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Prevalence and Biomarkers of Diabetic Kidney Disease in Diabetic Patients on Treatment in Buea and Ngaoundere, Cameroon

Mbarawa Marat Kofia Ibrahim[1, 2], Asongalem Emmanuel Acha[3], Nsagha Dickson Shey[4], Njouendou Abdel Jelil[2, 3], Assob Nguedia Jules Clement[2, 3, *]

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buea, Buea, Cameroon

[2]Medical Research and Applied Biochemistry Laboratory, Faculty of Health Sciences, University of Buea, Buea, Cameroon

[3]Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, Buea, Cameroon

[4]Department of Public Health and Hygiene, Faculty of Health Sciences, University of Buea, Buea, Cameroon

Email address:

mbarawamarat@gmail.com (M. M. K. Ibrahim), cpehw@yahoo.com (A. E. Acha), nsaghads@hotmail.com (N. D. Shey), ajnjouendou@gmail.com (N. A. Jelil), juleclement@gmail.com (A. N. J. Clement)

[*]Corresponding author

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Abstract: Diabetic Kidney Disease (DKD) is a complication of diabetes that often leads to the End Stage Renal Disease. It is characterised by the presence of persistent albuminuria and a reduction of the Glomerular Filtration Rate (GFR) in diabetic condition. No study has revealed the prevalence of DKD in Cameroon. This cross-sectional study was conducted in Buea and Ngaoundere to determine the prevalence of DKD and characterize its biochemical profile in diabetic population under medical care. A total of 250 diabetics were enrolled with a mean age of 56.78±12.06 years, out of which 59.6% were diagnosed with Chronic Kidney Disease (CKD), 32.8% presented micro-albuminuria and 3.6% were diagnosed with macroalbuminuria. The prevalence of DKD was 15.2% out of which 78.9% were females (p=0.002). The prevalence of the co-morbidity hypertension and DKD was 8.8%. Significant association was found between DKD and two variables: female gender (OR: 2.28 (1.21-4.29); p=0.002) and hyper-creatinemia (OR: 3.47 (2.13-5.66); p < 0.001). The high prevalence of micro-albuminuria found in this study may reflect a high frequency of micro-albuminuria in diabetic population in Cameroon. This study is the first, to assess DKD in Cameroon according to the ADA consensus on CKD and diabetes. The findings showed that, diabetic complication is a serious problem in Cameroon and, more actions should be taken to improve its management.

Keywords: DKD, Diabetes, Cameroon, GFR, ADA Consensus, KDIGO

1. Introduction

Diabetic Kidney Disease (DKD), a complication that occurs in about 20 to 40% of diabetic population [1] can progress to the End Stage of Renal Disease. It is more frequent in African-American individuals and, has been identified as one of the main causes of mortality due to complication of diabetes worldwide. DKD occurs in type 1 and type 2 diabetic patients with poor glycaemic control [2]. The pathophysiology of DKD occurs in the glomerulus by the expansion of mesangial, the thickening of the tubular, the loss of the endothelial fenestration and podocytes, the development of Kimmelstiel-Wilson nodules and, the appearance of the glomerular sclerosis [3]. Recent experimentations have shown the influences of inflammatory factors in the structural changes of glomerulus [4]. Tumour Necrosis Factor and Reactive Oxygen Species, activators of the nuclear factor kappa-light-chain-enhancer, the regulator of inflammatory factors, are associated with proteinuria. The persistence of immune response participates highly in the acceleration of kidney structure changes and the decline of

