



## Detection of Diabetic Kidney Disease among Sudanese Type 2 Diabetes Mellitus using biomarkers, Kosti Diabetic Center White Nile State, 2025.

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Received: 05/07/2025

Published: 30/09/2025

### Abstract

This study aimed to assess the spread out of diabetic kidney disease (DKD) among Sudanese Type2 diabetic patients by investigating level of blood glucose, Cystatin-C, and serum Creatinine concentration, as biomarkers. Midray BA-88A semi-automated chemical analyzer, spectrophotometer were used. Enzymatic spectrophotometric glucose oxidase method for determine glucose level, (Jaffe method) for determine creatinine concentration and the Genrui PA50 Specific Protein Analyzer Semi-automated instrument was Opened, The test item *CYS-C* and sample type (serum) at the item column were Selected for *CYS*-level. 400 samples were selected and organized as follows 250 as cases and 150 healthy individual persons for control purposes. The study concluded the mean age of diabetic patients with DKD (95) was  $63.7 \pm 12.95$ , and for those without DKD, 155 was  $59.7 \pm 13.11$ . There was a statistically significant difference in age between the two groups (P-value = 0.02). The mean fasting blood glucose levels for patients without DKD and with DKD were  $174.16 \pm 4.4$  and  $223.6 \pm 6.6$ , respectively. There was a statistically significant difference in fasting blood glucose levels between the two groups (P-value = 0.001). The mean duration of diabetes for patients without DKD was  $2.4 \pm 0.05$ , and for patients with DKD was  $2.7 \pm 0.07$ . There was a statistically significant difference in the duration of diabetes between the two groups (P-value = 0.001). The mean levels of Cystatin C for patients without DKD was  $1.5 \pm 0.13$  and patients with DKD was  $1.08 \pm 0.08$ , there was a statistically significant difference in Cystatin C levels between the two groups (P-value = 0.001). The mean concentration of Creatinine for patients without DKD was  $1.84 \pm 0.53$  and patients with DKD was  $1.27 \pm 0.47$  there was a statistically significant difference in Creatinine concentration between the two groups (P-value = 0.001).

**Keywords:** CKD, serum creatinine, blood glucose, Cystatin C

### Introduction

Diabetic kidney disease (DKD) is a major public health problem characterized by elevated urine albumin excretion or reduced glomerular filtration rate or both <sup>(1, 2, 3)</sup>. Persistent abnormalities of either urinary albumin excretion or eGFR, significant urinalysis abnormalities lasting three months lead to the diagnosis of DKD in people with diabetes <sup>(4,5)</sup>. It is a type of progressive kidney disease affecting approximately 40% of patients with diabetes and it is one of the most common causes of end-stage renal disease (ESRD) worldwide <sup>(6,7)</sup>. The complication of diabetes is considered to be the main cause of death among patients on dialysis in developed countries. While the overall prevalence of chronic kidney disease (CKD) in Sudan is estimated to range between 8–11%, the mortality risks of kidney disease increased by 31.1% in patients with diabetes <sup>(8-9)</sup>.

Hypertension and hyperglycemia accelerate the development and progression of DKD, they are key risk factors of DKD and may include long duration of diabetes; non-glycemic control, blood pressure, obesity, cigarette smoking and an extent to genetic predisposition <sup>(10,11)</sup>. Hyperglycemia contributes to development and progression of DKD via multiple molecular mechanisms such as gene polymorphism of angiotensin converting enzyme (ACE). Limited data are available regarding the risk factors associated with the development of diabetic kidney disease (DKD) among Sudanese adults with type 2 diabetes mellitus (T2DM) <sup>(12,13)</sup>.

Cystatin C is a low molecular weight protein that is an endogenous cysteine proteinase inhibitor and has a high correlation with GFR. Because of its small size Cystatin C is freely filtered by renal glomeruli and does not return to the blood stream and not secreted by renal tubules it has been suggested to be closer to the “ideal” endogenous marker <sup>(14)</sup>, and it is measured from blood stream by the glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline, the blood levels of Cystatin C raise. Plasma levels of Cystatin C are a more precise test of kidney function than plasma creatinine <sup>(15, 16)</sup>, Serum biomarkers, such as serum creatinine (SCr) and serum cystatin C (SCysC), have been widely used to evaluate renal function in patients who have chronic kidney disease (CKD) <sup>(16)</sup>.

**objective**



To assess the spread out of diabetic kidney disease (DKD) among Sudanese Type2 diabetic patients by investigating level of blood glucose, Cystatin-C, and serum Creatinine as biomarkers

### Material and Methods

#### Study design

An analytical case-control hospital-based study is designed.

