Int J Diabetes & Metabolism (2008) 16: 69-78

Risk factors of poor control of HBA1c and diabetic retinopathy: Paradox with insulin therapy and high values of HDL in African diabetic patients

B Longo-Mbenza,[1] MM Muaka,[2] G Mbenza,[3] S Mbungu-Fuele,[4] L Mabwa-Mbalanda,[4] V Nzuzi-Babeki,[4] J Mbadi-A-Sungu[4]

Division of Cardiology, Pathophysiology and Clinical Epidemiology[1]; University of Kinshasa; Division of Ophthalmology[2], Army Salvation Hospital, Kinshasa, Gombe[3];Lomo Medical Center, Kinshasa, Limete,[4] DRC

Abstract

Objective: To determine the risk factors of poor control of glycated haemoglobin and diabetic retinopathy. The agreement between poor control of glycated haemoglobin (HbA1c) >7% and poor control of glycemia ≥ 126 mg/dL to classify diabetic retinopathy was also assessed. **Design, settings and methods:** The study was a cross-sectional survey carried out on 300 African diabetic patients admitted to Lomo Medical Center, Kinshasa, Congo, between July 2005 and December 2007. Patients (150 type 1 and 151 type 2) were interviewed and underwent a complete medical assessment. HbA1c levels, anthropometry, blood pressure components, lipid profile, type of diabetes, severity and complications were determined for each patient. All patients were examined for evidence and severity of diabetic retinopathy by an ophthalmologist. **Results:** The rates of arterial hypertension, uncontrolled hypertension, poor control of HbA1c, poor control of glycemia, higher pulse pressure and diabetic retinopathy were 73.3%, 81.8%, 68%, 57%, 47.7% and 33.3%, respectively. Type 1 diabetes, diabetes duration ≥ 4 years, female sex, underweight, diabetic retinopathy, diabetic nephropathy, elevated total cholesterol and higher levels of HDL-cholesterol were significantly associated with poor control of HbA1c. There was a poor agreement of 52% and kappa statistic of 0.19 ($p<0.0001$) between poor control of HbA1c and poor glycemic control to classify diabetic retinopathy. In all diabetic patients, aged ≥60 years, female sex, diabetes duration ≥4 years, type 1 diabetes, higher pulse pressure, underweight, poor control of HbA1c, smoking, stroke, diabetic nephropathy and low HDL-cholesterol are significantly associated with the presence and the severity of diabetic retinopathy. However, in 87 diabetic patients with a history of intravenously administered insulin, duration diabetes ≥ 4 years and good control of HbA1c <7% are significantly associated with the presence of diabetic retinopathy. There was a J-shaped relationship between poor control of glycemia ≥126mg/dL and the severity of non proliferative diabetic retinopathy. **Conclusion:** Urgent and efficient diabetes care and diabetes monitoring are needed in sub-Saharan Africa.

Keywords: *Africa, diabetes mellitus, glycated haemoglobin, diabetic retinopathy, risk factors.*

Introduction

Sub-Saharan Africa (SSA) now faces a double disease burden, with emerging non communicable diseases (NCD), such as arterial hypertension, stroke, coronary heart disease and diabetes mellitus (DM) added to the challenges in infectious diseases.[1] In a survey conducted in 2005 in SSA, it is estimated that 16% people had DM.[2,3]

The escalating prevalence of type 1 and type 2 DM, risk factors and chronic complications of DM are now established. These include 7-52% for diabetic retinopathy (DR), 6-30% for diabetic nephropathy (DN), and 1-5% for macroangiopathy.[4,5] DR, DN, chronic renal failure, neuropathy, age ≥ 60 years, smoking, DM duration ≥ 4years and higher pulse pressure are identified risk factors of stroke in diabetic Africans,[6] whereas higher pulse pressure is a risk factor of diabetic cardiomyopathy[7] and DR[8] in Africans, respectively. Ethnic differences in the prevalence and severity of DM chronic complications[9] and low lipid profiles or indifferent role of dyslipidemia in the general population[10] or in NCD including DM[11, 12] are reported from SSA. Although genetic susceptibility may not be ruled out, lack of health care facilities to control of blood glucose, blood pressure and dyslipidemia might account for most of the differences with other populations around the world.

Lack of awareness by Africans and facilities for detection and monitoring of DM may contribute to the high prevalence of diabetic complications.[13] The high cost of insulin is the most important cause of lack of access to insulin in type 1 diabetics.[14] Moreover, a small percentage of type 2 diabetics require insulin when they become severely underweight and hyperglycaemic. There is, therefore, an urgent need for intravenous insulin (5-10 UI of Insulin per hour) to control hyperglycemia in these poorly

Received on: 28/01/2008
Accepted on: 18/06/2008

Correspondence to: Benjamin Longo-Mbenza, University of Kinshasa, BP 783, Kinshasa XI, DR CONGO, E-mail: longombenza@yahoo.fr, Fax: 0826705666

controlled African diabetic patients. In the Democratic Republic of the Congo (DRC), our country, where people with type 1 DM have access to insulin for less than 25% of the time,[14] the only equipment for continuous monitoring of glycated haemoglobin (HbA1c) in diabetics had been introduced in July 13th, 2005 in our LOMO MEDICAL Clinic, Kinshasa, Limete.

Data from the United Kingdom Prospective Diabetes study (UKPDS)[15] and the Diabetes control and complication trial (DCCT)[16] have shown that intensive control of glucose results in a 25-70% reduction in the number and severity of DM microvascular complications. In the UKPDS, control of high blood pressure (BP) reduced the risk of microvascular complications by 37% and death from type 2 DM-related disease by 32%.[17] Greater reductions from those with tight blood glucose control were observed. Furthermore, compared with diet/oral antidiabetic drugs regimen, insulin treatment conferred higher risk of DR in all Congolese diabetics (OR=2 95%CI 1.2-3.5; P<0.01) in general and in female diabetics (OR=2.7 95%CI 1.4-5.4 P<0.01) and type 2 diabetics (OR=2.4 95%CI 1.4-4.1; P<0.001) in particular.[8] Because the lack of data on HbA1c among Congolese diabetics, the present study was initiated with the aim of determining the association between cardiovascular disease (CVD) risk factors, nutritional status, DM duration, diabetic chronic complications with special reference to DR, insulin treatment and HbA1c. The lipid and non lipid risk factors to DM complications were also assessed.

Materials and methods

Design, settings and study population

The study was a cross-sectional survey of DM patients consecutively assessed for DM chronic complications including stroke, arterial hypertension, DR, DN and other comorbidities in different clinics of DRC, between July 2005 and December 2007.

