

Renal Function Impairment in Patients with Type 1 Diabetes Mellitus in a Nigerian Tertiary Health Institution: A Preliminary Study

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ABSTRACT

Background: Chronic kidney disease (CKD) is a long term complication of Diabetes Mellitus (DM). We evaluated renal function in Type 1 DM patients in a tertiary health institution in Nigeria.

Methods: A case-control study. Fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) were measured. Stages of diabetic nephropathy were defined by estimated glomerular filtration rate, eGFR (CystatinC-based), albumin/creatinine ratio (ACR) and plasma kidney injury molecule 1 (KIM-1). Resistivity index (RI) was by renal Doppler ultrasound.

Results: Ten patients (aged 15 to 17 yr, IDDM duration 0.2 to 9 yr) were evaluated against ten age-matched controls. Recent and remote poor glycemic control were evidenced by FPG, mean 243.9 ± 105.84 mg/dl (13.55 ± 5.88 mmol/l) and HbA1c ($11.72 \pm 1.93\%$) respectively. Serum cystatin C was elevated (1.56 ± 0.78 mg/l versus 0.52 ± 0.11 , $p=0.017$). Six patients had a GFR <60 mL/min/1.73m². One patient had stage 1, 3 had stage 2, 4 had stage 3, 2 had stage 4 but none had stage 5 CKD; GFR decline occurred as early as 2.5 months. Three patients had normoalbuminuric renal insufficiency; ACR: 3 macroalbuminuric and 3 microalbuminuric. KIM 1 was elevated (3.76 ± 1.15 ng/ml versus 1.15 ± 0.09 , $p<0.001$); Two subjects had hypercholesterolaemia (one moderate and one severe); seven subjects had reduced HDL, much lower than the optimal/desirable

levels $15.83 - 30.12$ mg/dl ($0.41 - 0.78$ mmol/l) while only 3 had desirable levels, $39.77 - 43.63$ mg/dl ($1.03 - 1.13$ mmol/l); one had increased RI (≥ 0.7).

Conclusion: Renal function impairment is prevalent in T1D subjects and commences as early as two and a half years of disease. Poor glycemic control is contributory.

Keywords: Type 1 DM, Chronic kidney disease, Resistivity index, Albumin creatinine ratio, Cystatin C, Kidney injury molecule 1

INTRODUCTION

Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects $\leq 40\%$ of T1D and type 2 diabetic patients (T2D)[1]. People with T1D are at increased risk of developing renal function impairment and end stage renal disease (ESRD)[2]. However, it takes years for microvascular complications in diabetes mellitus such as diabetic retinopathy and nephropathy to develop. Diabetic nephropathy usually develops approximately ten years after the diagnosis of T1D and three to five years after the diagnosis of T2D[3,4]. The trend of decline in eGFR and proteinuria in patients with T1D can be used to predict the risk of ESRD.

However, renal disease complicating diabetes has been associated with the duration of diabetes and evident microvascular disease[2,5].

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GFR decline in patients with T1D and proteinuria are used to predict the risk of ESRD[2]. Proteinuria occurs in 15–40% of patients with type 1 diabetes, with a peak incidence around 15–20 years of diabetes[5-7]. Urine albumin and glomerular filtration rate (GFR) are markers of kidney disease. Spot urine ACR is an alternative to the albumin excretion rate (AER) because of a high correlation between AER and ACR. Thus, ACR estimates 24-hour urine albumin excretion.

Cystatin C is an important marker in detecting early kidney dysfunction in both T1D and T2D compared to creatinine based formulae. It is also an important predictor of cardiovascular disease in patients with diabetes. It has been demonstrated that cystatin C proved to be more reliable than 24-hour creatinine clearance and was superior to plasma creatinine as well as the Cockcroft-Gault estimation[8]. KDIGO current guideline incorporates cystatin C based formulas in addition to creatinine and GFR estimating formulae when the latter are less accurate as in severe muscle wasting when tubular secretion of creatinine is affected by drugs[9].

