Diabetic Nephrophathy in patients with diabietes mellitus type-2

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The study concluded that lipid profile and high blood pressure can cause complications in Sudanese patients with type -2DM like nephropathy.

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Diabetic Nephropathy in Patients with Diabetes Mellitus Type-2

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_Diabetic Nephropathy in Sudanese Patients with Diabetes Mellitus Type-2

by:

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Dedication

To my husband Abu-Bakr
To my daughter Maab
To my sisters and brothers
to my mother
to my father

Acknowledgement

I am grateful to Dr. Nasser Aldeen Mohammad Sheriff for his perfect supervision and guidance to this work from the start point up to the end point; also I am very thankful to all the participants involved in this study. Special thank to Mr. Mosab Nouraldein Mohammed for his support in editing and publishing processes.

Abstract

Kidney Function assessment is important in diabetic patients and early detection of diabetic nephropathy in preclinical stage of disease will contribute to decreasing morbidity and mortality rates.

This study focused on the determination of risk factors that may lead to diabetic nephropathy in type-2 DM patients in Khartoum state.

Early morning urine samples and blood samples were collected from 50 diabetic patients, their age range was from 30-65years.All the 50 subjects did not suffer from urinary tract infection, heart, liver or renal diseases.

Micro albumin measured in urine samples using Immunoturbidimetric method to show an early indication of deteriorating renal function and increased vascular permeability. While HbAlc was assayed using Labona Check^{TM}A1c HbA1c Analyzer. Lipid profile was assayed using colorimetric assay kits.

Microalbumin concentration showed significant differences between diabetic patients, where 58%of the study group shown elevated MAU (diabetic nephropathy)38% of them are males and20% are females, and 42% of study group without elevated Microalbumin (non-nephropathy) 14% of them are males and 28%of them are females. Furthermore, the study showed significant difference (PV=<0.05) in blood pressure (systolic and diastolic), T. cholesterol, TG, and LDL between nephropathy and non-nephropathy groups, which indicate these markers have influence in appearance of MAU in diabetic patients.

There was insignificant difference in age, duration of DM, BMI, HDL, and glycemic control between nephropathy and non-nephropathy.

The study concluded that lipid profile and high blood pressure can cause complications in Sudanese patients with type -2DM like nephropathy.

Introduction and Literature review

Diabetes mellitus is a chronic disease that affects the lives of millions around the world. It is a global epidemic with devastating humanitarian, social and economic consequences. The disease claims as many lives per year as HIV/AIDS and places a severe burden on healthcare systems and economies everywhere, with the heaviest burden falling on low- and middle income countries. Yet awareness of the global scale of the diabetes threat remains pitifully low [1].

The prevalence of DM has increased continually during the last years until it became as one of the big health problems in most countries especially; the low- and middle-income countries. This will have a major impact on the quality of life of hundreds of millions people and their families, overwhelm the capability of many national health-care systems, and impact adversely upon the economy of those countries that are in most need of development [2]. The prevalence of type-2 diabetes is rising at an alarming rate throughout the world, due to increase in life expectancy and obesity, and adoption of sedentary lifestyles [3].

Newly released statistics from the CDC illustrate that diabetes has risen by over 14% in the last two years in the U.S. The CDC estimates that 20.8 million Americans — 7% of the U.S. population — have diabetes, up from
18.2 million in2003 [4].

Diabetes was the sixth leading cause of death listed on the U.S. death certificates in 2002[5]. Mortality from communicable diseases in less developed countries is declining. In association with increasing diabetes prevalence, this will inevitably result in increasing proportions of deaths from CVD in these countries, as well as increased prevalence and associated consequences of other complications of diabetes [6].

The most common form of human diabetes is type-2 diabetes; this was previously referred to as non-insulin-dependent diabetes (NIDDM), maturity onset, or non-ketotic diabetes [7]. It is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell [8]. This type is highly associated with a family history of diabetes, older age, obesity and lack of exercise [9].

The danger of diabetes comes from complications of the disease. Kidney disease is a known complication of diabetes. DN is the major risk factor for death in DM [10]. The classical definition of DN is of a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining GFR and eventually ESRD [11].

DN occurs in approximately one third of individuals with Type-I diabetes, recent studies suggest that a similar proportion of type-2 diabetes patients develop this serious complication as well [12]. Therefore, renal function assessment is important in diabetic patients and indicators are needed to identify the early structural and functional changes in DN [10]. There is good evidence that early treatment delays or prevents the onset of DN, or diabetic kidney disease [13].

Urinary MAU is an established marker of early DN. MAU is defined as when urinary albumin excretion increases but remains undetectable by

conventional laboratory methods, such as routine urine testing strips [14].

Its presence is an indication of early glomerular dysfunction [15].

DN at this microalbuminuric stage is reversible with euglycaemic control. Therefore, it is pertinent to detect nephropathy at or before microalbuminuric stage [16].

Diabetes mellitus:

Definition and description of diabetes mellitus:

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [17]. Insulin is a hormone or chemical produced by cells in the pancreas. Insulin bonds to a receptor site on the outside of cell and acts like a key to open a doorway into the cell through which glucose can enter. Some of the glucose can be converted to concentrated energy sources like glycogen or fatty acids and saved for later use. When there is not enough insulin produced or when the doorway no longer recognizes the insulin key, glucose stays in the blood rather entering the cells, a condition called hyperglycemia [18].

The body will attempt to dilute the high level of glucose in the blood by drawing water out of the cells and into the bloodstream in an effort to dilute the sugar and excrete it in the urine. It is not unusual for people with undiagnosed diabetes to be constantly thirsty, drink large quantities of water, and urinate frequently as their bodies try to get rid of the extra glucose. This creates high levels of glucose in the urine [18].

At the same time the body is trying to get rid of glucose from the blood, the cells are starving for glucose and sending signals to the body to eat more food, thus making patients hungry. To provide energy for the starving cells, the body also tries to convert fats and proteins to glucose. The breakdown of fats and proteins for energy causes acid compounds called ketones to form in the blood. Ketones also will be excreted in the urine. As ketones build up in the blood, a condition called ketoacidosis can occur. This condition can be life threatening if left untreated, leading to coma and death [18].

Classification of diabetes mellitus

Four major types of diabetes have been defined by the NDDG and the WHO: insulin-dependent diabetes mellitus Type-I (IDDM), non-insulin dependent diabetes mellitus type-2 (NIDDM), gestational diabetes mellitus (GDM), and diabetes secondary to other conditions such as pancreatic disease, hormonal disease, drug or chemical exposure, insulin receptor abnormalities and certain genetic syndromes [19]

Type-I diabetes:

Type-I diabetes is also called juvenile-onset diabetes and accounts for only 5—10% of those with diabetes. It results from a cellular-mediated autoimmune destruction of the beta-cells of the pancreas. The rate of beta cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or others tress. Still others, particularly adults, may retain residual beta cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion. Immune

mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8^{th} 9^{th} decades of life. Autoimmune destruction of beta cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis [20].

Type- 2 diabetes:

Type-2 diabetes is also called adult-onset diabetes and accounts for 90— 95% of those with diabetes. It encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. Although the specific etiologies are not known, autoimmune destruction of beta cells does not occur [20].