African diabetic patients from Moanda, Boma, Matadi, and Kinshasa private health care centers (CMG, Litoyi Medical, Salvation Army, LOMO Medical Center) screened for detection of DR were eligible for participation in this study. In the absence of a computerized database in the majority of the clinics, every third diabetic patient was approached to participate during the study period. The sample size was calculated as follows: expected proportion (P) of DR of 0.30, desired precision or total width (W) of 0.10 and 95% confidence interval ($N=4x(1.96)^2xP(1-P).W^2$).

In total, 323 patients were contacted by the ophthalmogist, diabetologist and cardiologist, out of which, 300 patients (92.9%) agreed to enrol according to the Helsinki II Declaration. The study was approved by the Ethical Committee of LOMO Medical Center.

Methods and Procedures

After receiving informed and verbal consent, known diabetic patients in the study settings were interviewed by the investigators and information pertinent to their age, sex, cigarette smoking, DM type, DM duration, insulin treatment and chronic complications was collected.

Additionally, BP was measured by trained nurses using a standard mercury sphygmomanometer in the morning and prior to drawing blood samples in the sitting position. Height was measured without shoes, and weight recorded while wearing indoor clothing. Body mass index (BMI) was calculated. Fasting blood samples were taken to assess lipid profiles, blood glucose and glycated haemoglobin (HbA1c) levels.

Fasting plasma glucose concentration was determined by the glucose oxidase method (RTU, Biomérieux, Marcy l'Etoile, France) using an analyzer (Helios Epsilon Spectrophotometer, Thermo Electron Corporation, Pittsford, NY, USA). Total serum cholesterol (TC) (CHOD-PAP method, serum triglycerides (GPO-PAP method), and high-density lipoprotein-cholesterol (HDL-C) (IRC methods) for all the patients were determined using an analyzer (Helios Epsilon Spectrophotometer, Thermo Electron Corporation, Pittsford, NY, USA).

Glycated haemoglobin fractions were measured in fresh anticoagulated blood samples with both migration set-up using a semi-automated multi-parameter instrument (HYDRASYS system, Sebia, Evry, France) and densitometric scanning of unstained gels) (HYDRAGEL 7/15 HbA1c) performed on HYRYS Densitometer and gel carrier O and specific HbA1c (NGSP) software (HYRYS densitometer, Sebia, Evry, France). After including a control blood sample into each run of samples, relative concentrations (percentages) of three fractions in each hemolyzate were yielded by the Densitometer scanning as follows: the most cathodic corresponding to the minor glycated hemoglobins A_{1C} (HbA1c), and the most anodic being the main fraction containing A_0 and A_2 hemoglobins.

All patients were referred to the ophthalmologist (MV. M) and underwent detailed eye examination. After adequate mydriasis, fundus examination was performed in all patients using direct and indirect ophthalmoscopy through dilated pupils.

Definitions

Patients were considered to have a cigarette smoking habit if they were current smokers. BMI defined by weight in kg, divided by height in meters squared, served to characterize the nutritional status as follows: under weight (BMI < 18.5 kg/m²), healthy weight (BMI: 18.5-24.9 kg/m²), overweight (BMI: 25-29.9 kg/m²) and obesity (BMI ≥ 30 kg/m²).[18] Anemia was defined by hemoglobin concentration <12 g/dL).

DM was diagnosed in patients with fasting blood glucose ≥ 126 mg/dL (7 mmol/L).[19] The interval in years between the DM diagnosis date and the date of the present study defined DM duration. DR included non-proliferative DR (NPDR) (absent signs of DR or presence of microaneurysms, haemorrhage, hard exudates) and proliferative DR (PDR)

(newly formed blood vessels and/or growth of fibrous tissue into vitreous cavity).[20]

The WHO/ISH definition of arterial hypertension[21] was used in this study: systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg or under ongoing treatment with antihypertensive drugs. DN was diagnosed in the presence of proteinuria (urinary albumin excretion rate ≥ 200µg/min) from sterile random urine samples as microalbuminuria was not available.[22]

Long duration of DM was set at a level ≥ 4 years according to our previous data.[8] The shorter DM duration of ≤ 3 years (Tertile I of DM duration levels in the present study) is characterized by accelerating DR with faster progression. The tight glycemic control in these patients was impaired by poor control of HbA1c (>7%) versus good control of HbA1c (≤ 7%) and fasting blood glucose (≥126 mg/dL or 7mmol/L) according to the American Diabetes Association (ADA). Uncontrolled hypertension was defined by BP ≥ 140/90 mmHg. Ischemic stroke confirmed by Brain Computerized Tomodensitometry scanning was considered but not hemorrhagic stroke.

The levels of TC ≥ 200 mg/dL (HDL-C < 40 mg/dL (< 1.034 mmol/L) for men or < 50 mg/dL (< 1.29 mmol/L) for women and triglycerides ≥150 mg/dL (≥1.7 mmol/dL) was defined as hypercholesterolemia, low HDL-C, and hypertriglyceridemia,[23] respectively. Higher pulse pressure (PP=SBP-DBP ≥ 60 mmHg) was defined as pre-clinical atherosclerosis or arterial stiffness.[7]

Statistical analysis

Data were reported as proportions (%) for categorical variables and mean ± SD values for continuous variables. Chi-square tests and Fischer exact tests were used to ascertain the association between variables. P for trend was calculated to assess a dose-effect response relationship between categorical variables. Comparisons of means between groups were made using the Student t-test and ANOVA. Logistic regression models were used to estimate the simultaneous effect of several determinants on poor control of HbA1c (yes/no) and DR (yes/no) outcomes, respectively. P values <0.05 were considered to be significant. The SPSS statistical package (SPSS Inc, Chicago, Illinois, USA) for Windows, version 13, was used for the analysis.

Results

Clinical and laboratory characteristics of DM patients

A total sample of 300 African diabetic patients of both genders and DM types was selected. Of those, 152 (50.7%) were males and 148 (49.3%) were females. Their continuous characteristics are shown in Table 1 with a higher variability and mean of HDL-C, unexpected increasing age, very high levels of blood glucose, increased HbA1c, and shorter DM duration.