KIM-1, also known as hepatitis A virus cellular receptor 1 and T-cell immunoglobulin mucin 1, is a transmembrane glycoprotein. It is a newer and novel biomarker of renal proximal tubular pathology which is normally shed into the urine under conditions of proximal tubular damage. It was thus initially reported as urinary marker of proximal tubular dysfunction (usually corrected for urine creatinine excretion, mg of KIM-1/ mg of creatine). However, more recent studies have confirmed that KIM-1 shed from proximal tubular cells during injury also gets released into the circulation[10]. Plasma KIM-1 may be particularly suitable for detecting chronic ongoing injury[11]. There is a statistically significant correlation between urinary and plasma KIM-1[11].

In patients with renal dysfunction and CKD of various causes, blood KIM-1 positively correlated with increasingly advanced stages of disease (i.e plasma KIM-1 levels were negatively associated with eGFR). Baseline serum KIM-1 performed very well as a predictive biomarker for progressive kidney disease in a type 1 diabetic cohort after other common covariates, like urinary ACR, hemoglobin A1c, and eGFR, were taken into consideration[10]. In humans, blood KIM-1 levels are significantly elevated in the setting of acute kidney injury and CKD and predict progression of renal disease in T1D[10].

DM contributes immensely to the prevalence of CKD. Black race has been reported to increase the risk of microalbuminuria and proteinuria by at least 2-fold[12,13]. Even though T2D is somewhat prevalent among Nigerians, T1D is very rare. The prevalence of T1D in Nigerian children is 0.33/1000 although many or at least some cases may remain undiagnosed[14]. There is paucity of work done on renal function in children with T1D in Nigeria and other sub-Saharan nations. The few cases reported also face a lot of challenges ranging from inadequate/unavailability of Insulin, inadequate self blood glucose monitoring and dietary challenges[15]. A recent study from Kenya[16] reported poor metabolic control in children with T1D, this may presuppose that African children may develop chronic complications early, we therefore attempted a cross sectional evaluation of renal function and other coexisting or contributing biochemical derangements in T1D patients in a single tertiary health care institution in South-western Nigeria.

METHODS

This was a single-centre prospective case-control study conducted at a tertiary referral hospital in South-western Nigeria. All the available 10 T1D subjects attending the paediatric endocrinology clinic (which serves all patients with paediatric endocrine disorders among which is T1D) were evaluated. Ten age- and BMI-matched non-diabetic individuals were concurrently recruited as controls; this was done mainly to assay cystatin C and KIM 1 whose reference values had not been established previously. Five mls of venous blood was drawn from the subjects for FBG, HbA1c, lipid profile (totalcholesterol, TC; Triglyceride, TG; high density lipoprotein, HDL-C; and low density lipoprotein, LDL-C). Five mls of venous blood was also drawn from the controls mainly for assays of Cystatin C and KIM 1, and also FBG and lipid profile. Morning hour spot urine specimens were obtained and analyzed for albuminuria. A biochemical auto-analyzer (Cardiochek PA, USA) was used for FBG and lipid profile assays; HbA1c was analyzed using Polymer technology systems, Inc, USA biochemical autoanalyzer. Urinary ACR of the random/spot urine was determined using CLINITEK microalbumin (Siemens Healthcare Inc, Camberly, UK), ACR was calculated as albumin (mg)/creatinine (g).

The prevalence of stages of diabetic nephropathy was determined using the eGFR (assessed by Cystatin C-based equation), ACR, and Plasma KIM 1. Plasma cystatin C and KIM-1 were measured using ELISA methods (Aviscerabioscience Company Inc. USA).

The cystatin C values were used to calculate glomerular filtration rates with the aid of available on line paediatric GFR calculators (cystatin C-based equation). Morning hour urine ACR was calculated. An ACR of 30–299 mg/g defined microalbuminuria; macroalbuminuria (or simply albuminuria) was defined as an ACR ≥ 300 mg/g; nephrotic-range albuminuria was defined as an ACR ≥ 2.2 g/g; 0 – 30 mg/g was defined as normal.