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection [20]. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macro vascular and microvascular complications [20].

The resistance to insulin could result from defect at several levels of insulin action. a) Decreased insulin receptor binding which is attributable to a decrease in receptor number with no changes in receptor affinity and is believed to be secondary to down-regulation of the receptor by the elevated basal endogenous insulin level.

b) Decrease in the tyrosine kinase activity of the receptor [21].

Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal [20].

The risk of developing this form of 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 dyslipidemia, and its frequency varies indifferent racial/ ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of Type-I diabetes. However, the genetics of this form of diabetes are complex and not clearly defined [20].

Gestational diabetes mellitus (GDM):

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. GDM complicates 4% of all pregnancies in the U.S., resulting in135,000 cases annually. The prevalence may range from 1 to 14% of pregnancies, depending on the

population studied. GDM represents nearly 90%of all pregnancies complicated by diabetes. Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester [20].

The insulin:

Insulin is synthesized in the pancreas within the beta cells (ß-cells) of the islets of Langerhans. Within the islets of Langerhans, beta cells constitute 60—80% of all the islets cells. Insulin is composed of 51 amino acid residues and has a molecular weight of 5808 Da. In beta cells, insulin is synthesized from the proinsulin precursor molecule by the action of proteolytic enzymes, known as prohormone convertases (PCI and PC2), as well as the exoprotease carboxypeptidase E. These modifications of proinsulin remove the center portion of the molecule, or C-peptide, from the C- and N- terminal ends of the proinsulin. The remaining polypeptides (51 amino acids in total), the B and A chains, are bound together by disulfide bonds [22].

The actions of insulin on the global human metabolism level include: control of cellular intake of certain substances (most prominently glucose in muscle and adipose tissue), increase of DNA replication and protein synthesis via control of amino acid uptake and modification of the activity of numerous enzymes (allosteric effect) [22]. These effects are the response of the activation of the insulin receptor which belongs to the class of cell surface receptors that exhibit intrinsic tyrosine kinase activity. The insulin receptor is a heterotetramer of 2extracellular a-subunits disulfide bonded to 2 transmembrane b-subunits. With respect to hepatic glucose homeostasis, the effects of insulin receptor activation are specific phosphorylation events that lead to an increase in the storage of glucose with a concomitant decrease in hepatic glucose

release to the circulation. In most non hepatic tissues, insulin increases glucose uptake by increasing the number of plasma membrane glucose transporters [23].

Insulin is used medically to treat some forms of DM. Patients with Type-I Depend on external insulin (most commonly injected subcutaneously) for their survival because of the absence of the hormone. Patients with type-2 DM have insulin resistance, relatively low insulin production, or both; some type-2diabetics eventually require insulin when other medications become insufficient in controlling blood glucose levels [22].

Complications of diabetes mellitus:

Diabetes complications include both acute glycemic complications and chronic complications. The major aim of diabetes management is to prevent these complications [23].

Acute glycaemic complications:

The acute metabolic complications of diabetes include:

Diabetic ketoacidosis (DKA) DKA usually occurs in the context of total insulin deficiency, such as in type-l DM. It occurs rarely in type-2 DM under the stress of acute illness. When DKA occurs in patients with type-2, it may represent a transition to insulin deficiency. DKA may be the initial manifestation of diabetes, particularly for type-l, in 20-30% of cases of DKA. Precipitating factors for DKA in those with established diabetes include infection, other acute illnesses, lack of diabetes education and training, noncompliance, poor self-care, inadequate glucose monitoring, psychological problems, and indeterminate causes [19].

Very often, no known precipitating factor can be identified, but under these circumstances, it is most likely related to poor compliance, poor self-care habits, and unrecognized subclinical illness [19].

Hyperosmolar hyperglycemic nonketotic coma (I-IHNKC):

HHNKC is clinically defined by the presence of relative insulin deficiency and hyperglycemia, usually >1,000 mg/dl with associated elevated serum osmolality (>300 mosm/kg), dehydration, and stupor, progressing to coma if uncorrected, without the presence of ketosis or acidosis. These patients have sufficient circulating insulin to prevent lipolysis and ketosis [19]. patients with type-2 DM has insulin insensitivity. Hepatic glucose production is accelerated and hyperglycemia occurs. An increase in counter regulatory hormone (e.g., glucagons, catecholamines, cortisol, and growth hormone) secretion further increases hepatic glucose output and decreases extrahepatic glucose use. There is then increasing hyperglycemia, which is exacerbated by oral carbohydrate ingestion, because the extra glucose load cannot be metabolized. There is still sufficient insulin, however, to prevent accelerated lipolysis. The hyperglycemia leads to intracellular fluid loss and osmotic diuresis with dehydration, hem concentration, and further worsening of the hyperglycemia. Potassium is lost from cells and excreted in the urine. The loss of potassium conceivably may further inhibit insulin secretion [21].

HI-INKC occurs at about a tenth of the frequency of classical DKA; however, it carries a much higher mortality. This is consistent with the finding that most patients are older than 50 years of age. It usually occurs in patients with type-2 DM and often is the first indication that the patient has diabetes [21].

The precipitating factors for HI-INKC are multiple. Infection is the single most important factor, with presumed counter-regulatory hormone hyper secretion as the cause of the metabolic disturbance. Cardiovascular emergencies, such as a cerebrovascular accident and myocardial infarction, are the other major factors. The cerebrovascular accident is particularly important because it can cause severe hyperglycemia. This may be associated with an inability to drink, and hyper osmolality can ensue. Drugs such as corticosteroids and thiazides also precipitate HHNKC [211

Lactic acidosis (LA):

LA consists of elevated lactic acid (lactic acidemia, >2.0 mmol/L) with acidosis (pH <7.3) and without ketoacidosis. There may be low levels of ketones present. Approximately half of the reported cases of LA have occurred in patients with diabetes. Occasionally a combined LA and DKA may be present. In this situation, the presence of excess lactate may decrease production of acetoacetate, which is measured by dipstick methods for ketones, but beta hydroxybutyrate levels may remain elevated with an increased ratio of beta hydroxybutyrate to acetoacetate. Under the circumstances of combined LA and DKA, LA predominates by laboratory parameters and may mask an associated or underlying DKA [21].

Hypoglycemia:

Biochemically, hypoglycemia can be defined as any blood glucose level <50mg/dl. This definition is not satisfactory, however, because many episodes of hypoglycemia with blood glucose levels < 50 mg/dl go unnoticed by patients with DM, particularly during sleep, and because some patients can experience a seizure or severe neurologic impairment at blood glucose levels> 50 mg/dl [25].

Clinical definitions of hypoglycemia are preferred that depend on these verity and type of symptoms or signs. Mild hypoglycemia is defined as any episode associated with cholinergic symptoms such as diaphoresis or adrenergic symptoms such as tachycardia, palpitations, or shakiness. Moderate hypoglycemia is defined as an episode with some readily apparent neurologic dysfunction resulting from deficient glucose supply to the nervous system. Symptoms and signs of moderate hypoglycemia include decreased concentration, confusion, blurred vision, poor coordination, and somnolence. By definition, patients in this category can initiate self-treatment. In contrast, episodes of hypoglycemia in which the patient's neurologic status is so severely compromised that assistance by another person is required are defined as severe hypoglycemia. Symptoms and signs of severe hypoglycemia include seizure, loss of consciousness, severe disorientation, totally inappropriate behavior, and inability to be aroused from sleep [25].