renal function in diabetic condition. Clinically, DKD is defined by the presence of persistent albuminuria, added to a reduction of the Glomerular Filtration Rate (GFR) in diabetic condition. It is due to the haemodynamic dysfunction in the kidney, which has as consequence, the reduction of the vascular resistance in both afferent and efferent arterioles leading to intra-renal hydrostatic pressure, then, the disruption in the autoregulation of its system [2]. The presence of kidney dysfunctions in diabetic condition doesn't automatically indicate DKD. In type 1 diabetes, the histological changes are frequently seen in DKD condition but, in type 2, these could indicate other pathogenic conditions. Then, it is some time difficult to diagnose true DKD, Non-diabetic Kidney Disease or the co-existence of the two forms [4]. Despite the confusion within these diseases, a consensus has been achieved by the American Diabetes Association (ADA), the American Society of Nephrology (ASN) and the National Kidney Foundation (NKF) to validate an approach for the identification, diagnostic and management of DKD. Thus, the ADA consensus conference on CKD and diabetes recommended the diagnosis of DKD using laboratory assessment by the identification of the estimated GFR at a level less than 60 ml/min/1.73 m2 and, the presence of kidney damages, estimated by albuminuria greater or equal to 30 mg/g of creatinine [5]. This recommendation has facilitated the clinical identification of DKD and helped in the understanding of the epidemiology of this disease. Nowadays, the worldwide prevalence of DKD has not been fully estimated, however, data from studies carried out in countries or limited regions are available. In the USA in 2008, the prevalence of DKD was 3.3% in the general population and 34.5% in diabetic population [6]. This prevalence was 10.8% in the diabetic population in Saud Arabia [7] and 6.7% in Egypt [8]. However, no study has been carried out in Cameroon using the above mentioned ADA consensus recommendations for the identification of DKD cases [5]. This present study was therefore conducted to estimate the prevalence of DKD in diabetic population and, to characterize their biochemical profile.

2. Material and Methods

2.1. Study Site and Design

This cross-sectional and multi-centric study was conducted from January 2018 to April 2019 in Buea and Ngaoundere, the headquarters of South-West and Adamawa Regions of Cameroon respectively. In Buea, participants were recruited from the Buea Regional Hospital and the Lambe Diabetic Foundation of Buea while in Ngaoundere, they were from the Ngaoundere Regional Hospital.

2.2. Study Population and Recruitment Strategy

This study targeted individuals that were diagnosed diabetics in the 3 selected health facilities. Using a random sampling technique, we included patients who were aged 18 years and above, diagnosed with diabetes and who accepted to sign the consent form. Menstruating females were asked to return after their menstrual period. Individuals who performed intensive physical exercises within the previous 72 hours were also asked to return after 72 hours period after resting. During the study, kidney functions of participants were assessed. For individuals with reduced kidney function according to our method, another appointment was established after 3 months period for a second assessment.

2.3. Data Collection and Management

Socio-demographic and clinical data were collected using a questionnaire. During each visit, we collected 3 ml of fresh blood sample from each participant in a dried tube. The same day of the recruitment, patients received a clean, dried and sterile container with no preservative for urine collection. They were prior trained on how to collect the first morning urine, and bring on the next day to health facility. Blood and urine samples that were not analysed during the day of collection were stored in a freezer at 2-4°C for analysis within the next 24 hours, or in another big freezer at -70°C in the case of long term storage in cryo-tubes.

2.4. Biochemical Analysis

Biomarkers of kidney function were measured using the semi-automated spectrophotometer biochemistry analyser, MCL-302B. Fasting blood glucose (FBG), serum creatinine, urea, Low Density Lipoprotein (LDL) cholesterol, High Density Lipoprotein (HDL) cholesterol, total cholesterol and Triglyceride (TG) as well as urine creatinine and albumin were analysed using specific reagent kits. The kits used to measure FB, Creatinine, Urea and Albumin were purchased from Chronolab Company in Barcelona, Spain. Kits for LDL, HDL and TG were procured from Biolabo Company in Maizy, France.

2.5. Assessment of Albumin-Creatinine Ration (ACR)

Albuminuria was assessed by determining ACR in urine. Albuminuria was normal when the result of ACR was less than 30 mg/g. Values between 30 and 299 indicated micro-albuminuria. Results greater or equal to 300 mg/g were the indication of macro-albuminuria.