There were 62 anaemic patients in the study population (20.7%). The higher proportion (p<0.0001) of anaemic

Table 1: General characteristics of the study population (n=300)

Variable name	Mean ± SD (95%CI)
Age (years)	56 ± 16 (55-57)
DM duration (years)	4 ± 3 (2 – 5)
BMI (kg/m²)	28.2 ± 6.2 (27.7-28.7)
SBP (mmHg)	137.7 ± 19.6 (136.1-139.2)
DBP (mmHg)	79.2 ± 10.3 (78.4-80)
Pulse pressure (mmHg)	57 ± 18.3 (55.9-58.8)
Blood glucose (mg/dL)	199.3 ± 36.4 (194.3-201.3)
Total cholesterol (mg/dL)	197.3 ± 35.4
HDL-cholesterol (mg/dL)	101.1 ± 86.4 (31-115.7)
Triglycerides (mg/dL)	158.3 ± 100.3 (146-170.5)
HbA1c (%)	7.4 ± 1.4 (7.3 – 7.5)

Out of 171 patients with poor glycemic control (≥ 126 mg/dL), 129 had poor control of HbA1c, whereas 75 patients with poor HbA1c had good glycemic control (<126 mg/dL). Thus, the agreement between HbA1c test and fasting glucose test by classification DR was 52%, and kappa statistic was 0.19 (P<0.0001).

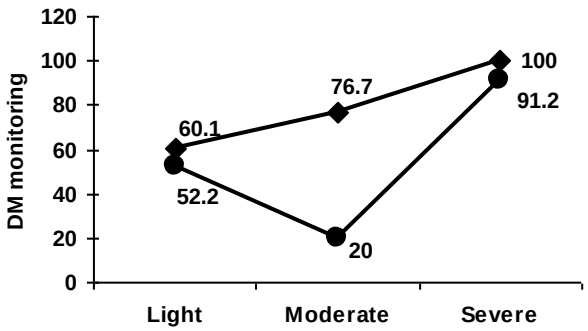

Figure 1: J-shaped relationship between poor glycaemic control and non proliferative DR severity (●—●) contrasting with dose-effect response relationship between poor control of HbA1c and non proliferative DR severity (◆—◆).

Table 2: Cardiovascular risk factors, DM types and DM chronic complications

Variable name	n (%)
Cigarette smoking	60 (20)
Type 1 DM	150 (50)
Type 2 DM	150 (50)
Total obesity	100 (33.3)
Arterial hypertension	220 (73.3)
Uncontrolled hypertension	180/220 (81.8)
Elevated total cholesterol	60 (20)
Elevated triglycerides	60 (20)
Low HDL- cholesterol	60 (20)
DR	100 (33.3)
DN	70 (23.3)
Stroke	81 (27)
Poor control of HbA1c	204 (68)
Poor glycemic control ≥126 mg/dL	171 (57)
Higher pulse pressure	143 (47.7)

patients was present among patients with bimodal curve of the most anodic application point including AO fraction and CO fraction (n=28/30 or 93.3%) in comparison with patients having normal profile of migration (n=32/270 or 11.9%), the difference being significant (p<0.0001). All of the anaemic patients had good control of HbA1c. However, anaemic patients were more prevalent (p<0.0001) among those with poor glycemic control (n=53/75 or 70.7%) than the sub-group with good glycemic control (n=9/129 or 7%).

The clinical spectrum of DM was associated with higher levels of arterial hypertension, uncontrolled hypertension, poor control of HbA1c, poor glycemic control, pre-clinical atherosclerosis and DR, and the presence of moderate increase of traditional cardiovascular risk factors, stroke and DN (Table 2).

Parameters associated with higher levels of HbA1c

Table 3 shows the levels of significance of associations between the HbA1c levels and other variables. Female sex, longer DM duration, DR, DN, cigarette smoking, elevated TC and uncontrolled glycemic levels were significantly associated with higher levels of HbA1c. Paradoxically, low HDL-C and total obesity were significantly associated with lower levels of HbA1c. However, the levels of HbA1c were not influenced by age, stroke, elevated TG, DM types, arterial hypertension, and uncontrolled hypertension.

In considering three different levels of BMI, a significant and negative relationship was shown between HbA1c, DM duration levels and those of BMI, whereas age, SBP, DBP and TG levels were significantly increasing with increasing levels of BMI (Table 4). TC, HDL-C, blood glucose and pulse pressure did not varied (ANOVA, p>0.05) between the three categories of the nutritional status (results not shown).

Using logistic regression and compared with DM type 2 (code=2), DM type 1 (code=1) was the only significant determinant of poor control of HbA1c (Y=0.908+0.389 DM types; SE=0.113, Wald=11.891, OR=1.5 95%CI 1.02-3.2; P=0.0006) independently for sex, cigarette smoking, nutritional status, DM duration, status control, total cholesterol and HDL-C.

Relationship between diabetic retinopathy and other variables

Not taking account insulin treatment, there was a significant association between age ≥60 years, female sex, DM duration ≥4 years, DM type 1, higher pulse pressure, underweight, poor control of HbA1c (>7%), cigarette smoking, stroke, DN, low HDL-C, and the presence of diabetic retinopathy (n=100), whereas the other associations were shown to be insignificant (Table 5).

Out of the 100 cases of DR, 87 cases (87%) had history of insulin treatment with lower (p<0.0001) levels of HbA1c (7.2 ± 1.4%) than those of 13 cases without a history of insulin treatment (9.5±1.4) but treated by oral hypoglycaemic agents. Thus, using logistic regression for a sub-group excluding DR cases without a history of insulin treatment in comparison with diabetics without DR, only a longer DM duration of ≥4 years (OR=3.2 95% 1.3-5.3;P<0.0001) and good control of HbA1c<7% (OR=1.4 95%CI 1.04-3.3; P=0.04) were significantly associated with the presence of DR after adjusting for all univariate risk factors of DR in the total study population. The 13 cases (13%) treated by oral hypoglycaemic agents were admitted with severe proliferative DR (PDR). The rest of DR cases (n=87) with a history of insulin treatment and non-proliferative DR had stages classified light for 23 patients (26.4%), moderate for 30 patients (34.5%), and severe for 34 patients (39.1%). Despite the increased rates of DR, poor control of HbA1c (n=14 for light, n=23 for moderate and n=34 for severe, P for trend=0.0001) and DM duration with the NPDR severity (3 ± 1 years for light, 4 ± 2 years for moderate, and 5 ± 2 years for severe; P for trend <0.0001), respectively, there was a J-shaped relationship between the rates of poor control HbA1c and those of NPDR (Fig. 1). All the 23 patients with moderate NPDR and poor control of HbA1c had good glycaemic control, whereas the 6 patients with moderate NPDR and poor glycaemic control had good control of HbA1c, and only 1 patient with moderate NPDR had both good control of HbA1c and good glycemic control.

