) لمرضي السكري و للاصحاء %(0) ,الاستون بنسبة %(60) لمرضي السكري و للاصحاء %(0), خلايا دم ابيض ميته بنسبة %(5) لمرضي السكري و%(5) للاصحاء ,خلايا دم احمر بنسبة %(5)لمرضي السكري و%(2) للاصحاء , املاحات كلي بنسبة%(66) لمرضي السكري و%(2) للاصحاء , خلايا خماير بنسبة%(2) لمرضي السكري و%(1) للاصحاء, خلايا جلد محيطه بنسبة%(11) لمرضي السكري و%(1) للاصحاء. ايضا علي معامل ارتباط 0.238 توجد علاقة ارتباط موجبه بين بين اليوريا وعدد سنوات الاصابه بالسكري, وعلي015 . - لا توجد علاقة ارتباط بين الكرياتينين وعدد سنوات الاصابه. وجد ان هنالك زيادة مؤثرة في مستوي كل من اليوريا والكرياتينين في الدم وفي البول في مستوي كل من الزلال السكر الاستون خلايا الدم \ الابيض الميته واملاحات الكلي في المصابين بمرض السكري النوع الثاني لاكثر من عشر سنوات. ولا يوجد اي تاثير او تغير في البول في كل من خلايا الدم الاحمر خلايا الخماير وخلاياالجلد المحيطه بالمسالك البوليه, ايضا وجد ان هنالك علاقة ارتباط قويه موجبه بين البولينا ومدة سنوات الاصابه بالسكري ولا توجد علاقة بين الكرياتينين ومدة سنوات الاصابه بالسكري. يوصي لمرضي السكري لاكثر من عشر سنوات بالفحص الدوري لوظائف الكلي والزلال في البول بالاضافة لفحص البول عام.

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Abbreviations

ADA: American Diabetes Association

AGAT: Areganine Guanidionacetic Acid Transferase

ATP: Adenosine Triphosphate

BMI: Body Mass Index

BUN: Blood Urea Nitrogen

CHD: Coronary Heart Disease

CK: Creatinine phosphate

Cr: Creatinine

CRF: Chronic Renal Failure

CT: Computed Tomography

DKA: Diabatics ketoacidosis

DM: Diabetes Melletus

DNA: Dinucluted Acid

ESRD: End Stage Renal Diseases

FBG: Fasting Blood Glucose

GAA: Guanidionacetic Acid

GAD: Glutamic Acid Decarboxylase

GAMT: Glycine Amino Transferase

GDM: Gestintial Diabetes Mellitus

GFR: Glomerular Filtration Rate

HNF: Hepatic Nuclear transcription Factor

Hb A1C: Hemoglobin A1c

ICD: International Classification of Diseases

IDDM: Insulin Dependant Diabetes Mellitus

IGT: Impaired Glucose Tolerance

IND: International Nomenclature of Diseases

LADA: Latent autoimmune Diabetes in Adult

MELAS: Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke like syndrome

MHC: Major Histo Compatibility

MODY: Maturity Onset Diabetes of Youth

MRADM: Malnutrition Related Diabetes Melletus

mRNA: Messenger Ribonucleic acid

NIDDM: Non Insulin Dependant Diabetes Mellitus

OGTT: Oral Glucose Tolerance Test

PCr: Phospho Creatinine

Pi: Phosphors

RBCs: Red Blood Cells

SD: Stander deviation

SPSS: Statistical Package for Social Sciences

TCF7L2: Transcription Factor 7-Like 2 gene

Chapter one

1. Introduction

1. Introduction

Diabetes mellitus is a syndrome with disordered metabolism and inappropriate hyperglycaemia due to either deficiency of Insulin secretion or to combination of insulin resistance or both and inadequate insulin to compensate (Tierney, *et al.,* 2002).

Type 2 diabetes mellitus is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells of pancreas. Type2 diabetes mellitus result rom complex interactions between environmental and genetic factors. Presumably, the disease develops when a diabetogenic lifestyle (ie, excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed on a susceptible genotype. The body mass index (BMI) at which excess weight increases risk for diabetes varies with different racial groups (Unger, *et al.*, 2010).

effects of diabetes Mellitus on renal function after 10 years onset include Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Nephropathy is the leading cause of chronic renal failure worldwide and is responsible for renal failure in about one third of patients who undergo dialysis.

It is suggested that patients with common risk factors including greater duration of diabetes, hypertension, poor metabolic control, smoking, obesity and hyperlipidemia are more prone to develop diabetic complications.

Diabetic nephropathy occurs in approximately one third types 2 Diabetics (Rehman, *et al*., 2005).

Assessment of a patient's renal function may be used for two different purposes. One is to diagnose impaired renal function, and the other is to

detect the presence of a progressive loss of renal function over time (Hsu, *et al.*, 2002).

in Sudan to the best of our knowledge there is lack of information regarding the impact of prolong type2 diabetes mellitus on the renalfunction. So this study is aims to estimation the renal function test described as serum creatinine, urea and urine general in diabeticspatient after more than ten years disease onset.

Chapter two

2. Literature review

2.1-Diabetes mellitus (DM)

2.1.1-Definition

The term diabetes mellitus describes a metabolic disorder of multiple aetiology char acterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs (Alberti, et al., 1998).

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state maydevelop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes. These include processeswhich destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues

resulting from insensitivity or lack of insulin (Diabetes Care , *et al.*, 1997).

2.1.2-Classification

2.1.2.1- Earlier classifications

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and, in modified form, in 1985.

The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition–related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM).

These were reflected in the subsequent International Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD–10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented acompromise between clinical and aetiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or aetiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary etiological classification (WHO, *et al.*, 1980).

2.1.2.2- Revised classification

The classification encompasses both clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia, as suggested by Kuzuya and Matsuda. The clinical staging reflects that diabetes, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology. The classification by aetiological type results from improved understanding of the causes of diabetes mellitus (Kuzuya, *et al*., 1997).

2.1.3- Aetiological types

The aetiological types designate defects, disorders or processes which often result in diabetes mellitus.

2.1.3.1-Type 1

2.1.3.1.1-Autoimmune Diabetes Mellitus

This form of diabetes, previously encompassed by the terms insulin–dependent diabetes, Type 1 diabetes, or juvenile– onset diabetes, results from autoimmune mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others . The rapidly progressive form is commonly observed in children, but also may occur in adults . The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease .

Others have modest fasting hyperglycaemia that can rapidly change

to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta–cell function, sufficient to prevent ketoacidosis, for many years . Individuals with this form of Type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis . At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C–peptide . Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85–90 % of individuals with Type 1 diabetes mellitus when fasting diabetic hyperglycaemia is initially detected . The peak incidence of this form of Type diabetes occurs in childhood and adolescence, but the onsetmay occur at any age, ranging from childhood to the ninth decade of life (Molbak, *et al*., 1994).

2.1.3.1.2-Idiopathic

There are some forms of Type 1 diabetes which have no known aetiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity.

This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis (Mclarty, *et al*., 1990).

2.1.3.1.3-Signs and symptoms

The classic symptoms of type 1 diabetes are as follows:

- Polyuria, Polydipsia, Polyphagia
- Unexplained weight loss

The onset of symptomatic disease may be sudden. It is not unusual for patients with type 1 diabetes to present with diabetic ketoacidosis (DKA) (Medtronic, *et al*., 2013).