2.6. Estimation of the GFR

The estimation of GFR was done using MDRD formula developed by Levey et Al. [9]. This formula has been taken in consideration in accordance with the indication of Levey where MDRD has been advised for people with diabetes.

2.7. Diagnostic of DKD

The diagnostic of DKD was done according to the recommendations from the consensus of ADA, ASN and NFK during the 2014 conference on CKD and Diabetes. There, DKD was considered present when eGFR repeated

twice, after 3 months interval period at least, was less than 60 ml/min/1.73m2 with micro-albuminuria or, persistent macro-albuminuria. We considered the presence of retinopathy coupled to micro-albuminuria as a marker of DKD in diabetic condition, without any other cause of CKD [5].

3. Ethical Consideration

The research protocol was approved by the Institutional Ethics Committee for Research on Human Health of the University of Douala. An Ethical clearance No 1157 IEC-UD/11/2017/T was granted for the study. Administrative clearance was obtained from the South-West and Adamawa Regional Delegations of Public Health. The Regional Hospital of Buea, the Regional Hospital of Ngaoundere and the Lambe Foundation of Diabetes of Buea, granted each an administrative authorization to carry-out this research.

4. Statistical Analysis

Data were entered into a spreadsheet of Microsoft Office Excel 2016 and analysed using SPSS 22.0. Descriptive statistics of variables were expressed as mean ± standard deviation (SD), frequencies and percentages. Chi-square or Fisher's exact tests were used to assess association between variables with 95% Confidence Intervals (CI). Student-t test was used to compare means of variables. Risk factors were assessed with Odds ratios (OR) and 95% CI. *P*-values < 0.05 were considered statistically significant.

Table 1. *Socio-demographic characteristic of the population in Buea and Ngaoundere centers.*

Variable	Buea N (%)	Ngaoundere N (%)	Total N (%)
Sex			
Female	58 (23.2)	52 (20.8)	110 (44)
Male	72 (28.8)	68 (27.2)	140 (56)
Marital status			
Single	23 (09.2)	17 (06.8)	40 (21.0)
Married	42 (16.8)	62 (24.8)	104 (41.6)
Divorced	16 (06.4)	5 (02.0)	21 (08.4)
Widow (er)	49 (19.6)	36 (14.4)	85 (34.0)
Religion			
Islam	10 (04.0)	78 (31.2)	88 (35.2)
Christianism	106 (42.4)	39 (15.6)	145 (58.0)
Others	14 (05.6)	3 (01.2)	17 (06.8)
Residence			
Urban	117 (46.8)	109 (43.6)	226 (90.4)
Rural	13 (05.2)	11 (04.4)	24 (09.6)
Age group (year)			
≤ 35	6 (2.4)	2 (00.8)	8 (3.2)
36 - 45	21 (08.4)	29 (11.6)	50 (20.0)
46 - 55	26 (10.4)	21 (08.4)	47 (18.8)
56 - 65	35 (14.0)	41 (16.4)	76 (30.4)
66 - 75	37 (14.8)	20 (08.0)	57 (22.8)
≥ 76	5 (02.0)	7 (02.8)	12 (4.8)
Overall	130 (52.0)	120 (48.0)	250 (100.0)

N: Frequency. %: Percentage.

5. Results

5.1. Demographic and Clinical Characteristics of Participants

During this study, we met 275 participants and excluded 25 after carefully cheeking according to our criteria and guidelines, explained above. The final number of participants enrolled was 50. As shown in table 1 above, the mean age was 56.78±12.06 years with a minimum of 28 years and a maximum of 89 years. The Clinical characteristics are presented in table 2 below. It can be seen that, the female population represented 56.0% with a mean's age of 56.2±10.8 years (CI: 54.4-57.8) while the male population had an average of 57.6±13.3 years (CI: 55.3-60.0). Obesity was prevalent at 63%, including pre-obesity and obesities of class I, II and III, with predominance in female population (P = 0.043). The age group 56-65 years was the more present (30.4%) and, more than half of the population (50.4%) had duration of diabetes less than 5 years. The prevalence of hypertension was 60.4% while, within sex groups, it was higher in males compared to females 63.6% and 57.8% (P=0.585) respectively.

