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Cystatin C and Chronic Complications of Diabetes Mellitus in a Subsaharian Population

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ABSTRACT

Background: Type 2 diabetes (T2D) is a chronic and progressive condition whose early management is crucial in preventing the occurrence of its chronic degenerative complications. Cystatin C (CysC) is a biomarker that may have a beneficial interest in the early detection of microvascular and macrovascular complications of T2D.

Methods: We carried out a cross-sectional analytic study at the National Obesity Center of the Yaounde Central Hospital. We recruited 135 patients with T2D and performed a neurologic physical exam using the Toronto clinical score, a fundoscopy, ECG, ABI, and the following paraclinicals: dosage of serum levels of Cystatin C, lipid profile (with determination of Atherogenic indexes), Creatinine (with calculation of glomerular filtration rate using CKD-EPI formula) and hs-CRP.

Results: Prevalences of diabetic retinopathy, nephropathy, neuropathy and PAD were 3.0%, 8.9%, 38.5%, 28.1% respectively. Electrocardiographic signs of myocardial ischemia were present in 5.9 % of the participants. We found 0.3[0.5-0.3] mg/l as median levels of the HDL-cholesterol and 0.9[1.2-0.7] mg/l for LDL-cholesterol. The median value of GFR was 105.7[119,0-85.7] ml/min/1.73m^2. The median serum CysC level was 0.8[0.9-0.6]mg/l and varied with age (p=0.01), A1C (p=0.016) and high blood pressure (HBP) (p=0.006). There was a relationship between serum CysC and diabetic nephropathy (p=0.01) and neuropathy (p=0.025). There was no significant relationship with diabetic retinopathy (p=0.225), PAD (p=0.169) and ECG signs of myocardial ischemia (p=0.669).

Conclusion: Chronic microvascular and macrovascular complications of type 2 diabetes are common and in our study are predominantly represented by diabetic neuropathy and PAD. Serum CysC can be useful in the diagnosis of chronic complications of T2D.

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Background and Rationale

Diabetes is a major health problem, the extent of which lies in its exponential growth and its evolution towards complications responsible for significant morbidity and mortality. It is also a disease that imposes an economic burden on our health systems. Since diabetes is a chronic and progressive condition over time, the implication for the practitioner is the use of multi-therapy, with the aim of achieving the therapeutic objectives set and preventing or early detecting the occurrence of chronic degenerative complications. Although the occurrence of chronic complications of diabetes depends on the quality of long-term glycemic control, there is also a significant part of genetics, especially with regard to diabetic nephropathy and retinopathy [1]. To reduce the incidence of these complications, it is important to prevent them, but also to diagnose them early, at a stage where the lesions are still reversible. It has been shown that some biological markers, both serum and urinary, can be detected before the expression of the first clinical signs of diabetes complications. These are very often inflammatory or endothelial dysfunction markers [2]. Cys is one of them and has recently presented an interest in early detection of chronic complications of T2D.

CysC is a protein inhibitor of proteinases, more specifically cysteine proteinase, which has an interest in cardiovascular risk assessment [3,4]. It is constantly synthesized by all the nucleated cells of the body, freely filtered at the glomerular level, completely reabsorbed at the tubular level. It is not significantly influenced by extra-renal factors such as age, sex and muscular mass, making it a better marker of kidney function than creatinine [5]. In addition, its serum levels vary according to race [6]. In daily medical practice, its dosage is not achieved because of difficulties related to availability, reproducibility and cost. Several studies have been conducted in the Caucasian and Black American populations to investigate the relationship between serum CysC and the various chronic complications of T2D, including diabetic retinopathy and nephropathy [7,8]. In Africa, a study in Tunisia showed a relationship between cystatinemia C and cardiovascular disease in T2D [9]. Given the variation in serum levels by race, no studies have investigated this relationship in the Black African population, reason why we carried out a study to investigate the relationship between serum cystatin c and chronic complications of type 2 diabetes.

Methods

Patients: we carried out a cross-sectional analytic study at the National Obesity Center of the Yaounde Central Hospital. We recruited 135 T2D patients, and investigated for cardiovascular risk factors, as well as risk factors for microvascular and macrovascular complications. For each volunteer, we performed a neurologic physical exam on them, using the Toronto clinical neuropathy score to diagnose diabetic neuropathy. We carried out the following investigations for each volunteer: a fundoscopy for diabetic retinopathy, ECG to look for signs of myocardial ischemia and Ankle-Brachial Index (ABI) to detect peripheral arterial disease (PAD).

