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# Neck Circumference as an Independent Cardiometabolic Risk Factor: A Cross-sectional Study in Kinshasa

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**Abstract:** Objective: To investigate the association between neck circumference (NC) and traditional cardiometabolic risk factors (CMRF) among adult's population at Kinshasa. Methods: A total of 400 participants were recruited. Spearman's correlation coefficient was employed to test the correlations between NC and CMRF. The association of NC with CMRF (dependent variables) was assessed by logistic regression. The receiver operating characteristics (ROC) curve analysis had allowed determining the cut-off points of NC to detect the presence of CMRF. Results: The average of age and WC was 55.4±12.0 years and 79.8±12.0 cm, respectively. The median value of BMI was significantly higher in women (24.6 kg/m²) than in men (22.6 kg/m²); whereas the median value of NC was significantly higher in men (37.8 cm) than in women (33.3 cm) (p < 0.001). In both men and women, NC was positively correlated with TC, LDL, TG and WC. Additionally, FPG and HDL were positively correlated with NC significantly. Moreover, there was a significant positive correlation between NECK and FPG but a significant negative correlation between NECK and HDLc among women. TG in men, raised TC, LDL and WC were found to be significantly associated with neck circumference with ORs 1.25 (95% CI: 1.08, 1.44), 0.67 (95% CI: 0.53, 0.85), 1.13 (95% CI: 1.02, 1.26), 1.27 (95% CI: 1.12, 1.45) in men versus 1.18 (95% CI: 1.03, 1.36) in women, 1.19 (95% CI: 1.06, 1.34) in men versus 1.21 (95% CI: 1.06, 1.38) in women and 1.18 (95% CI: 1.06, 1.36) in men versus 1.43 (95% CI: 1.23, 1.66) in women, respectively. Cut-off points for NC to identify CMRF were between 37.5 and 38 cm in men, 32.5 and 33 cm in women. Conclusion: NC is associated with CMRF, and could be a useful and accurate tool to identify high risk participants.

Keywords: Neck Circumference, Cardiometabolic Risk, Kinshasa

#### 1. Introduction

Cardiovascular disease (CVD) is the leading cause of disability adjusted life years worldwide, particularly in developing countries [1]. The global cardiovascular risk is the probability of suffering from a coronary event or stroke in a given period of time and in this sense it is an absolute risk, generally reported as percentage at 10 years. Usually risk

functions are used derived from longitudinal studies of healthy people at baseline [2]. They consider some factors that are coherently linked with events in population analyses: among these there are some metabolic factors (total cholesterol, HDL cholesterol, fasting blood glucose), some biological factors (blood pressure) and some lifestyle factors (tobacco smoking), all modifiable beyond those non-modifiable like age and gender. Upper-body subcutaneous

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adipose tissue, estimated by neck circumference (NC), is a unique fat depot that may confer additional risk for metabolic risk factors over generalized and central adiposity [3-6]. NC as an index for upper-body subcutaneous adipose tissue distribution has been evaluated in relation to cardiovascular risk factors, insulin resistance, and biochemical components of metabolic syndrome (MetS) [7-10]. However, epidemiological population-based studies on the clinical significance of NC in connection with CMRF in general people are lacking. The aim of this study was to investigate the association between NC and same traditional CMRF.

#### 2. Patients and Methods

#### 2.1. Study Population

This cross-sectional study was made in general and eligible population of Kinshasa. Data were collected between November and December 2019 in Congolese participants aged 20 to 80 years, who had lived in the community over 10 years. Participants who met any of the following criteria were excluded from the study: abdominal disease that might affect the distribution of fat; a known Diabetes mellitus (DM); goiter; history of using cortical steroids; current treatment with statins or glucocorticoid; Cushing's syndrome; and recent, substantial weight loss or weight gain.

#### 2.2. Sample Size

The minimal random sample size was calculated using the following formula:

$$n = \frac{(\mathbf{Z})^2 x (p)(q)}{(\mathbf{d})^2}$$

Z=parameter related to statistical risk of admitted error=1.96 for a 5% error risk

q=assumed proportion of the target population not having the problem (q=1 - p)

p=expected prevalence for MetS from a recent known prevalence of absolute accuracy=11% in Kinshasa Hiterland [11]. d=absolute accuracy=5%

$$n = \frac{(1.96)^2 x (0.11)(0.89)}{(0.05)^2} = 150$$

We add 25% possible loss which makes a minimal of 188 participants. A total of 400 participants were analyzed in this study.

#### 2.3. Ethical Consideration

The study design protocol was approved by the Ethics Committee of Lomo University of Research. Written informed consents were obtained from all patients. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

#### 2.4. Clinical and Anthropometric Evaluation

Waist circumference (WC) was measured using flexible

tape between the highest lateral edge of the right and left Ilium. NC was measured in the middle of the neck between the mid-cervical spine and the mid-anterior neck at 0.5 cm, so palpable, just below the laryngeal prominence. BMI was calculated as the weight in kilograms divided by the height in meters squared. WHR and WHtR were calculated as waist circumference divided by hip circumference and height, respectively. Blood pressure (BP) was measured 3 times in a sitting position after at least 15 minutes of rest using an electronic type blood pressure monitor (OMRON M3 IT). The average of 3 recorded systolic and diastolic BP values was used in the analysis.