#### Study area

The study was carried out at Kosti Teaching Hospital, Diabetic Center, Kosti city which is considered as the most important and biggest town at about 360 kilometer south to Khartoum, the capital of Sudan, in Latitudes (13.1522° or 13° 9' 7.9" north ) and Longitudes (32.6725° or 32° 40' 21" east) According to ([https://en.m.wikipedia.org/wiki/white\\_Nile\\_\(state\)\)](https://en.m.wikipedia.org/wiki/white_Nile_(state)))

#### Study duration

This study was carried out during December 2022 to 2025.

#### Study variables

##### Dependent variables

Fasting Blood Glucose, serum Creatinine, serum Cystatin C.

##### Independent variables:

Included: Age, gender, Residence, Level of education and Family history of DM

#### Study population

The study included adult Sudanese known as having type2 diabetes mellitus attended to Diabetic Center at Kosti teaching hospital for follow up.

#### Sample size:

400 samples was selected and organized as follows 250 as cases and 150 healthy individual persons for control purposes

#### Inclusion criteria

Type 2 Diabetic patients aged from 25-80 years of both gender and Healthy individuals were included as the control group.

#### Exclusion criteria

Patients with hypertension, thyroid disease, heart disease, and type 1diabetes, patients on dialysis or transplanted were excluded and those who refused to participate.

#### Ethical approval

Ethical approval for the study was obtained from the research board of Faculty of Medical Laboratory Sciences, University of EL Imam EL Mahdi.

#### Ethical permission

The permission was taken from the Local Kosti Diabetic Center authorities.

#### Informed consent

All patients were informed about the objectives of the study before sample collection; written informed consent for participation in the study was obtained from each participant.

#### Data analysis:

The data were analyzed by using SPSS (version 26; SPSS Inc., Chicago, IL) software and linear regression analysis.

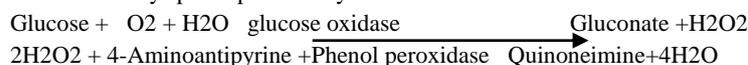
#### Sample collection

From each participant, blood and urine samples were collected, 5 ml of venous blood was collected, 2.5ml was taken into EDTA blood containers then stored in -80c for DNA Extraction and 2.5mL into plain containers, immediately separated after centrifugation at (3000rp/min for 5minutes) and tested for fasting blood glucose, serum Creatinine and serum Cystatin C levels.

#### Measurement of serum Fasting Blood Glucose:

##### Test principle:

Glucose level was determined by an enzymatic spectrophotometric glucose oxidase method. The basic principle is that, Glucose is oxidized by glucose oxidase (GOD) enzyme to produce gluconate and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The H<sub>2</sub>O<sub>2</sub> is then oxidatively coupled with 4 amino-antipyrene (4-AAP) and phenol in the presence of peroxidase (POD) enzyme to yield a red Quinoneiminequinoneimine dye that is measured at 500 nm with a spectrophotometer. Glucose in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.



The absorbance at 500 nm is proportional to concentration of glucose in the sample. The method has linearity from 0.0126 mmol/l (0.23 mg/dl) to 27.5 mmol/l (500 mg/dl).



**Procedure:**

	Blank	Standard	Sample
Glucose standard(S)	-	10µL	-
Sample	-	-	10µL
Reagent (A)	1000µL	1000µL	1000µL

After mixed and incubated for 10 minutes. The absorbance (A) of the standard and the sample were measured at 500 nm against the blank using Mindray BA-88A semi-automated chemical analyzer. The glucose concentration expressed as mg/dl. Quality control samples were run in the same way as patient samples; finally test results were documented on screen.

**Measurement of serum Cystatin C:**

**Test Principle:**

CYS-C of the sample has an agglutination reaction with the rabbit anti human CYS-C antibody latex particles suspension of the reagent. The reaction formed antigen-antibody complex, which has turbidity. With a certain amount of antibody, its turbidity is in direct proportion to the CYS-C of the sample. By detecting the reaction change at specific wavelengths and referring to the multi-point calibration curve, the CYS-C content in the sample can be calculated. The reagents are pre-calibrated, each specific calibration curve has been recorded into the magcard and each detection kits are allocated with one magcard.