Hypoglycemia associated with insulin therapy may be related to errors in dosage, delayed or skipped meals, exercise, and intensity of glycemic control, variation in absorption of circulating insulin from subcutaneous depots, variability of insulin binding, degradation, impairment of counter regulation, and possibly the use of human insulin. Impairment of counter regulation and autonomic neuropathy contributes to hypoglycemic unawareness, which further complicates insulin therapy in diabetes management and glycemic control. The frequency of hypoglycemic events is increased in diabetic patients who have renal, adrenal, or pituitary insufficiency [25].

Chronic complications of diabetes mellitus:

The chronic complications of DM are responsible for most of the morbidity and mortality associated with this disease [23]. The major chronic diabetes complications include both microvascular disease which affect capillaries and small blood vessels (diabetic retinopathy, nephropathy, and neuropathy), [24] and macro vascular disease which affecting large arteries (cardiovascular disease, peripheral vascular disease, and cerebrovascular disease). [20] The risk of developing any of these complications increases with longer duration of the disease [26].

The risk of developing complications is variable (table 1.1). For nephropathy, in particular, a strong but unknown genetic influence exists. The duration of diabetes, glycemic control, and hypertension are the strongest risk factors for microvascular disease; smoking, blood pressure, lipids, are the strongest risk factors for macro vascular disease [27].

The prevention of the chronic complications of diabetes involves not only glucose control but also specific risk factor modification and treatment strategies aimed directly at the prevention and treatment of chronic complications [23].

Table 1: Risk factors for the development of complications of diabetes

Factors	Microvascular disease	Macrovascular disease
Non-modifiable		
Genetic (susceptibility or protective)	++	++
Ethnic origin	+	+
Duration of diabetes	++	+
Modifiable or potentially modifiable		
Glycemic control	++	+
Blood pressure	++	++
Blood lipids	+	++
Smoking	+	++
BMI	+	+

+=Moderate risk factor; ++=strong risk factor. [26]

Macro vascular complications:

Cardiovascular Disease:

Cardiovascular disease remains a major problem for diabetic patients. Among type-2 diabetic USA patients, up to 75% of deaths are attributed to CVDs [28]. Myocardial ischemia due to coronary atherosclerosis commonly occurs without symptoms in patients with diabetes. As a result, multi vessel atherosclerosis often is present before ischemic symptoms occur and before treatment is instituted.

A delayed recognition of various forms of CHI) undoubtedly worsens the prognosis for survival for many diabetic patients. One reason for the poor prognosis in patients with both diabetes and ischemic heart disease seems to be an enhanced myocardial dysfunction leading to accelerated heart failure (diabetic cardiomyopathy). Thus; patients with diabetes are unusually prone to congestive heart failure. Several factors probably underlie diabetic cardiomyopathy: severe coronary atherosclerosis, prolonged hypertension, chronic hyperglycemia, microvascular disease, glycosylation of myocardial proteins, and autonomic neuropathy. Improved glycemic control, better control of hypertension, and prevention of atherosclerosis with cholesterol-lowering therapy may prevent or mitigate diabetic cardiomyopathy.

Several predisposing factors simultaneously affect the development of CVD and. Among these concomitant factors are obesity, physical inactivity, heredity, sex, and advancing age. To some extent, these predisposing factors exacerbate the major risk factors: dyslipidemia,15 hypertension, and glucose tolerance; and they may cause CVI) and DM through other pathways as well. To a large extent, both CVD and diabetes must be prevented through control of the predisposing risk factors.

Peripheral vascular disease:

The incidence of peripheral vascular disease in diabetic patients between the sexes is almost equal and occurs more commonly below the knees. Because atherosclerosis of large vessels, small vessels, or arterioles does not progress at the same rate, it is possible to have more severe disease in small vessels, leading to small patchy areas of gangrene of the foot or the toes in the presence of a palpable dorsal pedal or posterial tibial pulse. In addition, collateral circulation may develop poorly in diabetic patients. Calcification in the medium sized muscular arteries (Monck berg's sclerosis) is also increased in diabetic patients. This process involves degeneration of smooth muscle cells, followed by calcium deposition [24].The earliest symptom of peripheral vascular disease is intermittent claudication, which is characterized by pain on walking and is relieved by stopping. This usually begins in the calf, and may involve various muscle groups, depending on the location of the occlusion. A more serious symptom is rest pain, which usually is worse at night and may require narcotics for relief. Because rest pain often is relieved by sitting with the feet dependent, edema of the legs may occur in those patients who sleep in a chair [24].

Cerebrovascular disease:

Studies documented a strong association of diabetes with risk of stroke, especially strokes due to vascular disease and infarction. Most ischemic strokes in diabetic patients are due to occlusion of small paramedical penetrating arteries. The occlusions cause small infarcts within the white matter of the brain. Diabetic autonomic neuropathy may contribute to the development of cerebrovascular disease in people with diabetes. Elevated blood pressure is the major risk factor for stroke. Other risk factors for stroke, besides diabetes, include cigarette smoking and a high level of LDL cholesterol. Stroke is substantially increased in individuals who have other vascular diseases, especially coronary heart disease, left ventricular hypertrophy, atrial fibrillation, and peripheral vascular disease. Preventing stroke in people with diabetes is feasible through identifying and treating risk factors, especially hypertension, cigarette smoking, and high LDL cholesterol [19].

Microvascular complications:

Diabetic neuropathies:

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can develop nerve problems at any time, but the longer a person has diabetes, the greater the risk. An estimated 50 % of those with diabetes have some form of neuropathy, but not all with neuropathy have symptoms. The highest rates of neuropathy are among people who have had the disease for at least 25 years. Diabetic neuropathy also appears to be more common in people who have had problems controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, in overweight people, and in people over the age of 40 [30].

Diabetic retinopathy:

Diabetic retinopathy is the most common diabetic eye disease and a leading cause of blindness in American adults [31]. Most patients develop diabetic changes in the retina after approximately 20 years. Over time, diabetes affects the circulatory system of the retina [32]. The earliest phase of the disease is known as background diabetic retinopathy. In this phase, the arteries in the retina become weakened and leak, forming small, dot-like hemorrhages. These leaking vessels often lead to swelling or edema in the retina and decreased vision [32].

The next stage is known as proliferative diabetic retinopathy. In this stage, circulation problems cause areas of the retina to become oxygen-deprived or ischemic. New, fragile, vessels develop as the circulatory system attempts to maintain adequate oxygen levels within the retina. This is called neovascularization. Unfortunately, these delicate vessels hemorrhage easily. Blood may leak into the retina and vitreous, causing spots or floaters, along with decreased vision. In the later phases of the disease, continued abnormal vessel growth and scar tissue may cause serious problems such as retinal detachment and glaucoma [32].