Diabetes duration

Compared with DM duration of diabetics without disorders, those patients with poor control of HbA1c (5 ± 2 yrs vs. 3 ± 1 yr; p<0.0001), DR (4±2 yrs vs. 2±1 yrs; p<0.0001), stroke (5±3 yrs vs. 3±1yrs; p<0.0001), and DN (5±3 yrs vs. 3±1 yrs; p<0.0001), were significantly longer. The same situation was observed for DM type 1 vs. DM type 2 (5±3 yrs vs. 3±1 yr; p<0.0001). On the contrary, elevated TG (3±1 yr vs. 4±2; p<0.0001), low HDL-C (3±1 yrs vs. 4±2; p<0.0001), uncontrolled hypertension (3±1 yrs vs. 4±3; p=0.015), and overweight (3±1 yrs vs. 4±3 yrs; p<0.0001) had shorter DM duration than their counterparts without disorders. Moreover, DM duration of patients with a smoking habit (4±2 yrs vs. 4±3 yrs; p=0.153) and elevated TC (3±2 yrs vs. 3±2 yrs; p=0.886) were not different from those of their counterparts without disorders.

Lipid profile and other DM comorbidities

There was no significant association between sex, cigarette smoking, DN, DM types, stroke, DR, uncontrolled hypertension and lipid profile in almost situations, albeit increasing TC was associated with cigarette smoking (p<0.05) and increasing TG was associated with overweight (p<0.0001), stroke (p<0.01) and uncontrolled hypertension (p<0.01) (Table 6). However, HDL-C levels were negatively associated with DR (P<0.0001), but paradoxically were positively associated with overweight (p<0.0001).

Discussion

Diabetes care in the sub-Saharan Africa context

This study reports data from African diabetic patients without appropriate care, but with emerging high cardiovascular risk. Dramatically increased levels of arterial hypertension, uncontrolled hypertension, poor control of HbA1c, poor glycaemic control, emerging rates of DM chronic complications are an Africa-wide concern.[2-9]

Table 3: Mean values of HbA1c according to cardiovascular risk factors and DM chronic complications

Variable name comparisons	HbA1c mean ± SD %	P value
Sex		
Men vs. women	7.4 ± 1.4 vs. 8.7 ± 1.4	<0.0001
Age		
≥60 yrs vs. <60 yrs	7.3. ± 1.2 vs. 7.4 ± 1.5	0.517
DM duration ≥ 4 yrs vs. 4 yrs	8.9 ± 1.5 vs. 7.4 ± 1.3	<0.0001
DR (yes vs. no)	7.8 ± 1.5 vs. 7.2 ± 1.3	<0.0001
Stroke (yes vs. no)	7.4 ± 1.4 vs. 7.4 ± 1.4	0.974
DN (yes vs. no)	7.7 ± 1.6 vs. 7.4 ± 1.4	0.033
Smoking (yes vs. no)	7.8 ± 1.7 vs. 7.3 ± 1.3	0.005
Low HDL-C (yes vs. no)	7.3 ± 1.4 vs. 7.7 ± 1.4	0.023
Elevated TG (yes vs. no)	7.5 ± 1.4 vs. 7.5 ± 1.4	0.819
Elevated TC (yes vs. no)	7.7 ± 1.4 vs. 7.3 ± 1.4	0.042
Total obesity (yes vs. no)	7.2 ± 1.3 vs. 7.5 ± 1.5	0.030
DM type 2 vs. type 1	7.4 ± 1.3 vs. 7.5 ± 1.5	0.517
Hypertension (yes vs. no)	7.6 ± 1.3 vs. 7.5 ± 1.4	0.821
Hypertension control (yes vs. no)	7.2 ± 1.4 vs. 7.5 ± 1.4	0.100
Glycemic control no vs. yes	7.7 ± 1.5 vs. 7.4 ± 1.4	0.030

Table 4: Opposite dose-effect response relationship between HbA1c levels, certain cardiovascular risk factors, and increasing levels of BMI (nutritional status)

Nutritional status BMI kg/m²	DM duration years	HbA1c %	Age years	SBP mmHg	DBP mmHg	TG mg/dL
Underweight <18.5	5 ± 3	8.6 ± 1.6	49 ± 17	130.5 ± 18.1	75.5 ± 8.9	110.8 ± 65
Healthy weight 18.5-24.9	4 ± 2	7.4 ± 1.3	58 ± 16	139 ± 17.6	79.6 ± 9.6	157.9± 115.6
Overweight ≥25	3 ± 1	7.2 ± 1.3	61 ± 13	144.1 ± 20	82.6 ± 11	195.8 ± 92.7
ANOVA, P	<0.0001	0.038	<0.0001	<0.0001	<0.0001	<0.0001

Table 5: Significant relationships between the presence of diabetic retinopathy (DR) and certain variables

Variable name	Presence of DR (n=100) n (%)	Absence DR (n=200) n (%)	P value
Women/Men	80/20 (80)	68/132 (68)	<0.05
Age≥ 60 years	81 (81)	104 (52)	0.019
DM duration ≥ 4 years	63 (63)	46 (23)	<0.0001
DM type 1/type 2	94/6 (94)	56/144 (28)	<0.0001
Cigarette smoking	50(50)	10 (5)	<0.0001
Underweight	88(88)	48(24)	<0.0001
Poor control of HbA1c>7%	95(95)	109(54.5)	<0.01
Higher pulse pressure	90(90)	53 (26.5)	<0.0001
Low HDL-C	36(36)	24 (12)	<0.01
Stroke	65(65)	16 (8)	<0.0001
DN	56(56)	14 (7)	<0.0001

Table 6: Mean values of TC, HDL-C, and TG according to DM co-morbidities and complications

