Determination of Risk Factors of Diabetic Nephropathy in Sudanese Patients with Diabetes Mellitus Type-2 Khartoum State

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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 - 65 years. 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™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 and 20% 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 glycaemic 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.

Keywords: Diabetic Nephropathy; Diabetes Mellitus

Background

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 in 2003 [4].

Diabetes was the sixth leading cause of death listed on the U.S. death certificates in 2002 [5].

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].

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 of the Study

General objectives:

- 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 glycaemic control (HbAlc) on developing of diabetic nephropathy.
- To determine the effect of duration of diabetes on progression of diabetic nephropathy.

Material and Methods

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 April 2014.

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 haemoglobinopathies, 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 (5 ml) was taken from study participants, from the directly into 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.

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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:

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

Cholesterol + O_2 $\xrightarrow{\text{CHO}}$ Cholestrol-3-one + H_2O_2

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

Reagent preparation

The reagent and the STD are ready for use.

Specimen

Serum, heparinized plasma.

Pipetting scheme

Pipette in to cuvette	Reagent blank	Sample/STD
Sample/STD	-	10 µl
Reagent (RGT)	1000 µl	1000 µl

Calculation of cholesterol concentration:

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

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

Normal range: < 200 mg/dl.

Measurement of triglyceride: Enzymatic test for triglyceride Reaction principle

Triglyceride $\xrightarrow{\text{LPL}}$ glycerol + FAs Glycerol + ATP GK glycerol + ADP

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

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H_2O_2 +4-aminophenazone +4cholorophenol $\xrightarrow{\text{Pod}}$ quinamine + $4H_2O$
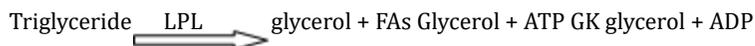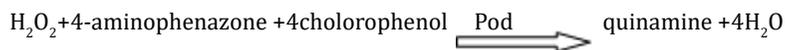
Reagent preparation

The reagent and the STD are ready for use.

Specimen

Serum and heparinized 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°C or 5 minutes at 37°C. Measure the absorbance of the Sample/STD against the reagent blank within 60 minutes.

Calculation of triglyceride concentration:

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

Normal range

- Females: 35 - 135 mg/dl
- Males: 40 - 160 mg/dl

Measurement of HDL: Enzymatic test for HDL Reaction principle

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

Cholesterol-ester + H_2O cholinesterase $\Longrightarrow$ Cholesterol Cholesterol+1/2O+ H_2O

Choloxidase Cholesterol+ H_2O_2 $\Longrightarrow$ $2H_2O_2$ +4aminoantipyrinephenol peroxidase quinamine + $4H_2O$
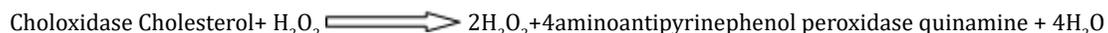
Method

Sample 0.5 ml precipitated (HDL cholA) 0.2 ml. 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	-	-	100 µl
Reagent A (T.cholestrol)	1.0 ml	1.0 ml	1.0 ml

Incubate at 6 - 25°C for 30 minutes or at 37°C for 10 minutes.

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

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Calculation:

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

Normal value: < 35 mg/dl.

Measurement of LDL

Will be calculated by the fallowing formula (Friedwald equation): LDL concentration = Total. Cholesterol - (Triglyceride + HDL)

Normal range: < 100 mg/dl.

Measurement of HbA 1 C
Labona Check™ Alc HbA1c Test Kit Test
Principle

The Labona Check™ Alc is about affinity assay. Labona Check™ 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™ AlcHbAlc analyzer, the ratio between them being proportional to the percentage of glycated hemoglobin in the sample.

Test procedure

1. Add 5 μl 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 collect 25 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 the R2/Reagent to the cartridge. Allow the sample to soak into the membrane for about 10 seconds.