Diabetic nephropathy

Structure and function of the kidney:

The structural and functional unit of the kidney is the nephron, (figure 2.1). A nephron is composed of a glomerulus (the filter), a proximal convoluted tubule (primary site of reabsorption) is the longest section of the nephron and their walls are formed by a low columnar epithelium. The eosinophilia cells of the epithelium have a wide brush border (long microvilli) and are active in endocytosis., A long loop of Henle (thin-walled descending loop and thick wall descending loop), a distal convoluted tubule (secondary site of reabsorption), and a collecting tubule and collecting ducts (sites of water reabsorption and urine concentration). Most regions of each nephron are closely associated with the bloodstream [33].

Blood first enters the kidney through the renal artery, which branches into a network of tiny blood vessels called arterioles. These arterioles then carry the blood into the tiny blood vessels of the glomerulus. It is here, in the renal corpuscle, where filtration occurs. The glomerulus filters proteins and cells, which are too large to pass through the membrane channels of this specialized component, from the blood. These large particles remain in the blood vessels of the glomerulus, which join with other blood vessels so that the proteins remain circulating in the blood throughout the body. The small particles (e.g., ions, sugars, and ammonia) pass through the membranes of the glomerulus into Bowman's capsule. These smaller components then enter the membrane enclosed tubule in essentially the same concentrations as they have in the blood. Hence, the fluid entering the tubule is identical to the blood, except that it contains no proteins or cells [34].

The tubule functions as a dialysis unit, in which the fluid inside the tubule is the internal solution and the blood (in capillaries surrounding the tubule) acts as the external solution. Particles may pass through the membrane and return to the blood stream in the process known as reabsorption. The reabsorption of many blood components is regulated physiologically. Alternatively, particles may pass through the membrane from the blood into this tubule in the process known as secretion. The most important particles that are secreted from the blood back into the tubules are 1-1+ and K+ ions, as well as organic ions from foreign chemicals or the natural byproducts of the body's metabolism [34]. The blood components that remain in the nephron when the fluid reaches the collecting duct are excreted from the body. The collecting duct from one nephron meets up with many others to feed into the ureter. The ureters (one from each kidney) enter the bladder, which leads to the urethra, where the liquid waste is excreted from the body. Hence, the material that is filtered and secreted from the blood into the tubule is less than the amount that is reabsorbed into the blood, which is ultimately excreted from the body

[34]. From the overview of kidney function above, it is clear that blood components (e.g., water, ions, and sugars) must be able to pass between the nephron tubules and the blood-filled capillaries surrounding them. But the phospholipid bilayer membranes are not permeable to polar molecules, because the interior lipid region of the membrane is nonpolar. Thus, the polar components of blood could not cross the membranes surrounding the tubules unless these membranes contained special channels to allow the passage of polar species. The channels required to allow the passage of polar blood components are formed by proteins embedded in the phospholipid-bilayer membrane. Proteins that form channels in the membrane typically have membrane spanning cylindrical shapes: there is a hydrophobic surface that can interact with the "tail" region of the phospholipid-bilayer membrane and a hollow internal core that forms the pore. These proteins form a "tunnel" from the aqueous phase on one side of the membrane to the aqueous phase on the other side of the membrane. The size of the tunnel determines the size of the particles that will be able to pass through the channel. If the internal core of the

protein channel is lined with hydrophilic amino-acid residues, then the channel allows passage of polar or charged particles between the two aqueous sides of the membrane. These channels may be left open continuously, or they may be opened and closed by elaborate cellular gating mechanisms. In either case, passage of particles through the membrane is dictated by the size, shape, and polarity of the channel [34]

Definition, stages and clinical features of diabetic nephropathy:

DN has been defined by the presence of proteinuria >0.5 g/24h. This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria, or macroalbuminuria [35]. In the early 1980s, studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional methods, were predictive of the later development of proteinuria type-2 diabetic patient. This stage of renal involvement was termed MAU or incipient nephropathy [36]. DN is usually accompanied by hypertension, progressive rise in proteinuria, and decline in renal function [36]. DN is a spectrum of progressive renal lesions ranging from renal hyper filtration to end stage kidney disease. The earliest clinical evidence of nephropathy is the presence of MAU (table 1.2). It occurs in 30% of Type-I diabetics 5 to 15 years after diagnosis but may be present at diagnosis in type-2 diabetics as the time of onset of type-2diabetes is often unknown. MAU progresses to overt proteinuria over the next 7t010 years (figure 2.2). Once overt proteinuria develops, renal function progressively declines and ESRD is reached after about 10 years [37].

Table 2: Stages and clinical features of diabetic nephropathy [37]

Stage 1	•Glomerular hypertension and hyper filtration • Normoalbuminuria: urinary ACR< 30 mg/g • Raised GFR, normal serum CR
Stage 2	• "Silent phase" (structural changes on biopsy but no clinical manifestations) • Normoalbuminuria
Stage 3	•MAU: urinary ACR between 30 and 300 mg/g • Normal serum CR • There may be increased blood pressure
Stage 4	•Overt "dipstick positive" proteinuria (macroalbuminuria) urinary ACR > 300 mg/g • Hypertension • Serum CR may be normal • Increase in serum CR with progression of nephropathy
Stage 5	•End stage renal failure • Requiring dial sis or transplant to maintain life

Risk factors of diabetic nephropathy

Cross-sectional and longitudinal studies have identified some factors associated with a high risk of nephropathy: elevated blood pressure and glycosylated hemoglobin and cholesterol concentrations, smoking, advanced age, high level of insulin resistance, male sex (the risk is lower among women, at least before menopause), and possibly high dietary protein intake [391. In clinical practice, the finding of a family history of cardiovascular events is a simple but powerful indicator of renal risk [39]. 1.4.2.2.3.4. Hypertension and diabetic nephropathy

Hypertension is common in diabetic patients, even when renal involvement is not present. About 40% of Type-I and 70% of type-2 diabetic patients with normoalbuminuria have blood pressure levels >

140/90 mmHg [40]. High blood pressure may contribute to the onset and progression of micro- and macro vascular complications. Indeed, an estimated 35 to 75% of diabetic complications can be attributed to hypertension [41]. Treatment of hypertension dramatically reduces the risk of cardiovascular and microvascular events in patients with diabetes [35]. In the UKPDS, a reduction from 154 to 144mmHg on systolic blood pressure reduced the risk for the development of MAU by 29% [42]. Blood pressure targets for patients with diabetes are lower (130/80mmHg) than those for patients without diabetes [42]. In the HOT study, a reduction of diastolic blood pressure from 85 to 81 mmHg resulted in a 50%reduction in the risk of cardiovascular events in diabetic but not nondiabetic patients [43].

Isolated systolic hypertension has been attributed to the loss of elastic compliance of atherosclerotic large vessels. In general, the hypertension in patients with both types of diabetes is associated with an expanded plasma volume, increased peripheral vascular resistance, and low renin activity. Both systolic and diastolic hypertension markedly accelerates the progression of diabetic nephropathy, and aggressive antihypertensive management is able to greatly decrease the rate of fall of GFR. A major aspect of initial treatment should consist of lifestyle modifications, such as weight loss, reduction of salt and alcohol intake, and exercise [44].