Renal Doppler examination using a D-C7 Mindray ultrasound machine with probe frequencies of 3.5-5 MHz was conducted on subjects after an overnight fast of at least 8 hours to minimize the limiting effect of intra abdominal gas. The interlobar or arcuate arteries in different regions (upper, middle and lower poles) of the right kidney were chosen for this study due to less variability in their wave form. The probe was gently placed over the flanks for insonation and the kidney was first visualized as a longitudinal image. Spectral waves were obtained with the subjects counseled to remain still and hold their breath in gentle end inspiration. The Doppler sample volume was set at 2-4mm gate just appropriate to be

placed in the mid portion of the diameter of the vessel to be insonated. The waveforms were optimized for measurement using the lowest pulse repetition frequency possible without aliasing (to maximize waveform size), the highest gain without obscuring background noise and the lowest wall filter (50HZ). Because of the variability of renal Doppler index with cardiac cycle, a minimum of 4 identical consecutive spectral waveforms was obtained for analysis of the resistivity index[17]. Independent samples T-tests were used to compare the mean values of the subjects and the controls

RESULTS

The recruited 10 subjects (6 males, 4 females) had mean age of 15.8 ± 1.55 years (range: 12-17 yr); mean weight 42.5 ± 8.32 kg (range: 30-55 kg); mean height 1.54 ± 0.17 meters (range: 1.26 - 1.71 m); mean BMI 18.07 ± 3.07 Kg/m² (range: 14.1-25.2 kg/m²); and mean duration of DM was 3.12 ± 2.61 years (range: 0.2 – 9yr). The recruited controls were age-, height- and BMI-matched with the patients as shown in Table 1. Recent poor glycaemic control in the patients was shown by hyperglycaemic state, FBG mean 243.9 ± 105.84 mg/dl (13.56 ± 5.88 mmol/l; range: 3.6 - 20mmol/l); remote poor glycaemic control was equally obvious by the mean HBA1c, $11.72 \pm 1.93\%$ (range: 8.5 -13%), Table 2.

Table 1: Socio-demographic characteristics of the subjects and controls

Variable	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Subjects Mean \pm SD	Controls Mean \pm SD	p value
Gender	M	F	F	M	M	F	M	F	M	M	M:F(3:2)	M:F(1:1)	-
Age	17	16	15	16	15	16	17	17	17	12	15.8 ± 1.55	16.5 ± 2.84	0.363
Weight	50	36	35	44	37	55	53	45	40	30	42.5 ± 8.32	57 ± 8.93	0.009
Height	1.7	1.6	1.4	1.6	1.6	1.7	1.7	1.6	1.3	1.3	1.54 ± 0.17	1.61 ± 0.08	0.308
BMI	17.7	18.2	14.6	17.2	14.1	18.8	18.6	17.4	25.2	18.9	18.07 ± 3.07	21.86 ± 2.87	0.117
Duration	3	0.5	9	4	4	4	4	2	0.5	0.2	3.12 ± 2.61	NA	NA

S1 –S10= Codes for subjects 1-10; M= Male; F= Female; Age in years; Weight in kilogram (Kg); Height in meters (m); BMI= Basal metabolic index (Kg/m²); Duration of diabetes in years; NA= Not applicable.