2.1.3.1.4-Diagnosis

Diagnostic criteria by the American Diabetes Association (ADA) include the following:

- A fasting plasma glucose (FPG) level ≥126 mg/dL (7.0 mmol/L), *or*
- A 2-hour plasma glucose level ≥200 mg/dL (11.1 mmol/L) during a 75-g oral glucose tolerance test (OGTT), *or*
- A random plasma glucose ≥200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis (Diabetes Care *et al* 2010).

2.1.3.1.5-Lab studies

A fingerstick glucose test is appropriate for virtually all patients withdiabetes. All fingerstick capillary glucose levels must be confirmed in serum or plasma to make the diagnosis. All other laboratorystudies should be selected or omitted on the basis of the individual clinical situation. An international expert committee appointed by the ADA, the European Association for the Study of Diabetes, and the International Diabetes Association recommended the HbA_{1c} assay for diagnosing type 1 diabetes only when the condition is suspected but the classic symptoms are absent (Diabetes Care, *et al.*, 2009).

2.1.3.1.6-Screening

Screening for type 1 diabetes in asymptomatic low-risk individualsis not recommended. However, in patients at high risk (eg, those who have first-degree relatives with type 1 diabetes), it may be appropriate to perform annual screening for anti-islet antibodies before the age of 10 years, additional screening during adolescence **(**Vehik, *et al.*, 2011).

2.1.3.1.7-Genetic factors

Although the genetic aspect of type 1 DM is complex, with multiple genes involved, there is a high sibling relative risk**.** Whereas dizygotic twins

have a 5-6% concordance rate for type 1 DM, monozygotic twins will share the diagnosis more than 50% of the time by the age of 40 years.

For the child of a parent with type 1 DM, the risk varies according to whether the mother or the father has diabetes. Children whose mother has type 1 DM have a 2-3% risk of developing the disease,

whereas those whose father has the disease have a 5-6% risk. When both parents are diabetic, the risk rises to almost 30%. In addition, the risk for children of parents with type 1 DM is slightly higher if onset of the disease occurred before age 11 years and slightly lower if the onset occurred after the parent's 11th birthday (Redondo, *et al*., 2008).

The genetic contribution to type 1 DM is also reflected in the significant variance in the frequency of the disease among different ethnic populations. Type 1 DM is most prevalent in European populations, with people from northern Europe more often affected than those from Mediterranean regions. The disease is least prevalent in East Asians (Borchers, *et al*., 2010).

Genome-wide association studies have identified several loci that are associated with type 1 DM, but few causal relations have been established. The genomic region most strongly associated with otherautoimmune diseases, the major histocompatibility complex (MHC), is the location of several susceptibility loci for type 1 DM—in particular, class II HLA DR and DQ haplotypes (Erlich, *et al*., 2008).

2.1.3.2-Type 2

Diabetes mellitus of this type previously encompassed non–insulin–dependent diabetes, or adult–onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. People with this type of diabetes frequently are resistant to the action of insulin. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive.

This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes **.** Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Defronzo, *et al*., 1997). There are probably several different mechanisms which result in this form of diabetes, and it is likely number of people in this category will decrease in the future as identification of specific pathogeneticprocesses and genetic defects permits better differentiation and a more definitive classification with movement into "Other types".

Although the specific aetiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes. The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (Campbell, *et al*., 1993).

Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region**.** Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection.

Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels inthese diabetic patients would be expected to result in even higher insulin values had their beta–cell function been normal **.** Thus,insulin secretion is defective and insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycaemia but is not restored to normal **.**The risk of

developing Type 2 diabetes increases with age, obesity, and lack of physical activity **.**It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia. Its frequency varies in different racial/ethnic subgroups (Zimmet, *et al.*, 1992).

It is often associated with strong familial, likely genetic, predisposition**.** However, the genetics of this form of diabetes are complex and not clearly defined. Some patients who present with a clinical picture consistent with Type 2 diabetes have autoantibodies similar to those found in Type 1 diabetes, and may masquerade as Type 2 diabetes if antibody determinations are not made. Patients who are non–obese or who have relatives with Type 1 diabetes and who are of Northern European origin may be suspected of having late onset Type 1 diabetes. Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion (Valle, *et al.*, 1997).

2.1.3.2.1-Signs and symptoms

Many patients with type 2 diabetes are asymptomatic. Clinical manifestations include the following:

- Classic symptoms: Polyuria, polydipsia, polyphagia, and weight loss
- Blurred vision
- Lower-extremity paresthesias
- Yeast infections (eg, balanitis in men) (Busko, *et al.*, 2014).

2.1.3.2.2-Diagnosis

Diagnostic criteria by the American Diabetes Association (ADA) include the following:

- A fasting plasma glucose (FPG) level of 126 mg/dL (7.0 mmol/L) or higher, or

- A 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), *or*A random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis Whether a hemoglobin A1c (HbA1c) level of 6.5% or higher should e a primary diagnostic criterion or an optional criterion remains a point of controversy.

Indications for diabetes screening in asymptomatic adults includes the following:

- Sustained blood pressure >135/80 mm Hg
- Overweight and 1 or more other risk factors for diabetes (eg, first-degree relative with diabetes, BP >140/90 mm Hg, and HDL < 35 mg/dL and/or triglyceride level >250 mg/dL) ADA recommends screening at age 45 years in the absence of the above criteria. (American Diabetes Association, *et al*., 2012).

2.1.3.2.3-Pathophysiology

Type 2 diabetes is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance. Elevated levels of free fatty acids and proinflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat (Unger, *et al*., 2010).

A role for excess glucagon cannot be underestimated; indeed, type 2 diabetes is an islet paracrinopathy in which the reciprocal relationship between the glucagon-secreting alpha cell and the insulin-secreting beta cell is lost, leading to hyperglucagonemia and hence the consequent hyperglycemia. For type 2 diabetes mellitus to occur, both insulin resistance and inadequate insulin secretion must exist. For example, all overweight individuals have insulin resistance, but diabetes develops only

in those who can not increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low for the level of glycemia.

With prolonged diabetes, atrophy of the pancreas may occur. A study by Philippe et al used computed tomography (CT) scan findings, glucagon stimulation test results, and fecal elastase-1 measurements to confirm reduced pancreatic volume in individuals with a median 15-year history of diabetes mellitus (range, 5-26 years).This may also explain the associated exocrine deficiency seen in prolonged diabetes (Philippe, *et al.*, 2011).

2.1.3.2.4-Previous

Etiology

The etiology of type 2 diabetes mellitus appears to involve complex interactions between environmental and genetic factors. Presumably,

the disease develops when a diabetogenic lifestyle (ie, excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed on a susceptible genotype. The body mass index (BMI) at which excess weight increases risk for diabetes varies with different racial groups. For example, compared with persons of European ancestry, persons of Asian ancestry are at increased risk for diabetes at lower levels of overweight. Hypertension and prehypertension are associated with a greater risk of developing diabetes in whites than in African Americans. In addition, an in utero environment resulting in low birth weight may predispose some individuals to develop type 2 diabetes mellitus**.** Infant weight velocity has a small, indirect effect on adult insulin resistance, and this is primarily mediated through its effect on BMI and waist circumferenceAbout 90% of patients who develop type 2 diabetes mellitus are obese. However, a large, population-based, prospective study has shown that an energy-dense diet may be a risk factor for the development of diabetes that is independent of baselineobesity (Wang, *et al.*, 2008).