Variable name comparisons	TC mg/dL	HDL-C mg/dL	TG mg/dL
Sex			
Men	197.8±35.4	99 ± 75	158.1 ± 101
Women	190±36	94 ± 82	162.2 ± 90.4
	P=0.093	P=1.000	P=0.881
Smoking			
Yes	207.6±41.1	103 ± 98	179.1 ± 150
No	195.2±33.5	98 ± 79	154.1 ± 85.1
	P=0.028	P=0.892	P=0.119
DN			
Yes	201.3 ± 33.4	100 ± 87	175.1 ± 117.1
No	195.9 ± 36.1	99 ± 90	152.2 ± 93.1
	P=0.275	P=0.280	P=0.105
Total obesity			
Yes	199.5 ± 34.3	100 ± 93	195.8 ± 92.7
No	196 ± 36.4	68 ± 58	136.8 ± 99.5
	P=0.453	P<0.0001	P<0.0001
DM types			
Yes	195.5 ± 35.1	97 ± 70	161.8 ± 94.4
No	199.9 ± 35.5	99 ± 71	155.6 ± 106.8
	P=0.312	P=0.301	P=0.622
Stroke			
Yes	195 ± 35.3	94 ± 80	183.1 ± 128.2
No	198 ± 35.3	94 ± 77	147.6 ± 83.8
	P=0.504	P=1.000	P=0.009
DR			
Yes	202.1 ± 34.5	33 ± 30	148.9 ± 107
No	195.2 ± 35.7	101 ± 90	162.4 ± 97.2
	P=0.143	P<0.0001	P=0.317
Uncontrolled hypertension			
Yes	200.7 ± 32.7	102 ± 94	195.6 ± 148
No	197 ± 35.9	99 ± 71	149.9 ± 83.4
	P=0.505	P=500	P=0.004

The following factors may explain the inadequate diabetes care in SSA: lack of efficient health care system, preventive education programmes, diagnostic aids and drugs, trained health-care workers with competitive salary, community involvement, positive policy environment, and patient adherence, education, and empowerment.[24] These findings present an added challenge in these Congolese patients, who must compete for resources with malaria, tuberculosis, HIV/AIDS, underweight, and insulin treatment.[1,14] Furthermore, many Africans are unaware of their DM, attend the clinics very late or after a consultation with the traditional healers with high prevalence of DM complications.[13]

Thus it was timely to deal with glycated haemoglobin (HbA1c) in African diabetics as a useful measure of the efficacy of glucose-lowering therapy. HbA1c integrates the summary of circadian blood glucose during the preceding 2-3 months, equivalent to the lifespan of erythrocytes.[25] It is, therefore, recommended to be cautious in interpretating very low fasting glucose levels among anaemic diabetic Africans with poor control of HbA1c. A great proportion of blacks (2-30%) bear the haemoglobin S gene with shorter lifespan of erythrocytes.[26]

Risk factors of poor control of HbA1c

Female sex, longer DM duration, cigarette smoking, total hypercholesterolemia, elevated HDl-C, high blood glucose and underweight were identified as univariate risk factors of poor control of HbA1c. The decrease of oestrogens in female diabetics may explain their susceptibility to poor control of HbA1c. Smoking induces insulin resistance[27] and these cigarette smokers have elevated TC and DM as reported elsewhere.[28] In Africans, underweight is associated with inflammation (oxidative stress), arterial hypertension and insulin resistance or abdominal obesity.[3,29] Lower BMI<30 kg/m² was significantly and independently associated with poor control of HbA1c (OR:2.21; P=0.034) in type 2 diabetics from Tunisia.[30]

Although the rates of poor control of HbA1c in the African diabetics was similar for type 1 DM and type 2 DM in univariate analyses, the presence of poor control of HbA1c was only associated with type 1 DM (excess of multivariate risk of 20%) in independent manner with sex, smoking, nutritional status, DM duration, glycaemic status, total cholesterol and HDL-C. In West Africa, sex and age do not have significant effect on HbA1c levels, whereas correlation is very poor between BMI and HbA1c.[31] The longer

duration and fluctuations of insulin treatment in type 1 diabetics may explain their higher risk of poor control of HbA1c in comparison with type 2 diabetics. Indeed, the African type 2 diabetics with the best glycaemic control are on diet-only therapy because of possible insulin production.[32] Thus, poor control of HbA1c is presumed to be due to sub-optimal treatment with insulin or oral hypoglycaemic agents.

Consequences of poor control of HbA1c

There is evidence for a significant association of poor control of HbA1c and long-term microvascular complications in these African diabetics such as DR and DN. Literature reports have shown compelling evidence that diabetic microangiopathies can be reduced by tight glycaemic control.[15] However, the lack of significant association between poor control and stroke in this study confirms that the relation between macrovascular disease and hyperglycaemia is less clear than the relation to microvascular complications.[15]

DR was the most frequent chronic complication in this study. Its univariate risk factors included both non-modifiable and presentable factors which varied according to insulin treatment. This hospital-based survey reports a frequency of DR (33.3%) similar to the community-based DR prevalence of 31.6% reported recently in the same town.[8] The magnitude of DR in SSA is lower than the 75% with DR from US Caucasians[33] but within the interval of 15-52% of DR observed in developing countries of SSA[34,35] and Asia.[36,37] The increasing prevalence of DR in Africans treated with insulin (87%) may be explained by insulin therapy related immune vascularitis.[38]

When insulin treatment was not considered in univariate analyses for all diabetics patients, there was a significant correlation between female sex, aged ≥60 years, DM duration ≥ 4 years, DM type 1, higher pulse pressure (pre-clinical atherosclerosis), underweight, poor control of HbA1c (>7%), cigarette smoking, stroke, DN, low HDL-C and the presence of DR. The literature has reported varying results on the role of gender in the DR pathogenesis: male preponderance [38] or DR similarly common among males and females.[8,39] As already shown in our country,[40] DR, projected aging of Africans and vulnerability of females will amplify the burden of blindness.

The present findings have shown that DM duration, along with poor control of HbA1c (poor glycemic control), are the strongest determinant of the frequency of DR as reported elsewhere.[41] It is easy to understand the significant association of DR with preventable risk factors such as higher pulse pressure, underweight, stroke, DN, and low HDL-C related also to DM duration and well established associated with DR in our country[8,42] and elsewhere.[43,44] Increased levels of carbon monoxide and platelet anomalies explain the present significant association between cigarette smoking and DR.[45] Because smoking is a preventable (modifiable) risk factor, it could have enormous public health importance despite the lack of significant impact of smoking on DR prevalence in our previous study[8] and other studies[46] after controlling for confounders. DR and DN have common risk factors.[47]