Table 2: Indices of biochemical monitoring of diabetes in the subjects and controls

Variable	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Subjects Mean±SD	Controls Mean±SD	p value
FPG	11.9	19.2	18.9	19.7	13.6	7.6	7.8	13.3	3.6	20	13.56±5.88	4.88±0.37	0.001
HBA1c	13	13	13	10.8	13	8.6	8.5	13	12.6		11.72±1.93	-	-

S1 –S10= Codes for subjects 1-10; FPG= Fasting plasma glucose (mmol/l); HBA1c= Glycated haemoglobin (%);

The subjects had significantly higher mean serum cystatin C than the controls (1.56 ± 0.78 mg/l versus 0.52 ± 0.11 mg/l, $p = 0.017$). The mean eGFR in the subjects was significantly lower than in the controls (56.30 ± 25.17 ml/min/1.73m² versus 134 ± 22.47 ml/min/1.73m², $p < 0.001$), Table 3. Six subjects had an eGFR < 60 mL/min/1.73 m², implying moderate CKD. Considering CKD staging by eGFR, 1 patient had stage 1 CKD, 3 had stage 2, 4 had stage 3, 2 stage 4; no patient had stage 5 CKD, Table 3. Similarly, six subjects had abnormal protein excretion in urine: ACR values signified 3 subjects had macroalbuminuria, 3 had microalbuminuria while the remaining 4 had normal ACR. Three subjects had normoalbuminuric renal insufficiency. KIM 1 levels were significantly elevated in the entire subjects. The mean plasma KIM-1 in the subjects was statistically significantly higher than

in the controls (3.76 ± 1.15 ng/ml versus 1.15 ± 0.09 ng/ml, $p < 0.001$) Table 3. Two subjects had hypercholesterolaemia (one moderate and one severe); seven subjects had reduced HDL, much lower than the optimal/desirable levels 15.83 - 30.12 mg/dl (0.41 - 0.78 mmol/l) while only 3 had desirable levels, 39.77 - 43.63 mg/dl (1.03 - 1.13 mmol/l), Table 4 and Figure 1.

Dipstick proteinuria was positive in 4 subjects; 8 subjects had dipstick glycosuria as 1+ (mg/dl) in 1 patient, 3+ (500mg/dl) in 4 subjects, 4+ (1000mg/dl) in 3, but negative in 2 subjects. Urine ketone was trace in 1 patient and 1+ in another one patient. The radiological profiles of all the subjects are as shown in Table 4.

The median right renal RI value was 0.64 (range 0.51-0.77); only one of the subjects (S8, Tab. 4) had an elevated value of 0.77, when the standard cut-off value of 0.7[17] was used.

Table 3: Comparison of Biochemical Markers of Renal function in the Subjects and Controls

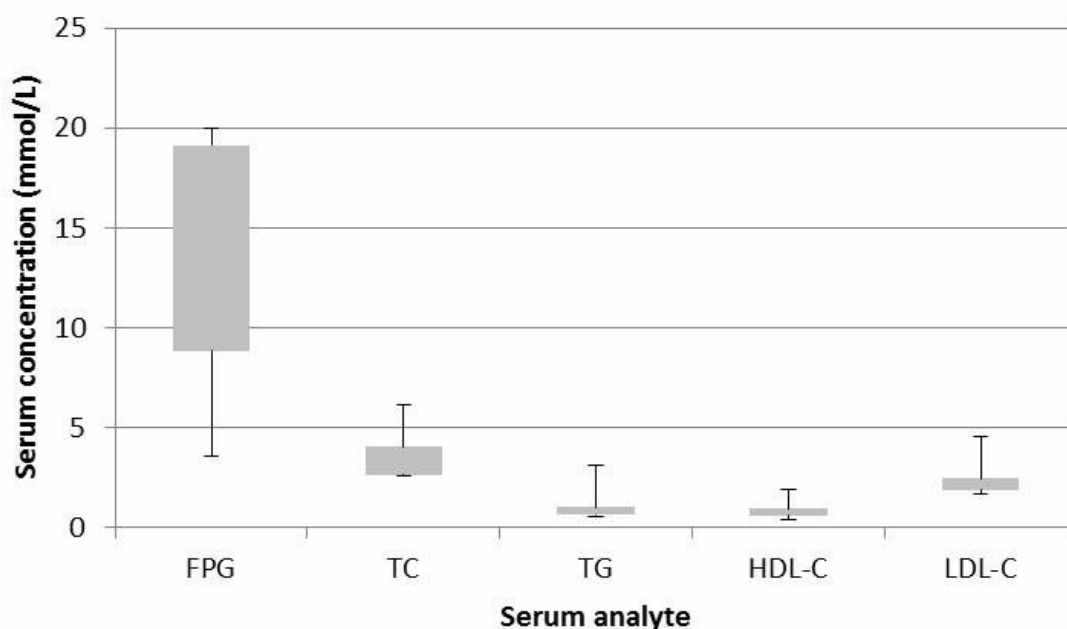
Variable	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Subjects Mean±SD	Controls Mean±SD	p value
Cyst C	0.84	1.51	3.20	2.56	6.18	0.62	1.60	1.28	1.60	1.20	1.56 ± 0.78	0.52±0.11	0.017
eGFR	83	48	24	29	61	110	46	56	46	60	56.3±25.17	134±22.47	<.001
ACR	>300	<300	<30	<30	<300	<30	<30	>300	>300	<300	-	-	-
KIM 1	6.1	3.0	1.8	4.6	6.4	3.8	3.6	3.2	2.6	2.5	3.76±1.52	1.15±0.09	<.001