Some studies suggest that environmental pollutants may play a role in the development and progression of type 2 diabetes mellitus.

A structured and planned platform is needed to fully explore thediabetes inducing potential of environmental pollutants (Hectors, *et al.*, 2011).

Secondary diabetes may occur in patients taking glucocorticoids or when patients have conditions that antagonize the actions of insulin

(eg, Cushing syndrome, acromegaly, pheochromocytoma).

2.1.3.2.5-Genetic influences

The genetics of type 2 diabetes are complex and not completely understood. Evidence supports the involvement of multiple genes in pancreatic beta-cell failure and insulin resistance. Genome-wide association studies have identified dozens of common genetic variants associated with increased risk for type 2 diabetes. Of the variants thus far discovered, the one with the strongest effect on susceptibility is the transcription factor 7–like 2 (TCF7L2) gene (Billings, *et al.*, 2010).

Identified genetic variants account for only about 10% of the heritable component of most type 2 diabetes. An international research consortium found that use of a 40-SNP genetic risk score improves the ability to make an approximate 8-year risk prediction for diabetes beyond that which is achievable when only common clinical diabetes risk factors are used. Moreover, the predictive ability is better in younger persons (in whom early preventive strategies could delay diabetes onset) than in those older than 50 years.

Some forms of diabetes have a clear association with genetic defects. The syndrome historically known as maturity onset diabetes of youth (MODY), which is now understood to be a variety of defects in beta-cell function, accounts for 2-5% of individuals with type 2 diabetes who present at a young age and have mild disease.

The trait is autosomal dominant and can be screened for through commercial laboratories (Miguel, *et al.*, 2011).

2.1.4- Other Specific Types

2.1.4.1- Genetic defects of beta–cell function

Several forms of the diabetic state may be associated with monogenic defects in beta–cell function, frequently characterized by on set of mild hyperglycaemia at an early age (generally before age 25 years). They are usually inherited in an autosomal dominant pattern. Patients with these forms of diabetes, formerly referred to as maturity– onset diabetes of the young (MODY), have impaired insulin secretion with minimal or no defect in insulin action.

Abnormalities at three genetic loci on different chromosomes have now been characterized. The most common form is associated with mutations on chromosome 12 in a hepatic nuclear transcriptionfactor referred to as HNF1" . Asecond form is associated with mutations in the glucokinase gene on chromosome 7p (Byrne, *et al.*, 1996).

Glucokinase converts glucose to glucose–6–phosphate, the metabolism of which in turn stimulates insulin secretion by the beta cell. Thus, glucokinase serves as the “glucose sensor” for the betacell. Because of defects in the glucokinase gene, increased levels of glucose are necessary to elicit normal levels of insulin secretion. A third form is associated with a mutation in the HNF4" gene on chromosome 20q **.** HNF4" is a transcription factor which is involved in the regulation of the expression of HNF1". A fourth variant has recently been ascribed to mutations in another transcription factor gene, IPF–1, which in its homozygous form leads to total pancreatic agenesis **.**Specific genetic defects in other individuals who have a similar clinical presentation are currently being defined. Point mutations in mitochondrial DNA have been found to be

associated with diabetes mellitus and deafness **.** The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A to G substitution. An identical lesion occurs in the MELAS syndrome(Mitochondrial myopathy,Encephalopathy, Lactic Acidosis, and Stroke–like syndrome); however, diabetes is not part of this syndrome, suggesting for unknown reasons different phenotypic expressions of this genetic lesion (Gruppuso, *et al*., 1984).

Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families. Such traits are usually inherited in an autosomal dominant pattern and the resultant carbohydrate intolerance is mild. Similarly, mutant insulin molecules with impaired receptor binding have been identified in a few families. These are also associated with autosomal inheritance and either normal or only mildly impaired carbohydrate metabolism (Sanz, *et al*., 1986).

2.1.4.2- Genetic defects in insulin action

There are some unusual causes of diabetes which result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the hyperglycaemia to symptomatic diabetes**.** Some individuals with these mutations have acanthosis nigricans. Women may have virilization and have enlarged, cystic ovaries. In the past, this syndrome was termed Type A insulin resistance. Leprechaunism and Rabson– Mendenhall syndrome are two paediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance**.**

The former has characteristic facial features while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia (Taylor, *et al*., 1992).

2.1.4.3-Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes.

Acquired processes include pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy**.** With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in beta–cell mass (Permert, *et al*., 1994).

If extensive enough, cystic fibrosis and haemochromatosis will also damage beta cells and impair insulin secretion**.** (Moran, *et al*., 1994)

Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcification on X–ray and ductal dilatation**.** Pancreatic fibrosis and calcified stones in the exocrine ducts are found at autopsy (Yajnik, *et al*., 1992).

2.1.4.4-Endocrinopathies

Several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases associated with excess secretion of these hormones can cause diabetes (e.g. Acromegaly, Cushing's Syndrome, Glucagonoma and Phaeochromocytoma)**.** These forms of hyperglycaemia typically resolve when the hormone excess is removed (Macfarlane, *et al*., 1997).

Somatostatinoma, and aldosteronoma–induced hypokalaemia, can cause diabetes, at least in part by inhibiting insulin secretion**.**

Hyperglycaemia generally resolves following successful removal of the tumour (Krejs, *et al*., 1979).

2.1.4.5- Drug– or chemical–induced diabetes

Many drugs can impair insulin secretion. These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance**.** In such cases, the classification is ambiguous, as

the primacy of beta– cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and pentamidine can permanently destroy pancreatic beta cells**.**

Fortunately, such drug reactions are rare. There are also many drugs and hormones which can impair insulin action. Examples include nicotinic acid and glucocorticoids (Assan, *et al*., 1995).

2.1.4.6-Infections

Certain viruses have been associated with beta–cell destruction.Diabetes occurs in some patients with congenital rubella**.** In addition, Coxsackie B, cytomegalovirus and other viruses (e.g. adenovirus and mumps) have been implicated in inducing the disease (Pak, *et al*., 1988).

2.1.4.7- Uncommon but specific forms of immune–mediated diabetes mellitus

Diabetes may be associated with several immunological diseases with a pathogenesis or aetiology different from that which leads to the Type 1 diabetes process. Postprandial hyperglycaemia of aseverity sufficient to fulfil the criteria for diabetes has been reported in rare individuals who spontaneously develop insulin autoantibodies**.** However, these individuals generally present with symptoms nof hypoglycaemia rather than hyperglycaemia. The “stiff man syndrome” is an autoimmune disorder of the central nervous system, characterized by stiffness of the axial muscles with painful spasms **.** Affected people usually have high titres of the GAD autoantibodies and approximately one-half will develop diabetes.

Patients receiving interferon alpha have been reported to develop diabetes associated with islet cell autoantibodies and, in certain instances, severe insulin deficiency (Fabris, *et al*., 1992).

Anti–insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby reducing the binding of insulin to target tissues**.** However, these antibodies also can act as an insulin agonist

after binding to the receptor and can thereby cause hypoglycaemia **.**

Anti–insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases **.**

As in other states of extreme insulin resistance, patients with anti–insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed Type B insulin resistance (Tsokos, *et al*., 1985).

2.1.4.8-Other genetic syndromes sometimes associated with diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin–deficient diabetes and the absence of beta cells at autopsy**.** Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness (Barrett, *et al*., 1995).