**Procedure**

The Genrui PA50 Specific Protein Analyzer Semi-automated instrument was Opened, The test item *CYS-C* and sample type (serum) at the item column were Selected. Lot at the batch No. column to enter the card-swiping interface was tick; the corresponding magcard was sated into the magnetic induction area. One cuvette and sterile tips, accurately 300µL from buffer solution, and then add 10µL from sample were taken. The cuvette was placed into the test channel. The instrument prompts (please add Antiserum) then accurately 100µL Antiserum added. The corresponding channel (Start bottom) immediately press and the instrument mix automatically. When the test finished the instrument automatically display and print the test result.

**Measurement of serum creatinine:**

**Principle**

Creatinine in the sample reacted with picric acid in alkaline medium forming a coloured complex (Jaffe method). The complex formation rate was measured in a short period to avoid interferences.



**Procedure**

The working reagent was prepared by mixing equal volumes of reagent A and reagent B. Sample type (serum), unit (mg/dL) and patient number were selected. 100µL from sample and 1000µL from working reagent, mixed gentially, then immediately was taken by Mindray BA-88A semi-automated chemical analyzer. Quality control samples were run in the same way as patient samples; finally test results were documented on screen.

**Results and Discussion**

**Results:**

The mean age of diabetic patients with DKD (95) was  $63.7 \pm 12.95$ , and for those without DKD, 155 was  $59.7 \pm 13.11$ . There was a statistically significant difference in age between the two groups (P-value = 0.02).Table (1) .

According to gender the percentage of males was 77 (30.8%) and females78 (31.2%) for without DKD and males51 (20.4%) and females 44 (17.6%) for those with DKD groups. There was no statistically significant difference in gender distribution between the two groups (P-value = 0.53). Table (1)

The mean duration of diabetes for patients without DKD was  $2.4 \pm 0.05$ , and for patients with DKD was  $2.7 \pm 0.07$ . There was a statistically significant difference in the duration of diabetes between the two groups (P-value = 0.001).

the table provided information on the residency for patients without DKD (urban96 (89.9%), rural59 (65.1%)) respectively and the residency for patients with DKD (urban49 (55.1%), rural 46 (39.9%)) respectively. There were no statistically significant difference in residency between the two groups (P-value = 0.10)

Table (1) showed the mean fasting blood glucose levels for patients without DKD and with DKD were  $174.16 \pm 4.4$  and  $223.6 \pm 6.6$ , respectively. There was a statistically significant difference in fasting blood glucose levels between the two groups (P-value = 0.001).

The mean levels of Cystatin C for patients without DKD was  $1.5 \pm 0.13$  and patients with DKD was  $1.08 \pm 0.08$ , there was a statistically significant difference in Cystatin C levels between the two groups (P-value = 0.001) table (1).

Table (1) provided the mean concentration of Creatinine for patients without DKD was  $1.84 \pm 0.53$  and patients with DKD was  $1.27 \pm 0.47$  there was a statistically significant difference in Creatinine concentration between the two groups (P-value = 0.001).

Table (1): Basic Demographics and clinical characteristics of type 2 diabetic patients with and without Diabetic Kidney Disease

Variables		T2DM without DKD n=155(62%)	T2DMwithDKD n=95 (38%)	p-value
Age (M ±SD)		59.7±13.11	63.7±12.95	0.02
Gender	Male	77 (30.8%)	51 (20.4%)	0.53
	Female	78 (31.2%)	44 (17.6%)	
Fasting Blood Glucose	(M ±SD)	174.16±4.4	223.6±6.6	0.001
Diabetic duration	(M ±SD)	2.4±0.05	2.7±0.07	0.001
Residency	Urban	96 (89.9%)	49 (55.1%)	0.10
	Rural	59 (65.1%)	46 (39.9%)	
Cystatin C	(M ±SD)	1.08±0.08	1.5±0.13	0.001
Creatinine	(M ±SD)	1.27±0.47	1.84±0.53	0.001

**Discussion:**

This analytical study was conducted in the Diabetic Center, Kosti Teaching Hospital in White Nile State, Sudan among 250 Type2 diabetic cases (115 male and 135 female) and 150 apparently healthy volunteers (84 male and 66 female) as the control group. Our results showed a significant increase in mean age of patients with DKD+ (59.7±13.11) and the patients without DKD-(63.7±12.95) (P-value 0.02), this result agreed with Roy S, Schweiker-Kahn O, et al.2021<sup>(17)</sup> and Farah, Randa I., et al. 2021<sup>(18)</sup>. who reported that the mean age of the patients with DKD+ was significantly greater than the patients without DKD-.while our results was disagree with Wang, Tingli, et al.2022<sup>(19)</sup> and Erdogmus, Siyar, et al. 2018<sup>(20)</sup>. According to the gender, our results revealed insignificant difference between patients with DKD+ and patients without DKD- (p.value 0.53) Table (1), this result agreed with that reported by Wei, Jing, et al.2022<sup>(21)</sup> and Roy S, Schweiker-Kahn O, et al.2021<sup>(17)</sup> and disagree with Yu, Margaret K., et al.2012<sup>(22)</sup> and Al-Ataa, H.M., 2020<sup>(23)</sup> whom revealed that there was significant difference of gender between DKD+ and DKD- .