Biochemical Assays: 5 milliliters of venous blood were collected by venipuncture in one dry tube. Serum was separated by centrifugation at 3000 rpm within 10 min. Biochemical assays were conducted using the autoanalyzer Cobas 501/6000, Roche Diagnostics, USA. Serum cystatin C was measured by particle-enhanced turbidimetric immunoassay using Tina-quant® Cystatin C reagent kits (Roche diagnostics, USA). Serum creatinine was determined by modified Jaffe kinetic method, follow by the calculation of GFR using the CKD-Epi formula. Lipid profile (with determination of atherogenic indexes using the Atherogenic Index of Plasma) and hs CRP were also performed. We also measured serum levels of Cystatin C, lipid profile (with determination of Atherogenic indexes), hs CRP, blood creatinin (with calculation of GFR).

Statistical Analysis: the data obtained was coded using the CsPro Version 7.1 software and analyzed using the *SPSS* Version 23 software. The Spearmn correlation coefficient was used and a p-value < 0.05 was set as statistically significant.

Ethic Statement: the study was carried out in strict compliance with the fundamental principles of the Helsinki Declaration. We have obtained research authorization from the regional ethics committee for human health research of the Centre.

Results

General Characteristics of the Study Population

We included 135 patients and the male/female ratio was 0.5. The median age was 54 [60-50] years and 104/135 (77%) were 50 years and older. The most common levels of education were high school (44.5%), primary school (31.1%) and university (18.5%). The predominant sectors of professional activity were unemployment (37.8%) and the private sector (17.7%). (Table 1) Clinically, the duration of diabetes progression was 4 [9-1] years. The most frequent circumstances of discovery of diabetes were polyuro-polydipsia syndrome (39.3%), incidental finding (37.8%) and voluntary screening (15.6%). Concerning the current treatment, most of the subjects were on OADs (62.2%). High blood pressure was found in 55/135 (40.7%) of the subjects, active smoking in 7/135 (5.2%) of the participants and harmful alcohol consumption in 22/135 (16.3%) of the patients. Sixty-four patients (47.4%) reported regular physical activity. As other co-morbidities, we found that 3/135 (2.2%) of the participants were HIV positive, 6/135 (4.4%) were carriers of liver disease with etiologies of hepatitis B and C viruses. No participant had received corticosteroid therapy in the 15 days prior to the consultation. The median BMI was 28.8 [32.4-25.2] kg/m² and patients were mostly obese (45.3%) (Table 2). The study of biological characteristics found a median A1C of 7.0 [8.8-5.5] % and 76/135 (56.3%) of the subjects had a glycemic imbalance. The median AIP was 0.6 [0.7-0.5]. The median values of hsCRP, GFR and Cys C were 1.8 [5.9-0.3] mg/l, 105.7 [119.0-85.7] ml/min/1.73m² and 0.8 [0.9-0.6] mg/l, respectively (Table 3).

Table 1: Sociodemographic Characteristics of the Population

Parameters	Categories	Effective	Percentage (%)
Gender (N=135)	Male	47	34.8
	Female	88	65.2
Age (years)	<50	31	23
	50-59	66	48.9
	≥60	38	28.1
Marrital status (N=135)	Married	90	66.7
	Single	13	9.6
	Divorced	5	3.7
	Widowed	19	14.1
	Separated	3	2.2
	Cohabitation	5	3.7
Level of education (N==135)	unschooling	8	5.9
	Primary	42	31.1
	High school	60	44.5
	University	25	18.5
Sectors of activity (N=135)	Unemployed	51	37.8
	Civil servants	19	14.1
	Private sector	24	17.7
	Retired	20	14.8
	Informal	19	14.1
	Others	2	1.5

Table 2: Clinical Characteristics of the Population

Parameters	Categories	Effective	Percentage (%)
Circumstances of Discovery (N=135)	Incidental	51	37.8
	Voluntary screening	21	15.6
	PU /PD	53	39.3
	Infection	3	2.2
	Coma	1	0.7
	Hospitalization	2	1.5
	Others	4	2.9
Actual treatment	None	8	5.8
	Dietetics	6	4.4
	OADs	84	62.2
	Insulin	18	13.3
	Insulin+OADs	19	14.1
BMI (kg/m²) (N=117)	Underweight	1	0.8
	Normal	25	21.4
	Overweight	38	32.5
	Mild obesity	36	30.8
	Moderate obesity	12	10.3
	Severe obesity	5	4.2

Table 3: Biochemical Characteristics of the Population

Parameters	Categories	Effective/ Median (IQR)	Percentage (%)
A1C (%)	< 7	59	43.7
	≥ 7	76	56.3

Prevalence of Chronic Complications of Diabetes

Among the microangiopathies, we found that 4/135 of the subjects had diabetic retinopathy, a prevalence of 3%. Nephropathy and neuropathy were found in 11/123 (8.9%) and 52/135 (38.5%) of the participants, respectively (Figure 1).