1.4.2.2.3.5. Pathophysiology of diabetic nephropathy

Type-2 diabetes causes growth of the kidney and enlargement of the glomeruli, which then are susceptible to damage [45]. Three major histological changes occur in the glomeruli in DN (l) mesangial expansion is directly induced by hyperglycemia, perhaps via increased matrix production orglycosylation of matrix proteins, (2) glomerular basement membrane thickening occurs, (3) glomerular sclerosis is caused by intraglomerular hypertension [46]. Glucose can bind irreversibly to proteins in the kidney and circulation to form so called advanced glycosylation end products (AGEs). AGEs can form complex cross links over years of hyperglycemia and can contribute to renal damage by stimulation of growth and fibrotic factors via receptors for AGEs [47].AGEs also can stimulate protein synthesis [48], further decrease degradability of the basement membrane [70]; increase its permeability [49] and causes endothelial dysfunction [49].Hyperglycemia increases the expression of TGFß in the glomeruli [47].TGFß appears to be crucial in the development of renal hypertrophy and accumulation of extracellular matrix [51].

Renal hypertrophy is an early event; irreversible changes such as glomerulosclerosis and tubulointerstitial fibrosis are preceded by hypertrophy [51]. Parallel to and to some extent concomitant with renal hypertrophy, hyper filtration and intrarenal hypertension develop in type-l [52] as well as in type-2 diabetes [53]. AngII contributes to the progression of DN [51]. High glucose stimulates the synthesis of angiotensinogen and Angll [52]. Angll preferentially constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressures.

In addition to its hemodynamic effects, Angll also stimulates renal growth and fibrosis through Angll type-I receptors, which secondarily upregulate TGF-ß and other growth factors [47].

Screening for diabetic nephropathy:

Detection of DN as early in the disease process as possible currently offers the best chance of delaying or possibly preventing progression to end stage disease. Thus screening for MAU and proteinuria in a structured, regular manner is recommended [54]. Most guidelines suggest annual screening, ideally using an early morning urine sample to avoid the variable effects of upright posture on albumin and other markers excretion [l l].

Microalbuminuria (MAU):

One of the earliest markers of DN is the presence of small amount of the protein albumin in the urine. This is called MAU (urinary albumin excretion of30-300 mg/24 hours) [55]. MAU is defined as when urinary albumin excretion increases but remains undetectable by conventional laboratory methods, such as routine urine testing strips [16]. MAU may progress over a span of a number of years to overt nephropathy characterized by the presence of larger amounts of the protein albumin leaking through the kidneys' filter mechanism into the urine. This is called macroalbuminuria (urinary albumin > 300 mg/24 hours). The presence of macroalbuminuria indicates more serious kidney disease [55].

Studies have shown that presence of MAU is reversible with interventions to tightly control blood sugar and blood pressure. MAU may sometimes resolve on its own. Specific medications including ACE inhibitors and ARBs have been shown to halt and reverse the presence of MAU and delay the progression to ESRD and the need for dialysis. In some cases, a combination of both agents is indicated to protect the kidneys [55]. Screening for MAU is recommended in all post-pubertal type-I with diabetes > 5 years duration and all type-2 diabetics on an annual basis [55].

Screening for MAU can be performed by three methods: measurement of the albumin to creatinine ratio (ACR) in early morning or random (untimed) spot urine collections, 24-h urine collection or timed urine collections (e.g. 4-11) [17].

The ACR is the preferred method as it does not require 24-11 or timed collections, it correlates with the 24-hour urine values over a large range of proteinuria, it is cheap to perform, and repeat values can be easily obtained to be certain that MAU, if present, is persistent. A patient is considered to have DN if 2 of 3 measurements of ACR are elevated above 30 to 300 mg/g [55].

Glycosylated hemoglobin (HbA1 c):

The aim of diabetic management is to maintain the blood glucose concentration with in or near the nondiabetic range with a minimal number of fluctuations. Glycosylated hemoglobin is the term used to describe the formation of a hemoglobin compound formed when glucose (a reducing sugar) reacts with the amino group of hemoglobin (a protein). The rate of formation is directly proportional to the plasma glucose concentrations. Because the average RBC lives approximately 120days, the glycosylated hemoglobin level at any one time reflects the average blood glucose level over the previous 2-3 months.

Therefore, measuring the glycosylated hemoglobin provides the clinician with a time averaged picture of the patient blood glucose concentration over the past 3months. [56] (Hemoglobin Alc (1-1bA1c), the most commonly detected glycosylated hemoglobin, HbA1c is a reliable of monitoring long term diabetes control rather than random plasma glucose. [56]

Lipid profile:

Lipids are organic substances present in all living organisms. They include Fats, Oils, Waxes and other related compounds. They are insoluble in water and hydrophobic and soluble inorganic solvents. They are heterogeneous group of compounds related to fatty acids and are not polymers, or they are esters of long chain fatty acids and alcohols. [57]

Although the term lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monotriglycerides phospholipids), as well as other sterol metabolites such as cholesterol. [58]

The main plasma lipids include:

•Fatty acids: in plasma only relatively small amount of fatty acids exists in the free unesterified form, most of which is bound to albumin. The majority of plasma fatty acids are instead found as a constituent of triglycerides or phospholipids. [56]

•Triglycerides (TG): as can be inferred from the name, triglycerides contain three fatty acids molecules attach to one molecule of glycerol by ester bonds.

Triglycerides containing saturated fatty acids pack together more closely and tend to be solid at room temperature. There is no charge group or polar hydrophilic groups in structure of TG, making it very hydrophobic and virtually water insoluble. [56]

Triacylglycerol stored in adipose tissue, are a major form of energy storage in animals. The adipocytes are designed for continuous synthesis and breakdown of triacylglycerol which controlled mainly by the activation of hormone sensitive enzyme lipase. The complete oxidation of fatty acids provides high calorie content, about 9Kcal/g, compared with 4Kcal/g for breakdown of carbohydrates and proteins. [59]

•Phospholipids: are similar in structure to TGs except they only have two esterified fatty acids. There several types of phospholipid head groups such as choline, inositol, serine and ethanolamine, which are hydrophilic in nature. [56]

• Cholesterol: is an unsaturated steroid alcohol containing 4 rings (A, B, C and D), and it has a single C-H side chain tail similar to a fatty acid in its physical properties. The only hydrophilic part of cholesterol is the hydroxyl group in the ring A, there for cholesterol is also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids.

Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to fatty acid, in the same way as in triglycerides. In contrast to free cholesterol, there are no polar groups in cholesteryl esters, making them very hydrophobic, so they are not found on the surface of lipid layers but are instead in the canter of lipid drops, along with TGs.

Cholesterol is almost exclusively synthesized by animals, cholesterol is also unique in that unlike other lipids, it is not readily catabolized by the most cells and, therefore, does not serve as source for fuel. Cholesterol can however be converted in the liver to primary bile acids, such as cholic acid and chenodexycholic, which promote fat absorption in intestine by acting as detergents.