2.1.4.9-Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2-10% of all pregnancies and may improve or disappear after delivery**.** However, after pregnancy approximately 5-10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2**.** Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required.Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother.

Risks to the baby include macrosomia (high birth weight),congenital cardiac and central nervous system anomalies, and skeletal muscle

malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome.

Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment.

Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia (Rother, *et al*., 2007).

2.1.5-Diabetes complications

Although the pathophysiology of the disease differs between the types of diabetes, most of the complications, including microvascular, macrovascular, and neuropathic, are similar regardless of the type of diabetes. Hyperglycemia appears to be the determinant of microvascular and metabolic complications. Macrovascular disease may be less related to glycemia. Telomere attrition may be a marker associated with presence and the number of diabetic complications.

Whether it is a cause or a consequence of diabetes remains to be Seen (Testa, *et al*., 2011).

2.1.5.1-Cardiovascular risk

The risk for coronary heart disease (CHD) is 2-4 times greater in patients with diabetes than in individuals without diabetes. Cardiovascular disease is the major source of mortality in patients with type 2 diabetes mellitus. Approximately two thirds of people with diabetes die of heart disease or stroke. Men with diabetes face a 2-fold increased risk for CHD, and women have a 3- to 4-fold increased risk.

Although type 2 diabetes mellitus, both early onset (< 60 y) and late onset (>60 y), is associated with an increased risk of major CHD and mortality,

only the early onset type (duration >10 y) appears to be a CHD risk equivalent (Wannamethee, *et al*., 2011).

In patients with type 2 diabetes mellitus, a fasting glucose level of more than 100 mg/dL significantly contributes to the risk of cardiovascular disease and death, independent of other known risk factors.

This is based on a review of 97 prospective studies involving 820,900 patients. Data from a large population-based study affirms that worsening glycemic control appears to increase the risk of heart failure (Seshasai, *et al*., 2011).

Adolescents with obesity and obesity-related type 2 diabetes mellitus demonstrate a decrease in diastolic dysfunction.

This suggests that they may be at increased risk of progressing to early heart failure compared with adolescents who are either lean or obese but do not have type 2 diabetes mellitus**.**

2.1.5.2-Diabetic retinopathy

Diabetes mellitus is the major cause of blindness in adults aged 20-74 years in the United States; diabetic retinopathy accounts for 12,000-24,000 newly blind persons every year. The National Eye Institute estimates that laser surgery and appropriate follow-up care can reduce the risk of blindness from diabetic retinopathy by 90% (Shah, *et al*., 2011).

2.1.5.3-End-stage renal disease

Diabetes mellitus, and particularly type 2 diabetes mellitus, is the leading contributor to end-stage renal disease (ESRD) in the United States. According to the Centers for Disease Control and Prevention, diabetes accounts for 44% of new cases of ESRD. In 2008, 48,374 people with diabetes in the United States and Puerto Rico began renal replacement therapy, and 202,290 people with diabetes were on dialysis or had received a kidney transplant (Shah, *et al.*, 2011).

2.1.5.4-Neuropathy and vasculopathy

Diabetes mellitus is the leading cause of nontraumatic lower limb amputations in the United States, with a 15- to 40-fold increase in risk over that of the nondiabetic population. In 2006, about 65,700 nontraumatic lower limb amputations were performed related to neuropathy and vasculopathy.

2.1.5.5-Cancer

A 2010 Consensus Report from a panel of experts chosen jointly by the American Diabetes Association and the American Cancer Society suggested that people with type 2 diabetes are at an increased risk for many types of cancer. Patients with diabetes have a higher risk for bladder cancer, particularly those patients who use pioglitazone. Age, male gender, neuropathy, and urinary tract infections were associated with this risk (Colmers, *et al*., 2012).

In a meta-analysis of 20 publications comprising 13,008 cancer patients with concurrent type 2 diabetes, researchers found that patients treated with metformin had better overall and cancer-specific survival than those treated with other types of glucose-lowering agents. These improvements were observed across cancer subtypes and geographic locations. Risk reduction was significant among patients with prostate, pancreatic, breast, colorectal and other cancers, but not for those with lung cancer. However, it remains unclear whether metformin can modulate clinical outcomes in cancer patients with diabetes (Nelson, *et al*., 2013).

2.2-Urea

Urea has long been recognized as the principal end product of nitrogenous metabolism in mammals.Two major hepatic pathways exist for the production of urea in vertebrates. Urea is formed from NH_4^+ (or glutamine) and HCO_3 in the ornithine–urea cycle (Brown, *et al*., 1960).

2.2.1-Urea Cycle

The urea cycle or the ornithine cycle describes the conversion reactions of ammonia into urea. Since these reactions occur in the liver, the urea is then transported to the kidneys where it is excreted. The step wise process of the urea cycle is summarized in the graphic on the left. One amine group comes from oxidative deamination of glutamic acid while the other amine group comes from aspartic acid. Aspartic acid is regenerated from fumaric acid produced by the urea cycle. The fumaric acid first undergoes reactions through a portion of the citric acid cycle to produce oxaloacetic acid which is then changed by transamination into aspartic acid. Urea is removed efficiently by the kidneys.

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates.Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is then dissolved into the blood and further transported and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted in sweat. Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy.The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle.Ammonia (NH_3) is another common byproduct of the metabolism of nitrogenous compounds. Ammonia is smaller, more volatile and more mobile than urea. If allowed to accumulate, ammonia would raise the pH in cells to toxic levels (Gibb, *et al.*, 2009).

2.2.2-Metabolism

The handling of urea by the kidneys is a vital part of mammalian metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, that allows for re-absorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons, thus raising the osmolarity in the medullary interstitium surrounding the thin ascending limb of the loop of Henle, which in turn causes water to be reabsorbed. By action of the urea transporter 2, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. This mechanism, which is controlled by the antidiuretic hormone, allows the body to create hyperosmotic urine, that has a higher concentration of dissolved substances than the blood plasma. This mechanism is important to prevent the loss of water, to maintain blood pressure, and to maintain a suitable concentration of sodium ions in the blood plasmas (Sakami, *et al.*, 1963).

2.2.3-Measurement

Urea is routinely measured in the blood as: Blood Urea Nitrogen (BUN). BUN levels may be elevated (a condition called uremia) in both acute and chronic renal (kidney) failure. Various diseases damage the kidney and cause faulty urine formation and excretion. Congestive heart failure leads to a low blood pressure and consequent reduced filtration rates through the kidneys, therefore, BUN may be elevated. Urinary tract obstructions can also lead to an increased BUN. In severe cases, hemodialysis is used to remove the soluble urea and other waste products from the blood. Waste products diffuse through the dialyzing membrane because their concentration is lower in the dialyzing solution. Ions, such as Na^+ and Cl^- which are to remain in the blood, are maintained at the same concentration in the dialyzing solution - no net diffusion occurs. Jim Hardy, Professor of

Chemistry, The University of Akron (Kishimoto, *et al*., 2008).

2.2.4-Normal blood urea range

Normal blood urea is 3.5-6.5 mmol/litre (20-30mg/dl) (Vikas, *et al*., 2007).

2.2.5-Interpretation

BUN is an indication of renal health. Normal ranges 1.8-7.1 mmol/L.

The main causes of an increase in BUN are: high protein diet, decrease in Glomerular Filtration Rate (GFR) (suggestive of renal failure) and in blood volume (hypovolemia), congestive heart failure, gastrointestinal hemorrhage, fever and increased catabolism. The main causes of a decrease in BUN are: severe liver disease, anabolic state, syndrome of inappropriate antidiuretic hormone (Longo, *et al*., 2008).