Table 2. *Clinical characteristic of diabetics patients according to the gender.*

Clinical variables	Female N (%)	Male N (%)	Total N (%)	Statistics	
				X^2	*P*-value
Diagnosed hypertensive					
No	59 (23.6)	40 (16.0)	99 (39.6)	1.07	0.585
Yes	81 (32.4)	70 (28.0)	151 (60.4)		
Sub-total of diagnosed hypertensive	140 (56.0)	110 (44.0)	250 (100.0)		
BMI					
Underweight	3 (1.2)	1 (0.4)	4 (1.7)		
Normal weight	39 (15.7)	49 (19.7)	88 (35.3)		
Pre-obesity	68 (27.3)	40 (16.1)	108 (43.4)	11.44	0.043*
Obesity class I	14 (5.6)	14 (5.6)	28 (11.2)		
Obesity class II	10 (4.0)	4 (1.6)	14 (5.6)		
Obesity class III	5 (2.0)	2 (0.8)	7 (2.8)		
Sub-total BMI	140 (56.0)	110 (44.0)	250 (100.0)		
Duration of diabetes					
≤ 5 Years	77 (30.8)	49 (19.6)	126 (50.4)	3.31	0.190
6 to 10 Years	37 (14.8)	40 (16.0)	77 (30.8)		

Clinical variables	Female N (%)	Male N (%)	Total N (%)	Statistics	
				X^2	*P*-value
≥ 26 years	26 (10.4)	21 (8.4)	47 (18.8)		
Sub-total duration diabetes	140 (56.0)	110 (44.0)	250 (100.0)		

N: Frequency. %: Percentage. BMI: Body Mass Index. X^2: Chi Square test. df: Degree of Freedom.

5.2. Biomarkers of DKD in Diabetic Study Population

The mean of FBG was 164.56±72.90 mg/dL and females presented a higher value compared to males, with respectively 168.33±74.46 mg/dL (CI: 155.59-171.57) and 160.42±71.01 mg/dL (CI: 147.87-174.68). The mean value of serum creatinine was 0.94±0.32 mg/dL, for the entire population of study. During the evaluation of the main lipid markers, we found that Total Cholesterol was equal to 206.11±66.89 mg/dL, HDL Cholesterol equal to 66.67±46.54 mg/dL and TG equal to 177.18±76.56 mg/dL. LDL Cholesterol was at 103.7±73.32 mg/dL. According to the duration of diabetes, people with a duration greater than 5 years presented higher values of FBG, serum urea and serum HDL compared to those with duration of less or equal to 5 years. In another hand, serum creatinine, Total cholesterol, TG and LDL Cholesterol were elevated in people with duration of less or equal to 5 years, compared to those with duration of greater than 5 years (Table 3).

Table 3. *Description of biomarkers' means according to the duration of diabetes.*

Variable	People with diabetes ≤ 5 years		People with diabetes > 5 years		Statistics	
	Mean	SD	Mean	SD	t-test	*p*-value
FBG (mg/dL)	160.04	67.55	169.16	77.96	0.989	0.324
S. Creat (mg/dL)	0.951	0.3403	0.932	0.3091	0.481	0.631
S. BUN (mg/dL)	44.61	42.604	41.20	62.861	0.502	0.616
S. Urea (mg/dL)	65.77	56.657	70.96	67.050	0.661	0.509
TC (mg/dL)	209.37	63.618	202.79	70.179	0.777	0.438
TG (mg/dL)	180.69	72.653	173.62	80.476	0.729	0.467
HDL (mg/dL)	63.42	44.410	70.58	48.532	1.216	0.225
LDL (mg/dL)	109.81	77.763	97.49	68.271	10330	0.185

FBG: Fasting Blood Glucose. S. Creat: Serum Creatinine. S. Urea: Serum Urea. BUN: Blood Urea Nitrogen. TC: Total Cholesterol. TG: Triglycerides. HDL: High Density Lipoprotein. LDL: Low Density Lipoprotein. SD: Standard Deviation. df: Degree of freedom.