A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens, cholesterol also contributes in facilitating triglyceride transport in serving the fuel needs of the body and maintain cell membranes. Finally, a small amount of cholesterol, after first being converted to 7dehydrocholesterol, can also be transformed to vitamin on irradiation of the skin by sunlight. [56]

•Lipoproteins: as name implies, lipoproteins are composed of both lipids and proteins called apolipoprotiens. Because the main role of lipoproteins is delivery of fuel to peripheral cells, the core of the lipoprotein particle essentially represents the cargo that being transported by lipoprotein.

The various lipoprotein particles were originally separated by the ultracentrifugation into different density fraction, which are:

I. Chylomicrons: the largest and least dense of the lipoprotein, the principle role of chylomicrons is delivery of dietary lipids (triglyceride and cholesteryl ester) to hepatic and peripheral cells.

Il. Very—Low Density Lipoproteins: they the major carriers of endogenous (hepatic derived) triglycerides and transfer triglycerides from liver to peripheral tissue.

Ill. Low—Density Lipoproteins: they primarily form as consequence of the lipolysis of VLDL, is more cholesterol rich than other lipoprotein.

IV. High—Density Lipoproteins: the smallest and densest lipoprotein particle is synthesis by both the liver and intestine. Their function is to remove excess cholesterol from peripheral cells. [56]

A limited number of studies have examined the association between lipid and lipoprotein levels and diabetic nephropathy in Type 2 diabetes. There have not been any consistent findings in cross sectional studies (Seghieri et al, 1990; Serenities al, 1992; Nielsen et al, 1993). In a study in Finland of 133 people with Type 2 diabetes (Niskanen et al, 1990) people with persistent microalbuminuria (>30 mg/24h) had significantly lower levels of HDL and LDL cholesterol and higher triglycerides after 5-year follow-up (0.92±0.03 v 1.07±0.03mm01/L, p<O.05•, 3.65±0.22 v 4.00±0.11 mmol/L; and 4.24±0.90 v 2.35±0.16 mmol/L, p<0.05, respectively). [60]

Rationale

The prevalence of type 2 diabetes mellitus has been increasing significantly in all countries during the last century. By 2010, 220 million people in the world are projected to be afflicted by this disease. The importance of protecting the body from hyperglycemia cannot be overstated. The complications of hyperglycemia are diabetic nephropathy, neuropathy, retinopathy and cardiovascular disease.

One of the earliest markers of diabetic nephropathy is the presence of microalbuminuria (MAU), Once overt kidney failure has developed two years survival is approximately 50 ^{0}/0, MAU is associated also with cardiovascular disease in patients with diabetes and hypertension.

The Diabetes Control and Complications Trial (DCCT) showed a significant relationship between reduction in glycosylated hemoglobin (HbA1c) levels and the risk of micro vascular complications including chronic kidney disease (CKD).

In people with Type 2 diabetes, greater disturbance of lipid metabolism has been reported in association with increasing diabetic renal disease.

So this study intends to assess HbAlc, and lipid profile in DMtype2 patients as risk factors of developing future complications like nephropathy, and to prevent these complications which could be a physically, psychologically, socially, and finically a burden.

Objectives

General objective

To determine risk factors of diabetes nephropathy in type 2 diabetic patients in Sudanese in Khartoum state.

Specific objectives

- To estimate the effect of lipid profile (total cholesterol, triglyceride, LDL, HDL) on progression of diabetic nephropathy.
- To determine the effect of high blood pressure on progression of diabetic nephropathy.
- To identify the effect of body mass index on developing of diabetic nephropathy.
- To estimate the effect of glycemic control (HbAlc) on developing of diabetic nephropathy.
- To determine the effect of duration of diabetes on progression of diabetic nephropathy.

MATERIALS AND METHODS

Study approach: This was quantitative study

Study design: descriptive, analytical, case study, hospital based.

Study area:

The study was conducted in Khartoum state, and samples were collected from Medical and Health Services University of Khartoum, and ALacdmya hospital.

Study period:

The study was carried during the period from December 2013 to Apri12014.

Study population: Samples were collected from Sudanese patients with DMtype-2 as study group.

Inclusion criteria:

Sudanese patients with DM type2 their age ranged between 30-65 years old.

Exclusion criteria:

Individuals with conditions such as hemoglobinopathies, renal diseases, urinary tract infection and cardiac conditions were excluded from the study.

Sample size:

The study included 50 participants, 24 females and 26 males.

Ethical consideration:

According to research ethics, permission was taken from the hospitals for sample collection and consent was obtained from each study participants for collection of samples. The objectives of the study were explained to all study participants, also all participants were informed about their results under strict confidentiality.

Sampling:

After informed consent, and use of antiseptic for cleaning the skin sample of venous blood (5ml) was taken from study participants, from the directly in to a plain container and EDTA anticoagulant, also sample of urine was collected in clean and sterile container.

Tools of data collection:

Questionnaire was specifically designed to obtain clinical information about study participants

Methodology:

Instruments: a spectrophotometer was used for chemical analysis.

Materials required: syringes, plain containers, EDTA anticoagulants, alcohol swabs, marker pen, cotton, centrifuge, reagents, cuvettes, samples, and standards.

Measurement of serum total cholesterol:

Enzymatic test for cholesterol

Reaction principle:

$$\text{Cholesterol ester} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2CHO \longrightarrow \text{Cholestrol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-amino-phenazonephenol} \xrightarrow{POD} \text{quinoneimine} + 4\ H_2O$$

Reagent preparation:

The reagent and the STD are ready for use.

Specimen:

Serum, heparinized or EDTA plasma.

Pipetting scheme:

Pipette in to cuvettes	Reagent blank	Sample /STD
Sample /STD	-	1o µl
RGT	1000 µl	1000 µl

Mix, incubate 10 min at 20-25 ^{0}c or 5 min at 37 ^{0}c. Measure the absorbance of the Sample /STD against the reagent blank within 60min.

Calculation of cholesterol concentration:

$$C = 200 \times \frac{A\ A\ \text{sample}}{A\ A\ \text{sample}}\ [\text{mg/dl}]$$

$$C = 5.17 \times \frac{AA\ sample}{A\ ASTD}\ [mmol/l]$$

Normal range:

<200mg/dl

2.14 Measurement of triglyceride:

Enzymatic test for triglyceride Reaction

principle:

Triglyceride $\xrightarrow{LPL}$ glycerol + FAs

Glycerol + ATP $\xrightarrow{GK}$ glycerol + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{GPO}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 +4-aminophenazone +4cholorophenol $\xrightarrow{Pod}$ quaniamine +4H_2O

Reagent preparation:

The reagent and the STD are ready for use.

Specimen:

Serum, heparinized, or EDTA plasma.

Pipetting scheme:

Pipette in to cuvettes	Reagent blank	Sample /STD
Sample /STD	-	10 µl
RGT	1000 µl	1000 µl

Mix, incubate 10 min at 20-25 ^{0}c or 5 min at 37 ^{0}c. Measure the absorbance of the Sample /STD against the reagent blank within 60min.

Calculation of triglyceride concentration:

$$C = 200 \times \frac{AA\ sample}{A\ A\ sample}\ [mg/dl]$$

Normal range:

Females 35-135mg/dl

Males 40-160mg/dl

2.15. Measurement of HDL: Enzymatic test for HDL Reaction principle:

Sample precipitate Phosphotungstic and magnesium iron VLDL and LDL precipitate reaction.