Insulin treatment

Our findings clearly demonstrate that good control of HbA1c (pseudo-good control of blood glucose) and longer duration are independent risk factors of DR in patients with history of intravenous insulin treatment as reported in female diabetics and type 2 diabetics, albeit in type 1 diabetics in the same town [48] and in the literature.[49] High quantity of insulin administered intravenously as a challenge over a short period to Africans with severe and uncontrolled glycaemia,[50] rapid decrease of hyperglycaemia and following improved glycemic control,[51-54, 56, 57] insulin treatment-induced immunogenic vascularitis,[49] normoglycaemic re-entry,[55] inappropriate level of insulinemia for growth factor[55,58] and insulin-related hyperandrogenisation in type 2 diabetic women[59] may be incriminated. Type 2 diabetics using insulin in whom it is more difficult to achieve metabolic control usually present a significant decline in beta cell function.[60] Poor glycaemic control with oral agents and diet as well as delayed diagnosis of DM are usually the main reason to start insulin treatment and it is very unlikely that insulin per se could be implicated in the pathogenesis of DR. Indeed, in the UKPDS study,[15] the intensive glycaemic control obtained with insulin was associated with lower development of DR. Therefore, poorer glycaemic control prior to intravenously administration of insulin, anaemia (renal insufficiency) and longer duration of DM were probably the main reason for the association between DR and good control of HbA1c observed in this sub-group with history of insulin treatment and lower levels of HbA1c. In fact, percent agreement and kappa statistic between HbA1c levels and those of fasting blood glucose were poor in these diabetic Africans. The association of fasting plasma glucose, poor glycaemic control (≥126 mg/dL) with DR was not significant in the present study probably because of the remarkable effect of the presence of elevated frequency of DN itself (23.3%).

Lipid profile, shorter DM duration and inverse epidemiology

Ignorance, poverty, delayed diagnosis of DM, along with elevated TG, low HDL-C, total obesity, and uncontrolled hypertension may explain the shorter mean duration of DM (4±3 years) in this study in comparison with good control of DM and longer DM duration ≥ 20 years before DR incidence among diabetics from rich Western societies.[61] In these diabetic Africans, both rates of DR, DM duration and proportions of poor control of HbA1c are perfectly correlated in a dose-response manner with the severity of non proliferative DR as reported elsewhere.[8] However, the J-shaped relationship shown between NPDR severity and poor glycaemic control (≥126 mg/dL) is questionable as the majority with moderate NPDR had both poor control of HbA1c and good glycaemic control (<126 mg/dL). Studies of the utility of HbA1c compared with fasting plasma glucose (FPG) have used different assays, thereby making it difficult to assign an appropriate cut point. In addition, the

FPG and HbA1c tests are imperfectly correlated whereas HbA1c remains a valuable tool for monitoring glycaemia.[62]

The analysis of this study population showed that elevated total cholesterol correlated with smoking and higher levels of HbA1c. Low HDL-C correlated with the presence of DR. Elevated TG was associated with uncontrolled hypertension, overweight and stroke. As part of the metabolic syndrome, dyslipidaemia is already present in Africans with epidemiologic transition,[63] lack of hypertriglyceridemia, low values of TC and high levels of HDL-C in the general population.[10]

The paradoxical association between overweight, lower values of HbA1c and higher values of HDL-C is characteristic of inverse epidemiology being reported in sub-Saharan Africans with both physical activity, Westernization, traditional diet, cigarette smoking, and low prevalence of coronary heart disease. A positive association has been shown in African patients between total cholesterol, cardiovascular disease, and high values of HDL-C.[64-66] The significant relationship between shorter DM duration and low HDL-C may explain the association observed between low HDL-C and DR. The same significant relationship between shorter DM duration and elevated TG may explain also the association observed between elevated TG and stroke. Thus, dyslipidaemia will play a deleterious role on the chronic complications of DM among these ageing Africans with lifestyle changes (smoking, physical inactivity, high fat intake, high refined sugar intake).[67]

Limitations

This cross sectional study was limited to data available in hospital and not obtained prospectively. Comparisons cannot be made with community-based studies.

It is obvious that the majority of laboratories in SSA are unable to *perform* HbA1c *assays* routinely because of poverty and bad governance (no electricity, trained professionals, or freezers). Clinics for diabetic Africans should be helped to obtain equipment for HbA1c analysis.[68] African medical doctors have to be cautious [69,70] in interpreting HbA1c in situations of anaemia and shorter erythrocyte turnover with possible false negatives in sickle cell disease, thalassaemia and false positives in polyglobulinaemia and post-splenectomy syndrome. Interpretation of HbA1c levels in Africans needs quality control[71,72] with inclusion of a control blood sample into each run of samples. The measurements of HbA1c are accepted as a way of monitoring long-term glucose control during appropriate treatment of patients with DM.

Due to the resolution and sensitivity limits of electrophoresis used in this study, it is possible that some abnormal hemoglobins may interfere with this method. Fetal haemoglobin migrates between fractions A_{1a} + A_{1b} when its level is ≥1% in infants, pregnant women, sickle cell disease, beta-thalassaemia giving falsely elevated levels of HbA1c.[73]

Bimodal application point means that abnormal hemoglobins SO and CO migrate more anodically than AO.[73] In such cases, only the relative percent of HbA1c has been calculated relative to haemoglobin AO while eliminating the abnormal fractions in these diabetic patients. Falsely elevated levels of HbA1c due to continuous uptake of glucose by the red blood cells were avoided in this study by samples and kits properly stored. Low levels of triglycerides and bilirubin may interfere with HbA1c quantification (carbamylated or acetylated hemoglobins)[74,75] in these diabetic Africans.

Conclusions

Diabetes care in these African diabetic patients is not adequate as shown by very high rates of poor control of HbA1c, poor glycaemic control and DM chronic complications. Type 1 DM, longer DM duration ≥4 years, female sex, underweight, diabetic retinopathy, diabetic nephropathy, elevated total cholesterol, and higher levels of HDL-C are significantly associated with poor control of HbA1c. There is a poor agreement between poor control of HbA1c and poor glycemic control in the presence of DR. Levels of HbA1c are not influenced by age, stroke, elevated triglycerides, DM types, arterial hypertension and uncontrolled hypertension.

In all patients aged ≥ 60 years, female sex, DM duration ≥ 4 years, type 1 DM, higher pulse pressure, underweight, poor control of HbA1c, cigarette smoking, stroke, DN, low HDL-C are significantly associated with DR. DM duration ≥ 4 years and good control of HbA1c <7% are also significantly associated with DR.

Availability of insulin, correct diabetes care, as well as control of HbA1c, glycemia, dyslipidemia, hypertension, and underweight are urgently needed to slow the onset of diabetic retinopathy, diabetic nephropathy and stroke in African diabetics.