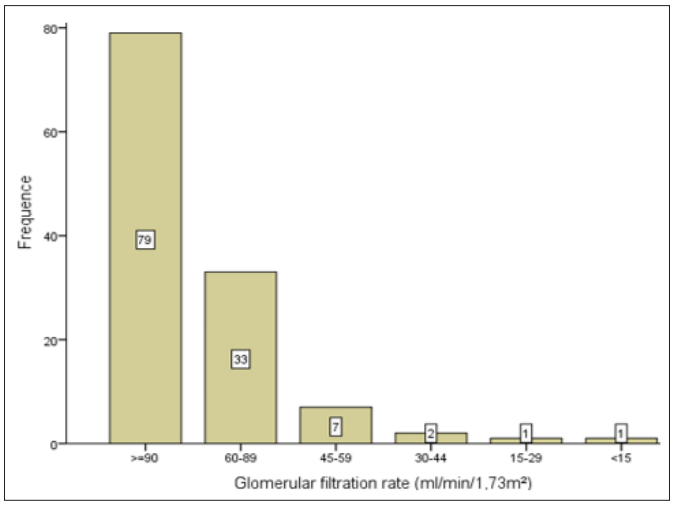

Figure 1: Prevalence of Diabetic Nephropathy

Among macrovascular complications, we found AOMI in 38/135 (28.1%) of subjects and electrocardiographic signs of myocardial ischemia in 8/135 (5.9%) of patients.

Distribution of CysC in the Population

We found a significant difference in serum CysC levels between the different age groups and a positive correlation (r=0.2, p=0.01). This difference was also found between the groups of patients with and without glycemic imbalance (r=-0.21, p=0.016) and those with and without HBP (r=-0.23, p=0.006) (Table 4).

Table 4: Distribution of CysC in the Population

Parameters	Categories	CysC (mg/l) Median (IQR)	r	p-value
Gender	Male	0.8[1.3-0.4]	0.04	0.615
	Female	0.8[2.8-0.4]		
Age groups (years)	<50	0.7[1.4-0.5]	0.2	0.01
	50-59	0.8[2.8-0.4]		
	≥60	0.9[1.8-0.4]		
Duration of Diabetes (years)	<5	0.8[2.8-0.4]	0.04	0.67
	≥5	0.8[1.8-0.4]		
A1C (%)	<7	0.8[2.8-0.4]	-0.21	0,016
	≥7	0.7[1.4-0.4]		
HBP	Yes	0.8[2.8-0.4]	-0.23	0.133
	No	0.7[1.4-0.4]		
Active smoking	Yes	1.0[1.3-0.5]	-0.13	0,133
	No	0.8[2.8-0.4]		
Harmful alcohol consumption	Yes	0.8[1.3-0.4]	-0.05	0.133
	No	0.8[2.8-0.4]		
BMI	Underweight	1.1[1.1-1.1]	-0.07	0.413
	Normal	0.8[1.8-0.6]		
	Overweight	0.8[1.3-0.4]		
	Mild obesity	0.8[2.8-0.4]		
	Moderate obesity	0.8[1.3-0.5]		

Relationship between CysC and Chronic Complications of DM

We found a significant association between serum CysC levels and nephropathy (r=-0.34, p<0.01) and neuropathy (r=-0.193, p=0.025). Serum CysC levels were inversely correlated with GFR (Table 5).