5.3. Assessment of CKD in the Study Population

The assessment of kidney function, according to the estimation of GFR, shown general mean of the population at 100.57 ml/min/1.73m2 where male population presented a more elevated value compared to female population with respectively 112.00±47.11 ml/min/1.73m^2 (CI: 103.23-120.24) and 91.54±55.46 ml/min/1.73m2 (CI: 83.43-102.40). Micro-albuminuria was found in 32.8% of participants and 3.6% were diagnosed with macro-albuminuria (figure 1). According to the classification of CKD as recommended by KDIGO, using the MDRD formula, 59.6% of participants presented CKD. The different stages, 1, 2, 3a, 3b, 4 and 5 were counted with the respective cases of 28 (11.2%), 83 (33.2%), 33 (13.2%), 3 (1.2%), 2 (0.8%) and 0 (0.0%) (Figure 2).

Out of the 250 participants, 38 presented DKD which represented a prevalence of 15.2% where, 78.9% were females (P=0.002) (Table 4). The prevalence of the co-morbidity hypertension and DKD was 8.8% (P=0.732) and, 9.2% were people diagnosed DKD with diabetes duration of less than 5 years (P =0.175). Within the diagnosed DKD patients group, the prevalence of hypertension was found to be 28.9%. The mean FBG of people diagnosed DKD was lower than those without DKD (P=0.666) while serum creatinine was higher in people diagnosed DKD compared to those without DKD (P< 0.001). Total Cholesterol (P=0.900), TG (P=0.923) and LDL Cholesterol (P= 0.551) were elevated in people with DKD compared to those without DKD (Table 5). Associations were assessed between the presence of DKD and some variables using a multivariate analysis. Significant association was found between DKD and two variables, female gender (P=0.002) and hyper-creatininemia (P<0.001), with respectively OR of 2.28 (CI: 1.21-4.29) and 3.47 (CI: 2.13-5.66) (Table 6).