References

1. Beaglehole R, Yach D. Globalisation and the prevention and control of non-communicable disease: the neglected chronic diseases of adults. Lancet 2003; 362: 903-908.
2. Longo-Mbenza B, Vangu Ngoma D, Nahimana D et al.Enquête sur les facteurs de risque des maladies non transmissibles à Kinshasa, Capitale de la RD Congo. Selon l'approche STEPS de l'OMS. 2006.
3. Longo-Mbenza B. Vangu-Ngoma D. Nahimana D et al. Screen detection and the WHO Stepwise approach to the prevalence and risk factors of arterial hypertension in Kinshasa. Eur J Cardiovasc Prev Rehabil. 2008;15:503-508.
4. Mbanya JC, Sobngwi E. Diabetes microvascular and macrovascular disease in Africa. J Cardiovs Risk 2003; 10: 97-102.
5. Rolfe M. Chronic complications of diabetes in Africa. In Gill, Mbanya JC, Alberti KGMM (Eds). Diabetes in Africa. Cambridge: FSG Communications, 1997.

6. Longo-Mbenza B, Ngimbi RM, Ngoma DV, et al. Risk factors of stroke among Congolese black hypertensive diabetics. Ann Cardiol Angiol 2008;57: 37-43 .
7. Longo-Mbenza B, Bieleli E, Muls E, et al.The role of early hemodynamic impairment and disease duration on diabetic cardiomyopathy and hypertension in central Africans with atherosclerosis. J Diabetes Complicat 2002;16: 146-152.
8. Longo-Mbenza B, Nkondi-Mbadi-A-Sungu J, et al. Higher pulse pressure, systolic arterial hypertension, duration and family history of diabetes in Central Africans. Int J Diabetes & Metab 2008; 16: 17-23.
9. Kalk WJ, Joannou J, Ntsepo N, et al. Ethnic differences in the Clinical and Laboratory Associations with retinopathy in Adult onset diabetes: Studies in Patients of African, European and Indian Origins. J Int Med 1997; 241: 31-37.
10. Fezeu L, Balkau B, Kengne A, et al. Metabolic syndrome in a Sub-Saharan African setting: central obesity may be the key determinant. Atherosclerosis 2007;193:70-76.
11. Longo-Mbenza B, Luila El, Mbete P. Is hyperuricémie a risk factor of stroke and coronary heart disease among Africans? Int J Cardiol 1999; 71:17-22.
12. Isezuo SA. Is high density lipoprotein cholesterol useful in diagnosis of metabolic syndrome in native Africans with type 2 diabetes? Ethn Dis 2005; 15: 6-10.
13. Yudkin JS. Insulin for the World's Poorest Countries. Lancet 2000; 355:919-921.
14. IDF (International Diabetes Federation). Diabetes Atlas, 2nd ed, Brussels, IDF, 2003.
15. UKPDS. Intensive Blood-glucose Control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes: UKPDS 33. Lancet 1998; 352:837-853.
16. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications of insulin dependent diabetes mellitus. N Engl J Med 1993; 329:977-986.
17. UKPDS. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 Diabetes: UKPDS 38. BMJ 1998; 317: 703-712.
18. WHO. Obesity: Preventing and managing the global epidemic: Report of WHO consultation on obesity, 3-5 June 1997. Geneva: WHO, 2006.
19. Mayfield J. Diagnosis and classification of Diabetes Mellitus: New criteria. Am Fam Physician 1998; 58: 1-8.
20. Early treatment of diabetic retinopathy study research group. Grading diabetic retinopathy from stereoscopy colour fundus photographs: an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology 1991; 98: 786-806.
21. Guidelines Sub Committee.1999 World Health Organization. International Society Hypertension Guidelines for the management of hypertension. J Hypertens 1999; 17: 158-183.
22. Gross JL, De Azevedo MJ, Silveiro SP, et al. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care 2005; 28:164-176.
23. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome – a new worldwide definition. Lancet 2005; 366: 1059-1062.
24. Beran D, Yudkin JS. Diabetes care in sub-Saharan Africa. Lancet 2006; 368:1689-1695.
25. Pecorado RE, Chen MS, Porte D Jr. Glycosylated haemoglobin and fasting plasma glucose in the assessment of outpatient glycaemic control in NIDDM. Diabetes Care 1982; 5: 592-599.
26. Diallo D, Tchernia G. Sickle cell disease in Africa. Curr Opin Hematol 2002; 9: 111-116.
27. Atvalls, Fowellin J, Lager I et al. Smoking induces insulin resistance – a potential link with the insulin resistance syndrome. J Intern Med 1993; 233:327-332.
28. Zavaroni I, Bonini L, Gaspirino P et al. Cigarette smokers are relatively glucose intolerant, hyperinsulinemic and dyslipidemic. Am J Cardiovasc 1994; 73:904-905.
29. Kasiam Lasi On'kin JB, Longo-Mbenza B, Nge Okwe A, et al. Paradoxical relationship between high density lipoprotein cholesterol and metabolic syndrome in Africans with type 2 diabetes mellitus. Diabetes & Metabolism. In Press
30. Ben Abdelaziz A, Soltane I, Gaha K, et al. Predictive factors of glycemic control in type 2 diabetes mellitus patients in primary health care.Rev Epidemiol Sante Publique 2006;54: 443-452.
31. Adebisi SA, Oghagbon EK, Jimoh AK. Glycated haemoglobin and associated variables in diabetics: Ilorin experience. West Afr J Med 2003; 22: 318-320.
32. Otieno CF, Kariuki M, Ng'ang'a L. Quality of glycaemic control in ambulatory diabetics at the out-patient clinic of Kenyatta National Hospital, Nairobi. East Afr Med J 2003; 80:406-410.
33. The Eye Diseases Prevalence Research Group. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol 2004; 122: 552-563.
34. Sidibe EH. Diabetic retinopathy in Dakar and review of African literature: Epidemiologic elements. Diabetes Metab 2000; 26: 322-324.
35. Rotchford AP, Rotchford KM. Diabetes in rural South Africa-an assessment of care and complications. JEMDSA 2002; 7: 60-65.
36. Rema M, Premkumar S, Anitha B, et al. Prevalence of diabetic retinopathy in urban India: The Chennai Urban Rural Epidemiology Study (URES) Eye Study. Invest Ophtalmol Vis Sci 2005; 46:2328-2333.
37. Jamal-U-Din, Qureshi MB, Khan AJ, et al. Prevalence of diabetic retinopathy among individuals screened positive for diabetes in five community – based eye camps in northern Karachi, Pakistan. J Ayub Med coll Abbottabad 2006; 18: 40-43.