Cholesterol-ester + H_2O cholesrtroase → Cholestrol

Cholesterol+1/2O+H_2O Choloxidase → Cholesterol+ H_2O_2

$2H_2O_2$+4aminoantipyrinephenol peroxidase → quaniamine + $4H_2O$

Method:

Sample 0.5ml precipitated (HDL cholA) 0.2ml

Mix thoroughly and let stand for 10 min at room temperature

Centrifuge at minimum for 10 min at 4000r.p.m.

Sample precipitate polyvinyl sulphate

	Blank	STD	Sample
Distal Water	100 µl	-	-
HDL STD	-	100 µl	-
Supernatant	-	-	100µl
Reagent A (T.cholestrol)	1.0 ml	1.0 ml	1.0 ml

Incubate at 6-25 ^{0}C for 30 minutes or at 37 ^{0}C for 10 minutes.

Measure the absorbance of the Sample /STD against the reagent blank at filter 500nm within 60 min.

Calculation:

$$C = \frac{\text{OD of test}}{\text{OD of STD}} \times \text{conc of STD} \times \text{DF}$$

Normal range:

35mg/dl

Measurement of LDL:

Will be calculated by the fallowing formula (Friedwald equation):

$$\text{LDL concentration} = \text{Total. Cholesterol} - \left(\frac{\text{Triglyceride}}{5} + \text{HDL}\right)$$

Normal range:

< 100mg/dl

2.17. Measurement of HbA 1 C:

Labona Check^{TM} Alc HbA1c Test

Kit Test principle

The Labona Check^{TM} Alc is about affinity assay. Labona Check^{TM} Alc HbAlc Test Kit consists of the cartridges, the RI/ Reagent and the R2/Reagent. the RI/ Reagent contains the agents that lyse erythrocytes and precipitate hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diol of glycated hemoglobin. When blood is added to the RI/ Reagent, the erythrocytes are lysed and all hemoglobin precipitates, as well as the boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction mixture is added to the cartridge and all the precipitated hemoglobin, conjugate-bound and unbound, remains on top of the filter. Any unbound boronate is removed with the R2/Reagent. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) color intensity respectively with the Labona Check^{TM} AlcHbAlc analyzer, the ratio between them being proportional to the percentage of glycated hemoglobin in the sample.

Test procedure

l. Add 5µl whole blood to the test tube pre-filled with the RI/ Reagent Mix well; leave the tube for minimum 2 minutes, maximum 3 minutes. Do not leave it more than 3 minutes.

2. Once the reaction mixture completed, shake the test tube once again for the components to be blended well. Open the tube and collect25 to the cartridge. Leave it for 10 seconds so as the applied sample to soak enough into the membrane.

3. When the sample is absorbed completely, 25k11 of theR2/Reagent to the cartridge. Allow the sample to soak into the membrane for about 10 seconds.

4. Once the s ample is absorbed completely, place the cartridge on the tray and then select "Analyzer" on the display. The tray shall be inserted into

Labona Check™Alc HbA I c

Analyzer. Reference range:

	NGSP	IFCC
Prediabetes	5.7- 6.47%	39 - 46mmol/mol
Presence of diabetes	>6.57%	≥48mmol/mol
Target in diabetes	<7.07%	<53mmol/mol

Measurement of Microalbumin:

ACCENT-200 MICROALBUMIN

DIAGNOSTIC KIT FOR DTEREMINATION OF ALBUMIN CONCENTRATION IN URINE AND CEREBEROSPINAL FLUID METHOD PRINCIPLE

Immunoturbidimetric method. Albumin in the sample forms with anti-albumin antibodies in the reagent an insoluble complex. The turbidity caused by the complexes is measured spectrophotometrically at 340nm and is proportional to the amount of albumin in the sample. PROCEDURE

These reagents may be used in automatic analyzers ACCENT-200 and ACCENT-200 IIGEN.

I -reagent and 2- reagent are ready to use. Before use mix reagent by gently inverting each bottle.

For reagent blank 0.9% NaCl is recommended.

CALCULATION

For the calculation of albumin 24hours quantity, multiply the concentration (mg/dl) with the volume (1) of the 24hours urines.

REFERENCE VALUES

urine	mg/24h	µg/min	mg/g creatinine
Normal	<30	<20	<30
Microalbumin	30-300	20-200	30-300
Clinical albumin (overt nephropathy)	>300	>200	>300

Cerebrospinal fluid, lumber 177 -251mg/L

Quality control

The precision and accuracy of all methods used in this study was checked each time a batch analyzed by using commercially prepared control sera. 2.20 Statistical analysis

The data which collected in this study analyzed using SPSS computer program. The mean and standard deviation of lipid profile (total cholesterol, triglyceride, LDL, and HDL), HbAlc, systolic, diastolic pressure, duration of diabetes, body mass index, age, and MAU were calculated. Statistical significance was analyzed using one-sample t test to compare risk factors in group land group2.The count and percentage of normal and abnormal results in patients group were calculated.

Results

In this descriptive case study, conducted in Khartoum hospitals in period from December2013 to Apri12014, blood and urine samples were collected from 50type2 diabetic patients to determine risk factors of diabetic nephropathy. The following results were obtained:

Table 1 shows number of study participants in groupl (Nephropathy) which includes 29 participants, minimum value of MAU in this group was 33mg/L, and maximum value was 278mg/L and mean was (122.04±66.41).
group2 (Non-nephropathy), minimum value of MAU was 2.0mg/L, and maximum value was 23.3mg/L, and mean for this group was (10.80±7.59).

Table 2 shows the present study included 50 patients distributed into 26 males comprised (52%) of study group, 19 males were in groupl and 7 males in group 2. 24 females comprised (48%) of study group, 10 females were in group 1 and 14 were in group 2.
Table 3 shows some of personal and clinical characteristics of type-2 diabetic patients. The means of age, BMI, systolic pressure and diastolic pressure are higher for patients group. The mean duration of diabetes approximately was eight years and the mean duration of pressure was two years ago in patient group. Table 1 shows mean age of two groups was not statistically different. In group1 age was 53.81 years and in group2 was 51.79 years. Also Mean BMI of nephropathy was 24.41 kg/m2 and non-nephropathy was 24.86 kg/m2 which were similar. There was significantly higher mean SBP in groupl (129.31 mmHg) compared to group 2 (122.86 mmHg). Mean of DBP in groupl was 83mmHg which was significantly different from groupl with 78.33mmHg. Mean duration

of Diabetes of group I patients was 11.49 years where there is no significant difference to group 2 patients where it was 9.47years. Mean HbAlc of group 1 patients was 10.05 % which is similar to group 2 patients HbA1c of 10.05 ^{0}/0. Mean T. cholesterol of group 1 patients was 185.55mg/dl that is significantly higher than group 2 patients which was
150.14 mg/dl. Mean of TG in groupl was 190.86mg/dl which was significantly higher than group2 was 111.76mg/dl. Mean of HDL in groupl was 60.90mg/dl where was no significant difference to group2 63.57mg/dl. Mean of LDL for groupl was 81.14mg/dl which was significantly higher than group 2 was 58.87mg/dl. In our study mean of SBP,DBP ，
T. cholesterol, TG, LDL were significant different on comparing the two groups and mean SBP and DBP of both group was found with in normal level and statistically different in the two groups.