S1 –S10= Codes for subjects 1-10; Cyst C= Cystatin C (mg/L); eGFR= Estimated glomerular filtration rate (ml/min/1.73m²); ACR= Albumin/creatinine ratio (mg/g); KIM 1= Kidney injury molecule 1 (ng/ml).

Table 4: Comparisons of biochemical indices of lipid metabolism and doppler ultrasonographic characteristics of the subjects and controls

Variable	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Subjects Mean±SD	Controls Mean±SD	p value
TC	2.6	3.1	5.7	2.6	3.8	6.1	4.1	2.8	3.1	2.6	3.63±1.28	3.64±0.53	0.963
TG	0.73	0.62	1.33	0.64	0.68	0.99	1.97	0.57	1.04	0.57	0.91±0.45	0.63±0.09	0.072
HDL-C	0.78	0.61	1.13	0.41	1.02	0.97	0.72	0.59	0.78	0.47	0.75±0.24	0.92±0.49	0.305
LDL-C	1.66	2.19	3.92	2.05	2.42	4.65	2.45	1.90	1.87		2.57±1.02	2.55±0.55	0.881
RR-RI	0.54	0.68	0.62	0.66	0.60	0.65	0.51	0.77	0.63	0.58	0.63±0.07	0.59±0.04	0.155

S1 –S10= Codes for subjects 1-10; TC= Total cholesterol (mmol/l); TG=Triglyceride (mmol/l); HDL-C= High density lipoprotein cholesterol (mmol/l); LDL-C= Low density lipoprotein cholesterol (mmol/l); RI= Right renal resistive index; NA= Not applicable

**Fig. 1:** Serum levels of markers of glycaemic and lipid control

DISCUSSION

DM is the commonest cause of CKD worldwide. Diabetic nephropathy is classically defined by the presence of proteinuria, in the absence of other renal disease. Albuminuria is a determinant of morbidity and mortality in patients T1D. GFR is the best indicator of overall renal function and dysfunction[18]; and is thus a necessity (either measured or estimated) in micro- and macroalbuminuric diabetic patients. Our finding of declined GFR with concurrent macroalbuminuria and microalbuminuria confirms that

renal dysfunction in T1D patients manifests as glomerular function impairment and proteinuria. Albuminuria has been associated with progression of diabetic nephropathy to ESRD[1]; it is also an independent predictor of cardiovascular disease, and all-cause mortality, both in patients with T1D and also in the general population[19].