38. Nagi DK, Pettitt DJ, Benneth PH, et al. Diabetic retinopathy assessment by fundus photograph in Pima Indians with impaired glucose tolerance and NIDDM. Diabet Med 1997; 14: 449-456.
39. Farhan H, Osama AM, Nasif A, et al. The prevalence of diabetic retinopathy and associated risk factors in type 2 diabetes mellitus in Al-Naeen (Kuwait). Middle East J Fam Med 2005; 3:1-4.
40. Mvitu M, Kimenyembo W, Kaimbo Wa Kaimbo D, et al. Frequency of visual impairment and blindness in Congolese people with diabetes. Diabet Med 2006; 23 (Suppl 4): 608-653.
41. Cai XL, Wang F, JI LN. Risk factors of diabetic retinopathy in type 2 diabetic patients. Chin Med J 2006; 119: 822-826.
42. Nkondi-Mbadi A, Nsungu J, Longo-Mbenza B, bungu-Fuele S, et al. Relation entre pression pulsée, troubles visuels et sévérité de la rétinopathie diabétique en Afrique sub-saharienne. J d'Ophtalmologie Française. In Press
43. Rema M, Pradeepa R. Diabetic retinopathy: An Indian perspective. Indian J Med Res 2007; 125:310.
44. Erasmus RJI, Alananu Ra, Bojuwoye B, et al. Diabetic retinopathy in Nigerians: relation to duration of diabetes, type of treatment control. East Afr Med J 1989; 66: 248-254.
45. Nielsen MM, Hjollund E. Smoking and diabetic microangiopathie. Lancet 1978; 2:533-537.
46. Klein R, Klein BE, Davis MD. Is cigarette smoking associated with diabetic retinopathy 1983; 118:228-238.
47. Araki A, Ito H, Hattori A et al. Risk factors for development of retinopathy in elderly Japanese patients with diabetes mellitus. Diabetes Care 1993; 16: 1184-1186.
48. Nkondi-Mbadi A, Nsungu J. Prevalence and risk factors of diabetic retinopathy in Kinshasa. MD Thesis, University of Kinshasa, 2005.
49. Kollarits CR, Kiess RD, Das A, et al. Diabetic retinopathy and insulin therapy in a rural diabetic population. Am J Ophtalmol 1984; 97: 709-714.
50. Gill G, Mbanya JC, Alberti KGGM. Diabetes in Africa. Cambridge: Biddles Ltd, 1997; 294.
51. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. New Engl J Med 1993; 329:977-986.
52. The KROC Collaborative Study Group. Blood glucose control and evolution of diabetic retinopathy and albuminuria. New Engl J Med 1984; 311: 365-368.
53. Henricsson M, Nilsson A, Janzon L, Groop L. The effect of glycaemic control and the introduction of insulin therapy on retinopathy in non insulin dependent diabetes mellitus. Diabet Med 1997; 14: 123-131.
54. Engerman R, Bloodworth JM, Nelson S. Relationship of microvascular disease in diabetes to metabolic control.Diabetes 1977; 26:760-769.
55. Keen H. Normoglycaemic re-entry and diabetic complications. Diabet Med 1984; 1: 85-87.
56. Chantelau E, Eggert H. Acceleration of diabetic retinopathy following improved glycaemic control: a report on 13 cases. Diabetologia 1997; 40: A501.
57. Lauritzen T, Larsen HW, Frost-Larsen K, Deckert T, and the Steno Study group. Effect of one year normal blood glucose levels on retinopathy in insulin dependent diabetes. Lancet 1983;1:200-204.
58. Paques M, Massin P, Gaudric A. Growth factors and diabetic retinopathy. Diabetes Metab 1997;23: 125-130.
59. Tron'ko MD, Khalanhot MD, Kravchenko VI, et al. Gender-related risk of non-fatal stroke, myocardial infarction and blindness in the type 2 diabetic patients depend on the type of treatment. Lik Sprava 2006; (1-2):23-27.
60. UKPDS Overview of 6 years therapy of type II diabetes: A progressive disease. Diabetes 1995; 44:1249-1258.
61. Murray McGavin DD. Diabetes and diabetic retinopathy. Comm Eye Health 1996; 9:49-50.
62. The expert committee on the diagnosis and classification of diabetes mellitus. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997; 20: 1183-1197.
63. Omran AR. The epidemiologic transition: a theory of the epidemiology of population change. The Milbank Memorial Fund Quarterly 1971; 49: 509-538.
64. Lepira FB, M'Buyamba Kabangu JR, Kayembe KP, Nseka MN. Correlates of serum lipids and lipoproteins in Congolese patients with arterial hypertension. Cardiovasc J South Afr 2005;16: 249-255.
65. Seedat YK, Mayet FGH, Latiff GH, Joubert G. Study of risk factors leading to coronary heart disease in urban Zulus. J Hum Hypertens 1993; 7:529-532.
66. Grinbaume A, Rosenthal T, Peleg E, Segal P. Ischemic heart disease risk factors in young black Africans from urban Zaire. J Hum Hypertens 1993; 7:555-557.
67. Murray CJL, Lopez AD.Global mortality, disability and the contribution of risk factors: Global Burden of Disease Study. Lancet 1997; 349: 1436-1442.
68. Rahlenbeck SI. Monitoring diabetic control in developing countries: a review of glycated haemoglobin and fructosamine assays. Trop Doct 1998; 28: 9-15.
69. Eaton SE, Felden SE, Huisman P. Glycated haemoglobin (HbA_{1c}) measurements in subjects with haemoglobin variants using the DCA 2000. Ann Clin Biochem 1997; 34: 205-207.
70. Weykamp CW, Penders TJ, Muskiet FA, Van Der Silk W. Glycohaemoglobin: a comparison of 12 analytical methods applied to lyophilized haemolysates by 101

laboratories in an external quality assurance program. Ann Clin Biochem 1993; 30: 169-174.
71. Jain M, McCall A, Riddle M. Standardization of hemoglobin A_{1c} values. Ann Intern Med 2000; 18: 676.
72. Marshall CL. Problems arising from the lack of a standardized method of performing hemoglobin A_{1c}. Am J Med Qual 2000; 15: 196.
73. Sebia. HYDRAGEL 7 & 15 HbA1c.Sebia, Evry, France, 2006.
74. Mayer TK, Freedman ZR. Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their clinical utility (critical review). Clin Chim Acta 1983;127:147-184.
75. Fluckiger R Harmon W, Meier W, et al. Hemoglobin carbamylation in uremia. N Engl J Med 1981; 304: 823-827.