#### 2.5. Biochemical Measurements

Peripheral venous blood samples were drawn after an overnight fast of at least 8 h. The blood samples for the plasma glucose test were collected into vacuum tubes with the anticoagulant sodium fluoride and centrifuged within 1 h after collection. Plasma fasting concentrations of Glucose (FPG), Total Cholesterol (TC), Triglycerides (TG), highdensity lipoprotein cholesterol (HDL) and Uric acid were measured using the standard procedure using a COBAS C111 France). Insulin was detected chemiluminescence method (Abbott i2000 SR, USA). Then, insulin resistance was estimated by the homeostatic model assessment (HOMA-IR) index: [FI (mIU/L) × FPG (mmol/L)]/22.5 [12].

#### 2.6. Definitions of Variables

High blood pressure (BP) was defined as BP 130/85 mm Hg or use of antihypertensive medication; low plasma high-density lipoprotein cholesterol (HDL-C) cholesterol as HDL-C  $<1.0\,$  mmol/L in men and HDL-C  $<1.3\,$  mmol/L in women. Raised plasma triglycerides (TG) was defined as TG  $<1.7\,$  mmol/L and raised fasting plasma glucose (FPG) as FG  $>5.6\,$  mmol/L1. Increased waist circumference was defined as WC  $>81\,$  cm in both males and females, raised total plasma cholesterol (TC) as TC  $>5.0\,$  mmol/L; and raised plasma low-density lipoprotein cholesterol (LDL-C) as LDL-C  $>3.0\,$  mmol/L.

#### 2.7. Statistical Analyzes

Data analyses were performed with the software package SPSS Statistics, Version 21 (IBM Corporation, Armonk, NY, USA). Normally distributed data were expressed as the means±SD, whereas continuous variables with a skewed distribution were summarized as the median with interquartiles range. To compare the differences between groups, one-way analysis of variance (ANOVA) was used for continuous variables with a Gaussian distribution, and the Mann-Whitney U test was used for variables with a skewed distribution. Spearman's correlation coefficient was employed to test the correlations between different variables. The association of NC (independent variables) with cardiometabolic risk factors (dependent variables) was assessed by logistic regression. The receiver operating

characteristics (ROC) curve analysis had allowed determining the optimal threshold of NC to detect the presence of cardiometabolic risk factors. A P value < 0.05 was considered statistically significant.

#### 3. Results

#### 3.1. General Characteristics of Participants

Table 1 shows the general characteristics of study population stratified by sex. Our survey included 400 participants (200 men and 200 women) with the mean age of

55.4 $\pm$ 12.0 years and the mean WC of 79.8 $\pm$ 12.0 cm. The median value of BMI was significantly higher in women (24.6 kg/m²) than in men (22.6 kg/m²) (p=0.002), whereas the median value of NC was significantly higher in men (37.8 cm) than in women (33.3 cm) (p < 0.001). WHtR, TC, LDL and TG were significantly higher in women. The majority of participants (83.8%) had low HDL. Increased WC, high BP, raised FPG, TC, TG and LDL were observed respectively at 37.3%, 34%, 62.8%, 21.3%, 37.5% and 31.3% in study population.

Table 1. General characteristics of study population by sex.