Our finding of GFR decline in subjects with less than one year disease duration: stage 2 CKD found in two subjects as early as 2.5 months to 4 years; stage 3 after 6 months to four years disease

and stage 4 occurring as early as 4 years duration implies that the onset of nephropathy in patients with T1D may be highly insidious and might have also begun prior to the usual research-established period in the course of the disease. This is obviously contrary to the report that it takes years for microvascular complications in diabetes mellitus such as diabetic retinopathy and nephropathy to develop. Therefore, there may be a need to re-evaluate the possible timings of onset of nephropathy in diverse populations suffering from T1D, since it had been previously documented that diabetic nephropathy usually develops approximately ten years after the diagnosis of T1D and three to five years after the diagnosis of T2D [3,4]. It may also suggest or buttress the fact that the course and complications of T1D are influenced by several other factors like race (especially black race being a higher risk of nephropathy and its faster progression to ESRD), ethnicity, genetic predisposition, environmental factors etc. The poor glycaemic control in our cohort may also be responsible for this early manifestation as opposed to the western world where good glycaemic control has been shown to lead to delay onset of complications [20]. The early decline in renal function in patients with T1D and proteinuria predicts the risk of ESRD [2].

The relatively high prevalence of moderate CKD (declined GFR) and albuminuria in this African population of study supports black race being an important risk factor for CKD [21]. So also is diabetic nephropathy reported to be more prevalent among African Americans, Asians, and Native Americans than Caucasians [22, 23], though the lifetime risk of nephropathy is estimated to be equivalent in T1D and T2D [24]. The high prevalence of microalbuminuria in our cohort also corroborates the report that black race increases the risk of microalbuminuria and proteinuria by at least 2-fold [12,13]. Six of our subjects have either micro or macro albuminuria. The Microalbuminuria precedes the development of macroalbuminuria and it predicts future nephropathy. The onset of macroalbuminuria in the absence of effective therapy is normally followed by a slowly progressive decline in GFR [25].

Only one of the subjects (S8, Tab. 1) had an elevated renal artery RI using a cut off value of 0.7 [17]. This subject was observed to have microalbuminuria with an eGFR of 56 ml/min/1.73m². All other subjects however had normal renal artery RI despite the fact that there were biochemical

features of renal insufficiency. This may be explained by the fact that the cut off value used is that established in the adult population. To the best of the authors' knowledge no cut-off value has been established for the pediatric population.

Furthermore, the very poor glycaemic and poor lipid control in these subjects would have contributed to the high prevalence of nephropathy in our subjects since these factors are determinants of microvascular complications of diabetes(diabetic nephropathy and diabetic retinopathy) which share common determinants, such as poor glycemic, blood pressure, and lipid control [18].

Early onset of GFR decline, everal months before 5 years in our study (two and a half months after diagnosis) negate the recommendation of 5 years after diagnosis before the first screening for microalbuminuria in patients with T1D [26]. This fact has been similarly stated in the report of EURODIAB IDDM complication study group, where it was demonstrated that in T1D the prevalence of microalbuminuria can reach 18% before 5 years, especially in those with poor glycemic and lipid control, with normal to high blood pressure levels, as is the case with the subjects in our study [27]. Therefore, we would suggest that, in T1D, screening for microalbuminuria should be performed as early as possible, if not immediately after diagnosis, especially in patients with poor metabolic control. This would be very valuable tool in preventing progressive renal function impairment and CKD in these patients. If microalbuminuria is absent, the screening must be repeated every 3-6 months.

One of the 3 subjects with macroalbuminuria (S1, Tab. 1) having only a stage 1 CKD (or reduced renal reserve) in terms of the GFR value suggests the possibility that mild reduction in GFR may not necessarily preclude or exclude overt proteinuria. Also, despite that microalbuminuria has been considered a risk factor for macroalbuminuria, not all patients progress to this stage and some may even regress to normoalbuminuria [28]. Therefore, there is need for both early biomarkers and early predictors of the risk of diabetic nephropathy to at least, supplement albumin excretion rate. Blood cystatin C and KIM-1 may play this role as they both become elevated as early as six months in the disease course after diagnosis; however, this needs to be substantiated by further research in a larger cohort because of our small sample size.