Table 1

Number, minimum, maximum, and mean ± Std. Deviation of MAU in groupl (nephropathy) and group2 (non-nephropathy).

	No	Minimum	Maximum	Mean ± Std.Deviation
Group l	29	33.0	278.0	122.04±66.41
Group 2	21	2.0	23.3	10.80±7.59

- The table shows numbers and mean of MAU in groupl and group2.
- Descriptive statistics used to estimate numbers and Mean ± Std.Deviation.

Table 2
Numbers and percentages of males and females in both nephropathy group and non-nephropathy group

Sex		Nephropathy	Non-nephropathy	
Male	Count	19	7	26
		38.0%		52.0%
Female	Count	10	14	24
		20.0%	28.0%	48.0%
Total	Count	29	21	50
		58.0%	42.00/0	100.0%

- The table shows numbers and percentages of males and females in both groups.
- Descriptive statistics used to estimate numbers and percentages.

Table 3

Comparison of the means of risk factors between nephropathy group and non-nephropathy group

Factors	Nephropathy group	Non-nephropathy group	P value
Age	51.79 ± 10.66 (30-65)	53.81 ± 9.52 (35-65)	0.193
BMI (kg/m^2)	24.41 ± 3.82 (17-36)	24.86 ± 3.51 (16-31)	0.677
Blood Pressure (Systolic)	129.31 ± 15.39 (115-160)	122.86 ± 12.90 (100-160)	O .041
Blood Pressure (Diastolic)	83 ± 9.29 (70-100)	78.33 ± 8.99 (60-100)	0.027
Duration of DM (years)	9.47 ± 5.48 (0.25-25)	11.49± 10.35 (0.58-35)	0.21
T.cholestrol (mg/dl)	185.55 ± 40.37 120-290)	150.14 ± 36.64 (98-267	0.003
Triglyceride (mg/dl)	190.86 ± 78.96 (115-402)	111.76 ± 33.16 66-200)	0.0000198
HDL (mg/dl)	66.90 ± 37 (20-208)	63.57 ± 39.31 (32-220)	0.761
LDL (mg/dl)	81.14±38.19 (13-155)	58.87 ± 28.71 (7.20-116)	0.029
HbAlc %	10.05 ± 2.35 (6.4-16.5)	10.05 ± 2.30 (7.70-16.5)	0.995

- The table shows the mean ±SD, minimum and maximum values, and probability (P).
- T-test was used for comparison.
- P-value <0.05 considered significant.

Discussion

In present study we found that factors like systolic blood pressure, diastolic blood pressure, serum cholesterol, TG and LDL were significantly higher in groupl in comparison to group2 patients suggesting that above mentioned factors are associated with progression of diabetic nephropathy.

Our study stated that number and percentage of male patients included in this study suffers from nephropathy more than female patients which suggest that males more susceptible to nephropathy than females and that can be due to females tend to control and pay attention to their condition more than males.

The current study found that serum total cholesterol, triglyceride, and LDL levels among diabetic patients with nephropathy showed significant difference in comparison with diabetic patients without nephropathy. As known diabetic patients suffer from greater-disturbance of lipid metabolism. Lipids induce glomerular and tubulointerstitial injury and damage glomerular capillary causing development of diabetic nephropathy.

In this study we found high blood pressure in nephropathy group compare to non-nephropathy group, suggesting that hypertension could be risk factor of nephropathy because elevated systemic blood pressure transmitted to glomerulus, contribute to glomerular hypertension and thus accelerate glomerular damage and renal impairment.

In our study, mean SBP was 129.31 mmHg, mean duration of DM was 11.49 years and mean HbA1c level was10.05 % while mean MAU was 122.04 mg/L in group 1 patients similar finding were observed by Dr. Shital S. Dodhia, etal. Who found mean SBP was 141.37 mmHg, mean duration of DM was 9.26 years and mean HbAlc level was 9.0% while mean GFR was 36.75 ml/min in type2 DM patients. (64)

We found also there was insignificant difference between group 1 and group2 in age, BMI, duration of DM, HDL, and glycemic control which contradict with above study findings mainly with influence of duration of DM and glycemic control, and this could due to the difference in period of carrying the studies out.

Study done by Fayza Ahmed, etal. The main objective was to determine the prevalence of microalbuminuria on urine samples of Sudanese type 2 diabetic patients (non- insulin-dependent) which is expressed by Albumin creatinine ratio (ACR). Across- sectional hospital based study was carried out in Elmusbah Medical Center, from November 2008 to March 2009, fifty-eight of type 2 diabetic patients studied included 29 females (aged 35 80 years) and 29 males (aged 43 - 88 years). Microalbuminuria was diagnosed in 26 (44%) patients. The prevalence of microalbuminuria was 8.66% from total populations at risk (N=300). The risk factors associated with microalbuminuria were found to be age, duration of diabetes, systolic and diastolic blood pressure (65). Another study done by M. V. Hospital for Diabetes & Prof. M. Viswanathan Diabetes Research Centre, WHO

Collaborating Centre for Research, Education & Training in Diabetes, Chennai, India. During median follow up of I l years in subjects with normal renal function at baseline, 44.1%. developed proteinuria at follow up. Glucose levels, HbA1c, systolic blood pressure (SBP), triglycerides, and urea levels were significantly higher at baseline among progressors than non-progressors. Progressors had a longer duration of diabetes and significant fall in estimated glomerular filtration rate (eGFR) levels at follow up. In Cox's regression analysis, baseline age, duration of diabetes, HbA1c, triglycerides, SBP significant association with baseline HbA1c and mean values of and presence of retinopathy showed the development of macroalbuminuria. (66)

Conclusion and recommendations

Conclusion:

In our study, it was found that persistent higher SBP (systolic blood pressure), DBP (diastolic blood pressure) with higher T. cholesterol, TG, LDL concentrations in Group 1 patients as compare to group2. Hypertension and abnormal lipid profile are the risk factors leading to progression of diabetic nephropathy. This study may allow one to gain deeper insight into the various differences that may occur to type 2diabetic and complications that can happen such as nephropathy.

Recommendations:

- Patients with type-2 diabetes particularly; patients with high risk for DN should be instructed to measure their blood pressure, and lipid profile regularly for prevention of DN.
- patients with risk of developing diabetic nephropathy should follow healthy life style in their diet, and exercise to maintain their health.
- Further studies are needed to investigate the other biochemical markers that help in early diagnosis of DN.
- It is important to increase the knowledge and awareness of diabetes patients in order to early-protect themselves from the complications of this disease. As a result, they will not face future adverse consequences.
- Adoption of non-invasive laboratory tests for the comfort of patients.

- It is important to develop statistical system at the hospitals and make annually report contains statistical information about diabetes mellitus and other diseases in Sudan.
- The field of early diagnosis for DN and diabetic complications in general deserves more studies since it is a very important subject, but unfortunately they are few in Sudan.

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