2.3-Creatinine

Ever since the discovery of phosphorylcreatine (PCr) in 1927 and of the creatine kinase (CK; EC 2.7.3.2) reaction in 1934, research efforts focused mainly on biochemical, physiological, and pathological aspects of the CK reaction itself and on its involvement in “high-energy phosphate” metabolism of cells and tissues with high-energy demands. In contrast, Cr (from greek kreas, flesh) metabolism in general has attracted considerably less attention.In recent years, however, a series of fascinating new discoveries have been made. For instance, Cr analogs have proven to be potent anticancer agents that act synergistically with currently used chemotherapeutics Cyclocreatine, one of the Cr analogs, as well as PCr protect tissues from ischemic damage and may therefore have an impact on organ transplantation. Circumstantial evidence suggests a link between disturbances in Cr metabolism and muscle diseases as well as neurological disorders, and beneficial effects of oral Cr supplementation in such diseases have in fact been reported. Oral Cr ingestion has also been shown to increase athletic performance, and it therefore comes as no

surprise that Cr is currently used by many athletes as a performance-boosting supplement. Some data suggest that Cr and creatinine (Crn) may act as precursors of food mutagens and uremic toxins. Finally, the recent identification, purification, and cloning of many of the enzymes involved in Cr metabolism have just opened the door to a wide variety of biochemical, physiological, as well as clinical investigations and applications **(**Conwayma, *et al*., 1996).

2.3.1 biosynthesis and Metabolism

In mammals, for instance, a complete urea cycle operates actively only in liver. The main site of Arg biosynthesis for other bodilytissues is, however, the kidney. Citrulline, synthesized in the liver or small intestine and transported through the blood, is taken up by the kidney and converted into Argmainly by the proximal tubule of the nephron. Arg formed within the kidney is then either released intothe blood and consumed by other tissues or used within the kidney itself for guanidinoacetate synthesis.

The transfer of the amidino group of Arg to Gly to yield L-ornithine and guanidinoacetic acid (GAA) represents the first of two steps in the biosynthesis of Cr and is catalyzed by L-arginine:glycine amidinotransferase. In mammals, pancreas contains high levels of both enzymes, whereas kidneys express fairly high amounts of AGAT but relatively lower levels of GAMT. On the contrary, livers of all mammalian species tested so far contain high amounts of GAMT but display only low levels of Cr and almost completely lack CK activity. On the basis mostly of these latter findings and of the fact that the rate of Cr biosynthesis is considerably reduced in nephrectomized animals, it was postulated, and is still largely accepted, that the main route of Cr biosynthesis in mammals involves formation of guanidinoacetate in the kidney, its transport through the blood, and its methylation to Cr in the

liver . Cr exported from the liver and transported through the blood may then be taken up by the Cr-requiring tissues (Fitch, *et al*., 1964).

comparison of the hepatic and renal venous levels with the arterial levels of Arg, GAA, and Cr suggested that in humans, the liver is the most important organ contributing to biosynthesis of both GAA and Cr, whereas the kidney plays only a secondary role. In accordance with these observations, immunofluorescence microscopy revealed significant amounts of AGAT not only in rat kidney and pancreas, but also in liver (mcguire, *et al*., 1986).

AGAT activity was detected in heart, lung, spleen, muscle, brain, testis, and thymus, and it has been estimated that the total amount of AGAT in these tissues approaches that found inkidney and pancreas. Although AGAT is absent from human placenta, the decidua of pregnant females displayed the highest specific AGAT activity of all rat tissues examined, implying a major involvement of this tissue in Cr biosynthesis during early stages of development. Likewise, Sertoli cells of rat seminiferous tubules, in contrast to germ cells and interstitial cells, were shown to synthesize guanidinoacetate and Cr from Arg and Gly. GAMT activity was also detected in rat spleen, heart, and skeletal muscle, in sheep muscle, as well as in human fetal lung fibroblasts and mouse neuroblastoma cells. Although the specific activities in these tissues are rather low, the GAMT activity in skeletal muscle was calculated to have the potential to synthesize all Cr needed in this tissue. One possible explanation is that the brain contains its own Cr-synthesizing machinery. A specific, saturable, Na1- and Cl2-dependent Cr transporter responsible for Cr uptake across the plasma membrane has been described for skeletal muscle, heart, smooth muscle, fibroblasts, neuroblastoma and astroglia cells, as well as for red blood cells and macrophages. Although the quantitative results of these latter studies differ to some extent, the highest

amounts of Cr transporter mRNA seem to be expressed in kidney, heart, and skeletal muscle; somewhat lower amounts in brain, small and large intestine, vas deferens, seminal vesicles, epididymis, testis, ovary, oviduct, uterus, prostate, and adrenal gland; and only very low amounts or no Cr transporter mRNA at all in placenta, liver, lung, spleen, pancreas, and thymus. An important aspect of Cr biosynthesis to add is that in humans, the daily utilization of methyl groups in the GAMT reaction approximately equals the daily intake of "labile" methyl groups (Met 1 choline) on a normal, equilibrated diet (Mudd, *et al.*, 1980).

2.3.2- Tissue distribution

The highest levels of Cr and PCr are found in skeletal muscle, heart, spermatozoa, and photoreceptor cells of the retina. Intermediate levels are found in brain, brown adipose tissue, intestine, seminal vesicles, seminal vesicle fluid, endothelial cells, and macrophages, and only low levels are found in lung, spleen, kidney, liver, white adipose tissue, blood cells, and serum. A fairly good correlation seems to exist between the Cr transporter mRNA level and total CK activity which, in turn, also correlates with the tissue concentration of total Cr (Berlet, *et al.*, 1979).

2.3.3-Regulation of metabolism

Cr biosynthesis is curtailed and the serum concentration of Cr is likely to be decreased, AGAT expression is upregulated. In contrast, an increase in the serum concentration of Cr, due either to an endogenous source or to dietary Cr supplementation, results in concomitant decreases in the mRNA content, the enzyme level, and the enzymatic activity of AGAT, thus suggesting regulation of AGAT expression at a pretranslational level (Guthmiller P, *et al.*, 1994). Growth hormone and Cr have an antagonistic action on AGAT expression, as evidenced by identical mRNA levels and enzymatic activities of kidney AGAT in hypophysectomized rats simultaneously fed Cr and injected with growth hormone compared

with hypophysectomized rats receiving neither of these compounds. AGAT levels in liver, pancreas, and kidney are also decreased in conditions of dietary deficiency and disease (fasting, protein-free diets, vitamin E deficiency, or streptozotocin- induced diabetes).

These findings seem, however, not to rely directly on the dietary or hormonal imbalance that is imposed. For example, insulin administration to streptozotocin-diabetic rats does not restore the original AGAT activity in the kidney. On the contrary, fasting and vitamin E deficiency are characterized by an increased blood level of Cr (Funahashi, *et al*., 1980).

2.3.4-Creatinine Metabolism and Renal Disease

The kidney plays a crucial role in Cr metabolism. On one hand, it is a major organ contributing to guanidinoacetate synthesis. On the other hand, it accomplishes urinary excretion of Crn, the purported end product of Cr metabolism in mammals. In chronic renal failure (CRF) rats, the renal AGAT activity and rate of guanidinoacetate synthesis are depressed . Accordingly, the urinary excretion of guanidinoacetate is decreased in a variety of renal diseases (Albanchaabouchi, *et al*., 1998).