Table 5: Correlation between CysC and Chronic Complications of DM

Parameters	Categories	CysC (mg/l) Median (IQR)	r	p-value
Diabetic retinopathy	Yes	1.3[2.8-0.6]	-0.105	0.225
	No	0.8[1.4-0.4]		
Diabetic nephropathy (GFR	≥90	0.7[1.4-0.4]	-0.34	<0.01
	60-89	0.8[1.3-0.6]		
	45-59	0.9[1.3-0.8]		
	30-44	1.6[1.8-1.3]		
	15-29	0.8[0.8-0.8]		
	<15	2.8[2.8-2.8]		
Diabetic neuropathy	Yes	0.9[2.8-0.4]	-0.193	0.025
	No	0.8[1.4-0.4]		
PAD	Yes	0.8[1.3-0.5]	0.119	0.169
	No	0.8[2.8-0.4]		
Myocardial ischemia	Yes	0.8[1.0-0.6]	-0.03	0.669
	No	0.8[2.8-0.4]		

Discussion

In the study population, neuropathy (38.5%) and PAD (28.1%) were found. This result is comparable to that of Moumbe & al who, in their cohort highlighted the presence of several chronic complications of T2DM, namely: neuropathy (40%), retinopathy (23.6%), CAD (23.6%), nephropathy (25%) and PAD (17.1%). These results raise questions about the follow-up of diabetic patients. It is recognized that glycemic imbalance is the major cause of the occurrence of complications, and it is therefore important that health care providers involved in the management of diabetes ensure the proper follow-up of patients. However, the different prevalence rates observed between this research and that of Moumbe & al could be explained by the different diagnostic methods used in the two studies. Indeed, the study of Moumbe et al was both retrospective and prospective and used GFR associated with 24h proteinuria as diagnostic criteria for nephropathy. Neuropathy was diagnosed by the monofilament test, PAD on the basis of symptoms and clinical examination and coronary artery disease via cardiac ultrasound and ECG performed in the prospective arm of the study [10].

We found that CysC varied significantly with certain cardiovascular risk factors such as advanced age (p=0.01), glycemic control (p=0.016) and HBP (p=0.006). This corresponds to the results of the study of Javier Cepeda et al, conducted in Spain [11]. Furthermore, variation with age was the only parameter that was consistent with the results of the study of Edinga & al in Yaounde [12]. The difference observed with the other parameters could be explained by the characteristics of the study population which was not exclusively carriers of T2D and certainly the black race.

Regarding the relationship between serum CysC and diabetic retinopathy, our study found CysC levels of 1.3 [2.8-0.6] mg/L in subjects with DR versus 0.8 [1.4-0.4] mg/L in those without DR. This difference was not significant (p = 0.225). This result is different from that of the study by Qian et al and Shijie Sun et al who found a significant association between these two parameters [13,14]. Knowing that from a pathophysiological point of view, there is a positive correlation between the duration of diabetes progression and the occurrence of chronic complications, the observed difference could be explained by the median duration of diabetes progression being lower in our study than in other studies.

CysC levels were elevated in subjects with diabetic nephropathy (1.1 [2.8-0.8] vs 0.8 [1.4-0.4]) and the relationship between the two parameters was found to be significant (p < 0.01, r=-0.34), a result similar to that of Jeon et al, who found that CysC is an early marker of nephropathy in T2DM [7]. Therefore, serum Cystatin C assay may be useful to screen, diagnose and manage diabetic nephropathy early.

CysC levels were elevated in patients with diabetic neuropathy (0.9 [2.8-0.4] vs 0.8 [1.4-0.4]) and a significant correlation was found (p = 0.025, r=-0.193). These results are comparable and similar to those of Yangun Hu et al who found a prevalence of 26.3% and high median rates in neuropathy carriers[15]. Although elevated cystatin C is associated with the presence of neuropathy, clinical assessment should not be overlooked when screening for this complication.

We found a 5.9% prevalence of CAD and a non-significant association with CysC (p = 0.669), a result that differs from that of Triki et al. This could be explained by their diagnostic criteria for CAD, which were based solely on signs of myocardial ischemia on the ECG in our study, whereas those set by Triki et al were a history of angina, myocardial infarction, valve disease, cardiomyopathy, arrhythmia, heart failure and PAD [9].

In our study, 28.1% of subjects had PAD defined by ABI<0.9. In these subjects, the median CysC level was 0.8 [1.3-0.5] mg/L vs. 0.8 [2.8-0.4] mg/L in patients without PAD. We did not find a significant relationship between CysC and PAD (p = 0.169). It could be suggested that CysC doesn't have a significant role in screening PAD in diabetics.

In conclusion, Cystatin C is a biomarker associated with some traditional cardiovascular risk factors. This biomarker could also

predict the occurrence of nephropathy and neuropathy in T2DM.

Conflicts of Interest
We declare no conflict of interest in this study.

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