Moreover, there is enough evidence suggesting that the risk for developing diabetic nephropathy starts when UAE values are still within the normoalbuminuric reference range [29-34]. Three of our subjects with moderate renal insufficiency/impairment in the presence of normal ACR are thus consistent with the phenomenon of nonalbuminuric or normoalbuminuric renal insufficiency in T1D patients. In other words, although the measurement of urine albumin excretion (UAE) is the cornerstone for the diagnosis of diabetic nephropathy, there are still some patients with either T1D or T2D who have decreased GFR in the presence of normal UAE [35, 36].

Normoalbuminuric renal insufficiency has been reported to be more common among female patients with longstanding T1D, with concurrent hypertension, and/or retinopathy [36, 37]. It is therefore not surprising that the only female (S3) among the three subjects in this category has the longest disease duration and the lowest GFR. Normoalbuminuric renal insufficiency is not limited to T1D patients, but has also been found in patients with T2D. For instance, in the third National Health and Nutrition Examination Survey (NHANES III), low GFR (<60 mL/min) was found in 30% of patients without micro- or macroalbuminuria and retinopathy³¹.

Our finding of prevalent moderate renal dysfunction/insufficiency and proteinuria in these subjects with below desirable or optimal levels of the protective lipoprotein (HDL-C) corroborates the fact that treatment of dyslipidemia (LDL cholesterol <100 mg/dl [$<2.6\text{mmol/l}$]) is one of the effective strategies for preventing the development of microalbuminuria, in delaying the progression to more advanced stages of nephropathy and in reducing cardiovascular mortality in patients with T1D and T2D [1]. Our findings showed that in patients with T1D, moderate renal dysfunction is highly prevalent, as established by raised serum cystatin C with declined GFR, proteinuria and elevated plasma KIM-1. Poor glycemic control, hypercholesterolaemia, and HDL-C below desirable/optimal levels are similarly evident.

LIMITATION OF STUDY

Considering the cross-sectional nature of our study, we could not specifically assess the time frames for the development and persistence of declined GFR of

<60 mL/min/ 1.73 m² (3 months needed to define CKD). Blood samples were collected only once for cystatin C and KIM-1 assays, with no further sample collection for repeat assays. Urines samples were also collected once for evaluation of albuminuria (ACR). However, our findings suggest definitive states of renal dysfunction in the patients.

CONCLUSION

It is concluded that renal function impairment is common in the cohort of T1D subjects studied and it has an early onset probably owing to the poor glycaemic control, we recommend early screening for detection of nephropathy after diagnosis of T1D to prevent the onset or halt the progression to more advanced stages. Adequate follow up and appropriate management alongside adequate glycaemic and lipid control will greatly reduce the incidence of nephropathy and its associated cardiovascular mortality in patients with T1D.

Conflicts of interest/ Disclosure: No financial or other forms of interest to disclose. "The results presented in this paper have not been published previously in whole or part, except in abstract form"

ACKNOWLEDGEMENTS

Oluwagbemiga Ayoola initially conceived the study idea and shared with Tewogbade Adedeji and Jerome Elusiyan. All authors were involved in proposal and protocol development, gaining ethical approval. Jerome Elusiyan handled patient recruitment, while Oluwagbemiga Ayoola and Tewogbade Adedeji handled data acquisition. Tewogbade Adedeji handled laboratory analysis and analysed the data. Tewogbade Adedeji wrote the first draft of the manuscript. All authors reviewed and edited the manuscript for its intellectual content and approved the final version of the manuscript.

We would like to thank all the parents of all the available 10 T1D patients (and the patients themselves) attending the paediatric endocrinology clinic for their cooperation with us during this study (or without whose consent and cooperation, this study would not have been possible). This research did not receive any grant from any funding agency in the private, public, commercial or not-for-profit organisations.

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