Although the serum concentration of guanidinoacetate was also shown to be decreased in both uremic patients and renal failure rats, it was found, in a few other studies, to be unchanged or even slightly increased. These conflicting results may be due to compensatory upregulation of guanidinoacetate synthesis in the pancreas, to different degrees of depression of urinary guanidinoacetate excretion, to unknown effects of peritoneal or hemodialysis, and/or to different stages of disease progression.

Similarly conflicting results were obtained for the serum concentration of Cr in uremic patients. It was found to be increased, unchanged, or even depressed relative to control subjects . The latter finding may be due to

dialysis of these patients, which was shown to decrease the serum concentration of Cr (Dedeyn, *et al*., 1995).

Both the erythrocyte concentration of Cr (even after hemodialysis) and the urinary excretion of Cr may be increased in uremia, although in one study decreased urinary Cr clearance was observed. In striated muscle of uremic patients, the concentrations of PCr and ATP are decreased ,whereas those of Cr and Pi are increased , thus suggesting that intracellular generation of high-energy phosphates is impaired. The most consistent, and clinically most relevant, findings are an increase in the serum concentration and a decrease in the renal clearance of Crn with the progression of renal disease **(**Bonas, *et al*., 1963).

Both the serum concentration of Crn and Crn clearance have been, and still are, widely used markers of renal function, in particular of the glomerular filtration rate (GFR). The validity of this approach critically depends on the assumptions that Crn is produced at a steady rate, that it is physiologically inert, and that it is excreted solely by glomerular filtration in the kidney. In recent years, these assumptions were shown to be invalid under uremic conditions, and several factors have been identified that may result in gross overestimation of the GFR .

For example, an increasing proportion of Crn in CRF is excreted by tubular secretion rather than glomerular filtration. Another factor contributing to the overestimation of the GFR seems to be degradation of Crn in the human and animal body (Caregaro, *et al.*, 1994).

Whereas the normal renal Crn clearance is ;120 ml/min, the renal and extrarenal Crn clearances in CRF patients were calculated to be ;3–5 and 1.7–2.0 ml/min, respectively. Therefore, Crn degradation may be negligible in healthy individuals, which led to the postulate that Crn is physiologically inert, but it may become highly relevant under conditions of impaired renal function.

As far as the serum and tissue concentrations as well as the urinary excretion rates of the potential Crn degradation products are concerned, they consistently indicate that the production of MG and creatol is increased in uremia (Ando, *et al*., 1979).

In CRF rats relative to controls, the concentration of MG was increased 3- to 18-fold in serum, blood cells, liver, muscle, colon, and kidney. In brain, on the other hand, MG concentration was increased only twofold, indicating limited permeability of the blood-brain barrier for MG. The same conclusion can be drawn from experiments on the effects of intraperitoneal injection of guanidino compounds into rats, which suggested a low permeability of the blood-brain barrier for MG, Crn, and guanidinosuccinic acid (GSA). Guanidine, 4-GBA, and GPA may be increased in serum and cerebrospinal fluid of uremic patients **.** as well as in serum, heart, skeletal muscle, brain, liver, kidney, and intestine of rats and mice with acute or chronic renal failure **.** Crn and its degradation products are likely to be of critical importance with regard to uremic toxicity (Levillain, *et al*., 1995).

2.4 Objectives

2.4-1 General objective

Study renal function in diabetic's patient more than 10 years on set.

2.4-2 Specific objective

1. To measurement blood urea.
2. To measurement serum creatinine level.
3. To estimation of urine general test.
4. To correlate the urea level with duration of the disease.
5. To correlate the creatinine level with duration of the disease.

Chapter three

3. Materials and methods

3- Materials and methods

3.1 Study Designed

This study was designed as case and control study.

3-2 Study area

This study is carried in Northern State, Aldaba in 2014.

3-3 Study population

One hundred participants (57 males and 43 females) were Divided into Tow groups fifty healthy volunteers as pre described by Physical examination and medical history (30 males and 20 females) were the control groups. Where as Fifty (27 males and 23 females), with more than ten years Type2 Diabetics' diseases onset who are under medical treatment Were defined as case groups.

3-4 Inclusions (Criteria to select samples)

The inclusions criteria those who are diabetes more than 10 years disease Prolong. Who under continuous medications.

3-5 Exclusions (Criteria to reject samples)

Those who are diabetes less than 10 years on set, or those with renal dysfunction in addition to those who have diabetics' complication or combined with other metabolic diseases. Any samples are collated or not enough is rejected.

3-6 Tools

Prepared questionnaire including data concerning diabetic's people and their diabetic's information (such as age, diabetic's history and urine general test).

3-7 Data collection

The data collection was carried between Februarys to June 2014.

3-8 Sampling Collection

2.5ml venous blood samples were obtained from each diabetic's and non diabetic's using standard venipuncture technique. Plasma specimens were collected as heparinized container after centrifugation at 3000 rpm for 5 minutes. Then biochemical parameters were estimated.

3-9 Ethical approval

All samples were collected from study population after its informed consent.

3-10 Estimation of urea

3-10.1 Principle of the method

Urea in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometer:

$Urea + H_2O \rightarrow 2NH + CO_2$

$NH + Salicylate + NaClO \rightarrow Indophenole$

3-11 Estimation of creatinine

3-11.1 principle of the method

Creatinine is sample react with picrate in alkaline forming a colored complex (Jaffe method). The complex formation rate is measured in a short period to avoid interference. Serum and plasma samples contain proteins that react in non specific way; nevertheless, the result can be corrected subtracting a fixed value. The use of the correction is known as Jaffe method compensated.

3-12 Examination of urine general tests

1. Collection of urine sample in clean dry container.
2. Examination of sugar, acetone, and albumin using urine strips.
3. Record result as +, ++, +++,…
4. Using clean dry centrifugation tube, centrifuged urine sample in centrifuge device at 3000r per 5 minuets.

5. Discharge supernatant and put deposit into clean dry slide.

6. examination for pus cell, RBCs, Calcium Oxalate, Epithelial cells, and yeast cells under microscope using 10X and 40X.

7. Record result as cell in high power per failed.

3-13 Statistical analysis:

Statistical analysis were performed using:

Statistical package for social sciences (SPSS) Versions 11.5 Paired sample T.test were used to Compare between means with P.value less than 0.05, Confidence value 95%. Also correlation were used in addition to percentages (distribution) were calculated.

Chapter four

4. Results

One hundred Sudanese people (57 males and 43 females) were Divided into tow group fifty healthy (30 males and 20 females) Individual with mean age (44.3±14.6) were the control group. Where as Fifty (27 males and 23 females), mean age (67. 42), mean fasting Blood glucose (201.52) mg/dl, mean duration 16.3 years, with more Than ten years type2 Diabetics' diseases on set were defined as case Group. See Table (4-1) Table (**4**-2) show At probability less than 0.05 in this study serum creatinine and urea were highly significant in Diabetics' compared to there copartner with p.value 0.000 and mean±SD (3.29±2.25 ,1.28±0.35) mg/dl and (63.88±3.38 , 21.28±5.48) mg/dl for Diabetics' and non Diabetics' patient respectively.