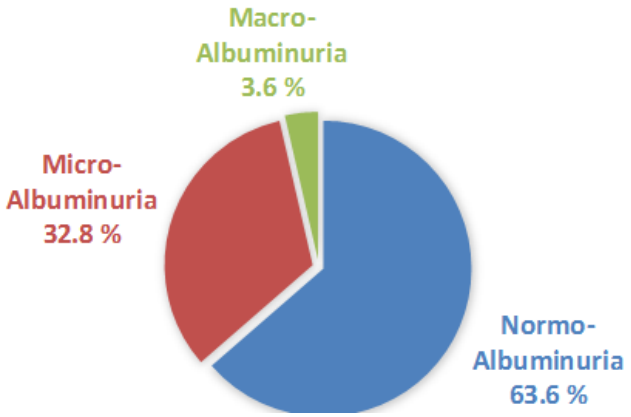

Figure 1. *Prevalence of micro- and macro-albuminuria in the study population.*

5.4. Prevalence and risk Factors of DKD in the Study Population

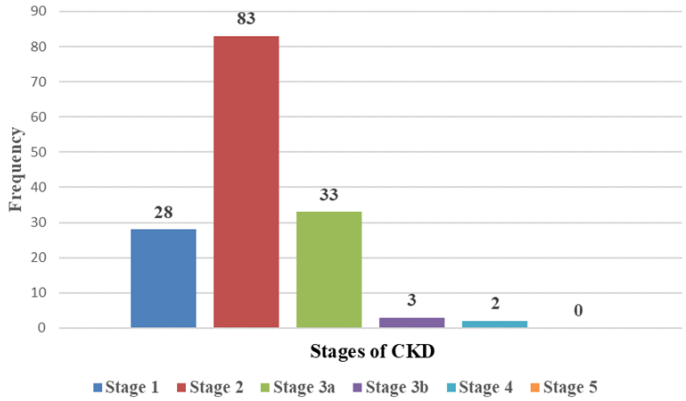

Figure 2. *Frequencies of different stages of CKD in the study population.*

Table 4. *Comparison of biochemical parameter's mean according to DKD status.*

Variable	People without DKD		People with DKD		Statistics		
	Mean	SD	Mean	SD	T-test	df	P-value
FBG (mg/dL)	165.53	70.59	160.28	83.07	0.44	248	0.666
S. Creat+ (mg/dL)	0.85	0.24	1.33	0.34	-10.89	248	< 0.001*
S. BUN (mg/dL)	44.53	58.24	35.76	22.20	1.00	248	0.317
S. Urea (mg/dL)	68.74	64.49	66.59	49.72	0.21	248	0.832
TC (mg/dL)	205.85	65.95	207.23	71.68	-0.12	248	0.900
TG (mg/dL)	176.96	75.81	178.17	80.65	-0.09	248	0.923
HDL (mg/dL)	68.08	49.06	62.06	33.16	1.00	95	0.317
LDL (mg/dL)	102.38	72.64	109.53	76.80	-0.57	248	0.551

FBG: Fasting Blood Glucose. S. Creat: Serum Creatinin. S. Urea: Serum Urea. BUN: Blood Urea Nitrogen. TC: Total Cholesterol. TG: Triglycerides. HDL: High Density Lipoprotein. LDL: Low Density Lipoprotein. SD: Standard Deviation. df: Degre of freedom. *: *P*-value < 0.05.

Table 5. *Characteristic of the population diagnosed with DKD.*

Variable		People with DKD N (%)	People without DKD N (%)	P-value
Sex				
	Female	30 (12.0)	110 (44.0)	0.001*
	Male	8 (3.2)	102 (40.8)	
Sub-total		38 (15.2)	212 (84.8)	
Diagnosed hypertensive				
	No	16 (6.4)	83 (33.2)	0.432
	Yes	22 (8.8)	129 (51.6)	
Sub-total		38 (15.2)	212 (84.8)	
Diagnosed DD				
	No	27 (10.8)	160 (64.0)	0.347
	Yes	11 (4.4)	52 (20.8)	
Sub-total		38 (15.2)	212 (84.8)	
Duration of diabetes				
	≤ 5 Years	23 (9.2)	103 (41.2)	0.119
	> 5 Years	15 (6.0)	109 (43.6)	
Sub-total		38 (15.2)	212 (84.8)	

N: Frequency. %: Percentage. DD: Diabetic Dyslipidemia, DKD: Diabetic Kidney Disease. *: *p*-value < 0.05 (*Fisher's Exact test*)

Table 6. *Multivariate analysis of possible risk factors for DKD.*

Variable	Adjusted OR (95% CI)	*p*-value
Female Sex	2.28 (1.21-4.29)	0.002**
Diagnosed hypertensive	1.05 (0.78-1.40)	0.760
Diagnosed DD	1.06 (0.85-1.32)	0.548
Duration of diabetes less than 5 years	1.30 (0.86-1.97)	0.119
Hyper-glycaemia	1.09 (0.83-1.43)	0.511
Hyper-Creatininemia	3.47 (2.13-5.66)	< 0.001**
Hyper-Total Cholesterolemia	1.07 (0.79-1.44)	0.648
Hyper-LDL Cholesterolemia	1.03 (0.84-1.25)	0.751
Hyper-HDL Cholesterolemia	1.14 (0.74-1.75)	0.536

Variable	Adjusted OR (95% CI)	*p*-value
Hyper- Triglyceridemia	1.12 (0.87-1.44)	0.311

LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein. DKD: Diabetic Kidney Disease. DD: Diabetic Dyslipidemia. CI: Confidence of interval OR: Odd Ratio. CI: Confidence of Interval.. **: P-value < 0.05.