We found a significant increase of the mean duration of diabetes among patients with positive DKD+ compared to patients with negative DKD- (P-value 0.001) Table (1). This result agreed with, Taha, T. A. A., et al.2022<sup>(24)</sup> and Farah, R.I., Al-Sabbagh, et al 2021<sup>(25)</sup>, Akhtar, Mohammed, et al.2020<sup>(26)</sup>. they revealed that Duration of diabetes has a direct effect on the progression of DKD among Sudanese with Type 2 Diabetes Mellitus, and Duan, Jiayu, et al.2023<sup>(27)</sup>. Reported that diabetic duration had a statistically significant effect on DKD. But this result disagrees with Abo-elnasr, Mohamed Sabry, et al.2020<sup>(28)</sup> who reported that when comparing both groups(DKD+ and DKD-) there was no statistically significant difference regarding DM duration.

In this study we reported that the fasting blood glucose level is a significant risk factor for DKD, (P-value 0.001) Table (1), this result agreed with Altemtam, N., Russell, J., & El Nahas, M.2012<sup>(29)</sup>, Tong, Xue, et al. 2020<sup>(30)</sup>. Gong, Lei, et al.2021<sup>(31)</sup> Ali, H., Abu-Farha, et al.2022<sup>(32)</sup>.

This study revealed significant increase in the mean Cystatin C levels among patients with positive DKD+ than the negative DKD- (p-value 0.001), table (1); The elevated Cystatin C levels in the patients with DKD group suggest that the diabetic patients with Diabetic Kidney Disease may be experiencing compromised kidney function and the difference in Cystatin C levels serve up an important marker for identifying and quantifying the severity of kidney dysfunction in diabetic individuals. Our results showed the Cystatin C is thought to be strong biomarker for DKD, this result is same as Liao, X., Zhu, Y. and Xue, C., 2022<sup>(33)</sup>, Mansour, Ahmed E., et al.2023<sup>(34)</sup>, Farah, Nizar M., et al.2023<sup>(35)</sup>as a result of Gupta, UshaKumari, et al.2022<sup>(36)</sup>. Ma, Cheng-Cheng, et al.2020<sup>(37)</sup>, and matching with Mohammed, N. B., et al.2014<sup>(38)</sup> they reported that among Sudanese patients with Type 2 Diabetes Mellitus, plasma levels of Cystatin C are significantly raised andlikewise Omer, Maha Ali, et al.2019<sup>(39)</sup>, Al-Rubeaan, Khalid, et al.2017<sup>(40)</sup> Abushama, Samah H., Ibrahim A. Ali, and Eltayeb Osman Elfaki.2020<sup>(41)</sup> demonstrated Cystatin C could be considered as a good candidate for the early detection of renal dysfunction in type 2 diabetic patients with normoalbuminuria.

The study found that the mean Creatinine concentration is significantly increased in DKD+than DKD- patients (P-value 0.001) table (1). These results agreed with many studies done in white Nile, like KararIi, Tarig, et al.2013<sup>(42)</sup> were found the higher levels of plasma Creatinine may be indicative of progressive renal disease and nephropathy among diabetic patients, in addition to Shigidi, Mazin MT, Wieam N. Karrar.2021<sup>(43)</sup>, SALIH, Ola Ahmed Faduly Mohamed, et al.2014<sup>(44)</sup>. Whereas Abeadalla, Abdarahiem A, et al. 2018<sup>(45)</sup>

**Conclusion:** The present study is concluded that:

1. DKD is a problem among Sudanese patients with Type 2 Diabetes Mellitus.
2. Serum Cystatin C and Creatinine levels were strong biomarkers for DKD.
3. Cystatin C was found to be the superior biomarker for early detection of DKD in Type 2 diabetic patients.

**Recommendations:**

Serum Cystatin C and Creatinine should be measured as parts of the routine assessment of renal function test for Type 2 diabetic patients.



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