Also estimations of some urine parameters of Diabetics' and non Diabetics' urine showed elevation and presence of albumin (35)% in Diabetics' and (1)% in non Diabetics', sugar (99)% for Diabetics' and (0)% in non Diabetics', acetone (60)% for Diabetics' and (0)% for non Diabetics', pus (55)% for Diabetics' and (5)% for non Diabetics', Calcium oxalate (66)% for Diabetics' and (1)% for non Diabetics', RBCs (5)% for Diabetics' and (2)% for non Diabetics', Yeast cells(2)% for Diabetics' and (1)% for non Diabetics', Epithelial cells (11)% for Diabetics' and (1)% for non Diabetics' Show table (4-3)**.**

Also Thise study showed positive correlation between urea and duration of diabetics' correlation coefficient .283. While creatinine records negative correlation with correlation coefficient -.015 see Figure (4-1) and Figure (4-2).

Table (4-1) Base line characteristics of study population

Parameters	**Diabetic's N=50**	**Nondiabetic's N=50**
Age in years (mean±SD)	67.42±8.860	44.30±14.612
Sex	Male: 27 Female:23	Male: 30 Female: 20
Disease duration in years (mean±SD)	16.34±3.756	0
Fasting blood glucose (mean±SD) mg/dl	201,52±26,274	94,04±13,711

Table (4-2) Serum urea and creatinine in study participant

Parameters	**Diabetic's N=50**	**Nondiabetic's N=50**	**p.value**
Urea (mg/dl)	63,88±3,38	21,28±5,489	*.000
Creatinine (mg/dl)	3.292±2.2599	1.280±.3569	*.000

*** significant**

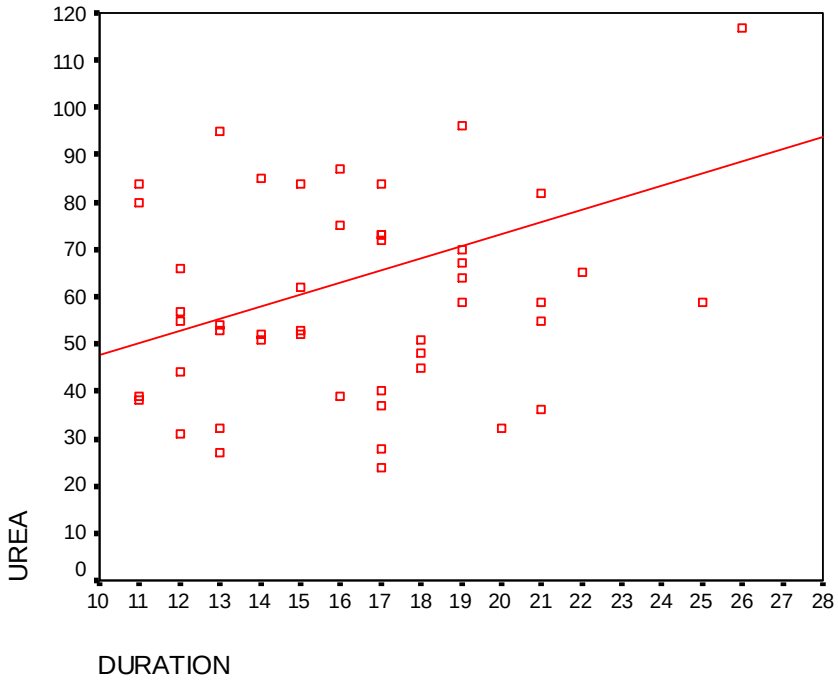
Table (4-3) presence of albumin, sugar, acetone, pus, Calcium oxalate, RBCs, Yeast cells, and Epithelial cells in urine sample of diabetic's and non diabetics

Parameters	**Diabetic's N=50**	**Nondiabetic's N=50**
Albumin %	35%	1%
Sugar %	99%	0%
Acetone %	60%	0%
Pus %	55%	5%
***RBCs %	5%	2%
Calcium oxalate %	66%	1%
Yeast cells %	2%	1%
Epithelial cells %	11%	1%

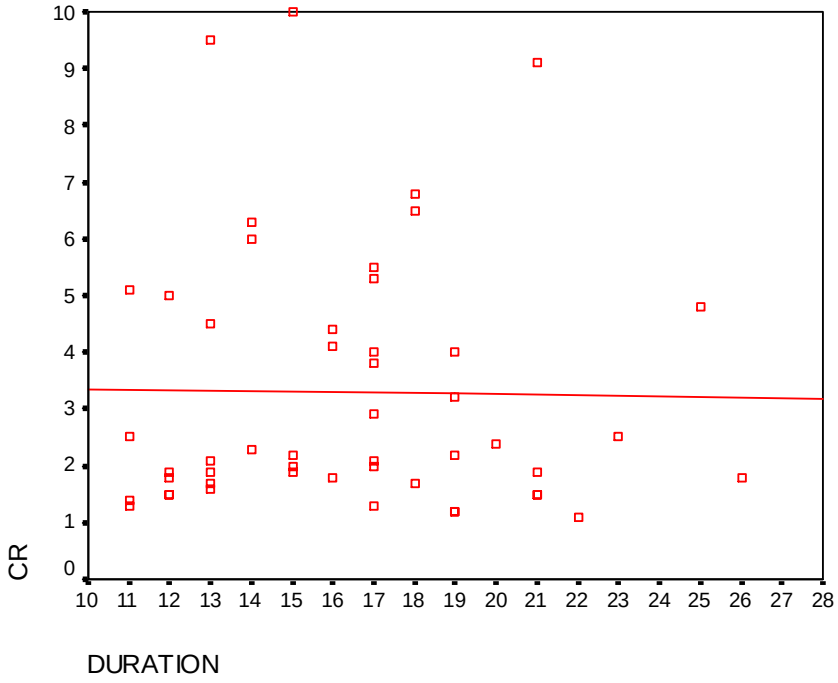
***** Red blood cells

Correlation between Duration and Urea level in diabetic's patient

Figure (4-1)



Correlation between Duration and creatinine level in diabatics patient Figure (4-2)



Chapter five

5. Discussion

5-1.Discussion

Results obtained from the present study showed that in addition to elevated blood sugar level in type 2 diabetes mellitus, plasma creatinine and urea concentration are also significantly increased in male and female diabetics compared with their levels in apparently healthy non-diabetic male and female controls.

This observation is in accord with the reports of Aldler, *et al.*,(2003), Judykay, *et al.*, (2007) and Wagle , et al., (2010). Aldler in their report submitted that raised plasma creatinine and urea levels in diabetic patient may indicate a pre-renal problem such as volume depletion.

Judykay in his submission suggested that high creatinine levels observed in diabetic patients may be due to impaired function of the nephrons. Judykay also posited that high urea levels in diabetes mellitus patients could be attributed to a fall in the filtering capacity of the kidney thus leading to accumulation of waste products within the system.

In addition, a report on the comparative study of serum sugar and creatinine levels in male and female type 2 diabetic patients showed that blood glucose and serum creatinine concentrations are elevated in type 2 diabetic patients compared with non-diabetic male and female controls Wagle, *et al.*, (2010).

Wagle , *et al.*, (2010) reports showed a progressive decrease in renal function in male and female diabetic patient as from age 40 years and beyond as a result of increased serum creatinine levels. Male diabetic patients were found to present significantly higher serum creatinine level than females.as well as increased serum creatinine, and decreased haemoglobin levels to predict the development of end stage renal disease in patients with type 2 diabetes and nephropathy.