6. Discussion

This present research has been done in diabetic people living in Buea and Ngaoundere, two regional headquarters of Cameroon, in Central Africa, to determine the prevalence of DKD and to describe some biomarkers related to this disease. The research has involved 250 participants from 3 selected health facilities. The assessment of DKD has followed the crucial recommendations of ADA consensus on diabetes and CKD [5].

From our study, we found that the prevalence of DKD is 15.2%. Prevalences of 6.7%, 10.3% and 34.5% have been found respectively in diabetic populations in Egypt [8], in the Kingdom of Saud Arabia (KSA) [6] and in the United States of America (USA). The heterogeneity in the prevalence found in our study with those countries is probably due to the ethnicity and racial influences associated to the development of DKD. Although studies suggested that, black peoples are more exposed developing DKD compared to others [11, 12]. The higher prevalence of DKD in USA compared to our study may be due to the complex socio-ethnoracial composition of this population and their lifestyle. The high prevalence of obesity and hypertension in the USA population with diabetes, which are respectively 85.2% [13] and 73.6% [14], added to metabolic syndrome and the American lifestyle, compared to the findings in our study population, with prevalence of obesity at 60.0% and hypertension at 60.4%, may be responsible to the difference between the two DKD prevalence's. The prevalence of DKD in this study was relatively similar to those obtained in Ethiopia and Tunisia, respectively 15.7% and 19.8% [15, 16]. In Tanzania, a prevalence of 24.7% has been found in 2013 [17]. This last one prevalence, as Mpondo et Al. said, may be attributable to the high prevalence of Schistosomiasis infection in the region of Tanzania, given its role in the etiology of renal disease [18-20].

According to the gender, DKD has been found positively associated with females, as reported in other studies [21, 22]. In a group of Indian living with diabetes, Verma et Al. reported that, the co-morbidity hypertension-DKD is prevalent at 45.4% [23]. However, in our study, we obtained a lower prevalence of this co- morbidity, at 8.4%. The higher prevalence obtained in Indian was due to the inadequate definition of the DKD status by the authors, who did not take in consideration the recommendations of the ADA consensus on diabetes and CKD in the diagnostic and management of DKD [5].

According to the KDIGO consortium, CKD has been found prevalent at 59.6%, staged from 1 to 5 [9]. This prevalence was high compared to the result reported by Choukem et Al. in 2012 in Yaounde, Cameroon, where a prevalence of 31% was obtained [24]. Differences happened between Choukem's study with ours, may be due to Cockcroft-Gault equation used as the methods to estimate the GFR there. Authors have reported the character of this equation to underestimate normal and high GFR compared to MDRD [25-27].

In this study, we diagnosed DKD according to the presence of persistent macro-albuminuria and microalbuminuria associated with retinopathy. Micro-albuminuria has been assessed in the diabetic population in Cameroon previously with a prevalence of 53.1% in Yaounde (1999) [28], and 34.6% in Bamenda in 2017 [29]. In agreement with these reports, the prevalence of micro-albuminuria found in our study confirms that micro-albuminuria is frequent among diabetics in Cameroon. Microalbuminuria, as an important risk factor for cardiovascular disease, and facilitator of the progression of diabetes to renal impairment, has to be integrated during routine investigation of diabetes complications in Cameroon [30, 31].

In this study, we used creatinine clearance alone, to estimate the GFR, according to the KDIGO guideline on CKD. However, a decreased eGFR is confirmed by an alternative filtration marker, cystatin C, to estimate another GFR. Nevertheless, the careful respect of some important guides of KDOQI guideline on DKD has facilitated the identification of DKD cases.

7. Conclusions

The study showed that the prevalence of DKD is 15.2% and women were more exposed to develop it compared to men. Micro-albuminuria has been also shown to be more prevalent in diabetic population. The prevalence of CKD in diabetic population is high. These findings showed that, diabetic complications remain a serious problem and more actions should be taken to improve on the management of diabetes in Cameroon.

Data Availability

The original data used to support the findings of this study will be provided upon request.

Conflict of Interests

All the authors do not have any possible conflicts of interest.

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