4. Once the sample 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 - 46 mmol/mol
Presence of diabetes	> 6.57%	> 48 mmol/mol
Target in diabetes	< 7.07%	< 53 mmol/mol

Measurement of Microalbumin
ACCENT-200 microalbumin
Diagnostic kit for determination of albumin concentration in urine and cerebrospinal fluid
Principle of the method

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 340 nm and is proportional to the amount of albumin in the sample.

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Procedure

These reagents may be used in automatic analyzers ACCENT-200 and ACCENT-200 IIGEN. 1-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.

Calculations

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

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 group 2. The count and percentage of normal and abnormal results in patients group were calculated.

Result

In this descriptive case study, conducted in Khartoum hospitals in period from December 2013 to April 2014, blood and urine samples were collected from 50 type 2 diabetic patients to determine risk factors of diabetic nephropathy. The following results were obtained.

Table 1 shows number of study participants in group 1 (Nephropathy) which includes 29 participants, minimum value of MAU in this group was 33 mg/L and maximum value was 278 mg/L and mean was (122.04 ± 66.41), group 2 (Non-nephropathy), minimum value of MAU was 2.0 mg/L, and maximum value was 23.3 mg/L, and mean for this group was (10.80 ± 7.59).

	No	Minimum	Maximum	Mean ± Std. Deviation
Group 1	29	33.0	278.0	122.04 ± 66.41
Group 2	21	2.0	23.3	10.80 ± 7.59

Table 1: Number, minimum, maximum, and mean ± Std. Deviation of MAU in group l (nephropathy) and group2 (non-nephropathy).
The table shows numbers and mean of MAU in group 1 and group 2.
Descriptive statistics used to estimate numbers and Mean ± Std. Deviation.

Table 2 shows the present study included 50 patients distributed into 26 males comprised (52%) of study group, 19 males were in group 1 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.

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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%

Table 2: *Numbers and percentages of males and females in both nephropathy group and non-nephropathy group.*
The table shows numbers and percentages of males and females in both groups.
Descriptive statistics used to estimate numbers and percentages.

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 group 1 age was 53.81 years and in group 2 was 51.79 years. Also Mean BMI of nephropathy was 24.41 kg/m^2 and non-nephropathy was 24.86 kg/m^2 which were similar. There was significantly higher mean SBP in group 1 (129.31 mmHg) compared to group 2 (122.86 mmHg). Mean of DBP in group 1 was 83 mmHg which was significantly different from group 1 with 78.33 mmHg. Mean duration of Diabetes of group 1 patients was 11.49 years where there is no significant difference to group 2 patients where it was 9.47 years. Mean HbAlc of group 1 patients was 10.05% which is similar to group 2 patients HbA1c of 10.05%. Mean T. cholesterol of group 1 patients was 185.55 mg/dl that is significantly higher than group 2 patients which was 150.14 mg/dl. Mean of TG in group l was 190.86 mg/dl which was significantly higher than group 2 was 111.76 mg/dl. Mean of HDL in group 1 was 60.90 mg/dl where was no significant difference to group 2 63.57 mg/dl. Mean of LDL for group 1 was 81.14 mg/dl which was significantly higher than group 2 was 58.87 mg/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.

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)	0.041
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 ± 78.96115-402)	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

Table 3: *Comparison of the means of risk factors between nephropathy group and non-nephropathy group*
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 group 1 in comparison to group 2 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.

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 was 10.05% while mean MAU was 122.04 mg/L in group 1 patients similar finding were observed by Dr. Shital S Dodhia., *et al*. 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 [17].

We found also there was insignificant difference between group 1 and group 2 in age, BMI, duration of DM, HDL, and glycaemic control which contradict with above study findings mainly with influence of duration of DM and glycaemic control, and this could due to the difference in period of carrying the studies out.

Study done by Fayza Ahmed., *et al.* 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 [18].

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.

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- 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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