In this study there was significantly increased of albumin in urine, This observation is in agreement with the reports of Middleton and Foley, *et al*., (2006) which suggested that People with diabetes (particularly type 2 diabetes) often develop kidney diseases other than diabetic nephropathy.

Kidney biopsy series in type 2 diabetes have found that non diabetic glomerular disease. In addition, there can be significant overlap While these biopsy series are biased (biopsies are usually done in people with diabetes when non diabetic renal disease is suspected), other studies have suggested that half of everyone with diabetes and significant kidney function impairment do not have albuminuria.

These studies suggest that testing for albuminuria may be insufficient in identifying all patients with diabetes who have renal disease. In addition to measurements of urinary albumin excretion, estimations of the level of kidney function and urinalyses are required to identify patients with kidney disease other than diabetic nephropathy. In most cases, the risk of end stage renal disease in diabetes does not appear to matter whether the renal diagnosis is one of diabetic nephropathy or an alternative diagnosis as management is the same. However, lists some concerning clinical and laboratory features that would lead to suspicion of a kidney disease unrelated to diabetes, requiring such a person to undergo additional testing or referral.

In this study we found that significantly increased of ca oxalate in urine , This observation is in accord with the reports of American Diabetes Services "Diabetes Increases the Risk of Kidney Stones" which submitted that Individuals with type 2 diabetes are at an increased risk for developing kidney stones in general, and have a particular risk for uric acid stones. Researchers at the Mayo Clinic followed 3,500 patients over a 20-year span and concluded that those with diabetes developed 40% more uric acid kidney stones than those without diabetes. People with type 2

diabetes have highly acidic urine, and this metabolic feature helps to explain their greater risk for developing uric acid stones. It was found that obesity and a diet rich in animal protein are related to abnormally acidic urine.

in this study we found that significantly increased of pus cell in urine Leucocyturia more than 5 pus cells/hpf was detected in 33.75% (n=27/80)of the Type 2 DM patients. In 20 patients with Type 2 DM, leucocyturia more than 5per high power field was detected in 30% (n=6/20). Our study can be compared with the study of Lerman-Garber et al which shows that the overall prevalence of leukocyturia (>5 cells/high power field (hpf)) was 46.5%.Patients with urinary tract infections were 7.5 times more likely to have leukocyturia, while a leukocyte count<5cells/hpf predicted the absence of urinary tract infections in 96% of the women.

This study also shows, that there is a relationship between leucocyturia >5 cells/high power field and positive urine culture reports.

in this study we found that is strong positive correlation between urea and duration and in anther hand no correlation between duration and creatinine This observation is in accord with the reports of KronholmE et al 2008 which submitted that An increasing number of epidemiological studies have indicated the presence of associations between duration and various health disorders, such as an overweight status, diabetes, hypertension, cardiovascular disease and mortality. On the other hand, epidemiological evidence concerning a relationship between duration and Chronic kidney disease is scarce. However, it remains unclear whether this relationship exists in persons with a long duration. Furthermore, the association between duration and urinary albumin excretion, a more sensitive marker of Chronic kidney disease than proteinuria, has not been ascertained to date.

The present study demonstrated that a shorter duration is significantly associated with higher urinary albumin-creatinine ratio levels, even after controlling for confounding factors. In addition, our findings also revealed an association between a longer duration and higher urinary albumin-creatinine ratio levels. Taken together, these findings indicate that there is a U-shaped relationship between sleep duration and albuminuria, thus implying that an inadequate duration may have a negative impact on albuminuria, which has been shown to be a risk factor for both renal failure and cardiovascular disease.

The strengths of the current study include the enrollment of a relatively large number of type 2 diabetic patients, which allowed for the statistical power to detect differences and adjustment for potential confounders. Additional strengths include the uniform collection of urine samples and the use of a standardized method for measurement. Furthermore, in the current study, the urinary albumin-creatinine ratio levels were determined using a quantitative method, while previous studies have assessed the presence of proteinuria using dip-stick tests. Therefore, the findings of the present study may have higher accuracy with regard to the relationship between duration and urinary parameters than previous studies. Moreover, this is the first study to examine the urinary albumin-creatinine ratio levels in relation to sleep duration in patients with type 2 diabetes.

5-2.Conclusion

The result concluded that there was statistically significant increased in seurm urea, creatinine. With presence of albumin, sugar, acetone, pus and Calcium oxalate in diabetics' urine. And also there was strong positive correlation between duration of diabetes mellitus and urea level while negative correlation between duration and creatinine level. There were no differences in RBCs, Yeast cells and Epithelial cells between diabetes and non diabetes.

5-3.Recommendations

In these study we reaffirmation Patients with type 2 diabetes more than ten years' disease on set should have regular screening for Renal function tests, regular screening for urine general testes, regular screening for blood pressure.

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Appendix(1)

بسم الله الرحمن الرحيم

Sudan University for science and technology

College of graduated studies

Questionnaire for diabetics

Personal data:

Name……. Data……...

Age……..... Sex……

Location……. Occupation……

Social status…… Marital status……

Tribe……

Medical data:

-Fasting blood glucose:……..

-Duration of disease (Years):…… ……..

-renal dysfunction: Yes:……No:…….

-Diabetics complication: Yes:……No:…….

-Chronic disease: Yes:……No:……

-any other metabolic disease: Yes:……No:……

Laboratory Investigation:

1- renal function test:…… ……

2- urine general test:……..

Appendix(2)

إسم المريض:.....

HB:.....…g/dl……%T.W.B.C……B.F.F.....F.B.G:……...mg/dl

Urea……..mg/dl Creatinine:…….mg/dl

Urine General **Colour……..** **Reaction……...** **Albumin……...** **Sugar……...** **Acetone……** **Bile pigment…….....** **Deposit H.P.F** **Pus……./H.P.F.** **R.B.Cs……/ H.P.F.** **Epith. Cells……** **Crystals……...** **Phosphate……** **Cast…….** **Trichomonas……...** **Ova……..** **Yeast…….....** **Bacteria……...** **Others……**	**Stool General** **Colour……..** **Consistency……** **Reaction……...** **Mucus……...** **Blood……...** **Worms……** **Microscopic Exam** **Pus……./H.P.F.** **R.B.Cs……/ H.P.F.** **Flagellate……** **Cyst…….....** **Ameba……...** **Worms……...** **Undigested Material……..** **Yeast…….....** **Bacteria…….....** **Ova……..** **Others……**

Appendix(3)

Estimation of urea procedure

1. Bring the reagents to room temperature
2. Pipette into labeled test tubes:

	Blank	Standard	Sample
Urea standard (S)	_	10µl	_
Sample	_	_	10µl
Reagent (A)	1.0ml	1.0ml	1.0ml

3. Mix thoroughly and incubate for 10 minutes at room temperature (16 to 25°c) or for 5 minutes at 37°c.

4. Pipette:

Reagent (B)	1.0ml	1.0ml	1.0ml

5. Mix thoroughly and incubate for 10 minutes at room temperature (16 to 25°c) or for 5 minutes at 37°c.

6. Read the absorbance (A) of the standard and the Sample at 600nm against blank. The colour is stable for at least 2 hours.

Appendix(4)

Estimation of creatinine procedure

1. Bring the reagents to room temperature

2. Pipette into cuvette: (Note1)

woraking reagent	1.0ml
Standard or Sample	0.1ml

3. Mix and insert cuvette into the photometer. Start stopwatch

4. Record the absorbance at 500 nm after 30 seconds (A1) and after 90 (A2).