Variable	All (n=400)	Men (n=200)	Women (n=200)	p
Age, year	55.4±12.0	55.9±12.2	55.0±11.9	0.334
BMI, kg/m <sup>2</sup>	23.4(19.6 - 27.0)	22.6(19.3 - 26.0)	24.6(21-28)	0.002
WC, cm	79.8±12.0	78.9±11.5	80.8±12.4	0.131
NC, cm	36.0(33.1 - 38.1)	37.8 (36.3 – 39.4)	33.3 (31.9 – 34.6)	> 0.001
WHtR	0.48 (0.42 - 0.56)	0.47(0.41 - 0.54)	0.48(0.44 - 0.57)	0.026
SBP, mmHg	128.6±28.1	129.3±28.1	127.9±28.3	0.440
DBP, mmHg	73.6±13.8	73.8±13.5	73.3±14.2	0.999
FPG, mmol/L	6.3(5.1 - 8.1)	6.2(5.0-9.0)	6.2(5.4 - 8.0)	0.702
HbA1c, %	6.6(4.0-11.4)	6.5(4.0-11.0)	6.8(4.0-12.0)	0.834
HOMA-IR	4.95 (3.03 – 9.12)	4.41(2.67 - 8.70)	5.33(3.15-9.50)	0.070
TC, mmol/L	3.5(3.1-4.8)	3.4(2.8-4.2)	3.7(3.2-5.2)	0.001
HDL, mmol/L	0.52(0.31 - 0.88)	0.53(0.31-0.91)	0.48(0.31-0.83)	0.398
LDL, mmol/L	2.3(1.5-3.3)	2.2(1.2-3.1)	2.5(1.7-4.0)	0.004
TG, mmol/L	1.4(0.9-2.0)	1.01 (0.82 - 1.88)	1.60(0.95 - 2.11)	> 0.001
TG/HDL	2.7(1.1-5.7)	2.4(1.0-4.9)	3.1(1.6-6.4)	0.009
BP > 130/85 mmHg	136 (34.0)	70 (17.5)	66 (16.5)	0.673
FPG > 5.6 mmol/L	251 (62.8)	128 (32)	123 (30.8)	0.605
TC > 5.0  mmol/L	85 (21.3)	32 (8)	53 (13.3)	0.009
Low HDL mmol/L	335 (83.8)	161 (40.3)	174 (43.5)	0.078
LDL > 3 mmol/L	125 (31.3)	52 (13.0)	73 (18.3)	0.023
TG > 1.7 mmol/L	150 (37.5)	61 (15.3)	89 (22.3)	0.004
WC > 81 cm	149 (37.3)	68 (17.0)	81 (20.3)	0.179

### 3.2. Correlation Between NC and Cardiometabolic Risk Factors

Table 2 demonstrates the correlation between NC with same cardiometabolic risk factors. In both men and women, NC was positively correlated with TC, LDL, TG and WC. Additionally, in women NC was positively correlated with FPG and negatively with HDL significantly. Compared with WC and WHtR, those correlations were advanced, excepted TC and LDL in women.

Table 2. Correlation analysis between NC, WC and WHtR with CMRF factors by sex.

	NC	WC	WHtR
Men (n=200)			
SBP	0.018	$0.209^{b}$	0.182 <sup>a</sup>
DBP	-0.038	0.040	0.104
FPG	0.115	$0.192^{b}$	0.163 <sup>a</sup>
TC	0.291 <sup>b</sup>	$0.249^{b}$	0.166 <sup>a</sup>
HDL	-0.012	-0.038	-0.079
LDL	0.242 <sup>b</sup>	0.221 <sup>b</sup>	0.175 <sup>a</sup>
TG	0.165 <sup>a</sup>	0.127	0.061
Women (n=200)			

	NC	WC	WHtR
SBP	0.137	$0.260^{b}$	0.127
DBP	-0.049	0.148 <sup>a</sup>	0.060
FPG	0.295 <sup>b</sup>	$0.189^{b}$	0.135
TC	0.165 <sup>a</sup>	0.201 <sup>b</sup>	0.069
HDL	-0.231 <sup>b</sup>	-0.141 <sup>a</sup>	-0.071
LDL	0.198 <sup>b</sup>	$0.229^{b}$	0.090
TG	0.244 <sup>b</sup>	0.080	0.014

a: p<0.05, b: p<0.01.

### 3.3. Association Between NC with Cardiometabolic Risk Factors

Hyperglycemia and low HDL in women, TG in men, raised TC, LDL and WC were found to be significantly associated with neck circumference from logistic regression analysis with ORs 1.25 (95% CI: 1.08, 1.44), 0.67 (95% CI: 0.53, 0.85), 1.13 (95% CI: 1.02, 1.26), 1.27 (95% CI: 1.12, 1.45) in men versus 1.18 (95% CI: 1.03, 1.36) in women, 1.19 (95% CI: 1.06, 1.34) in men versus 1.21 (95% CI: 1.06, 1.38) in women and 1.18 (95% CI: 1.06, 1.36) in men versus 1.43 (95% CI: 1.23, 1.66) in women, respectively (see Table 3, Figure 1, Figure 2).

**Table 3.** Regression analysis to identify cardiometabolic risk factors associated with neck circumference (continuous independent variable) compared between men and women.

D 1 4W 111	NC (1 (2 )	Men		Women	Women		
Dependent Variables	NC (tertiles)	OR (95% IC)	р	OR (95% IC)	р		
	T1	1	-	1	-		
DD > 120/95	T2	2.06(1.00-4.25)	0.050	1.38(0.65-2.93)	0.401		
BP > 130/85 mmHg	T3	1.28(0.61-2.69)	0.521	1.86(0.89 - 3.88)	0.099		
	All	1.04(0.94 - 1.16)	0.450	1.13(0.99 - 1.30)	0.064		
	T1	1	-	1	-		
FPG > 5.6 mmol/L	T2	0.83(0.41-1.68)	0.598	2.09(1.04-4.20)	0.038		
FPG > 5.6 mmol/L	T3	0.92(0.45-1.88)	0.812	3.24(1.57 - 6.70)	0.002		
	All	1.03(0.93-1.15)	0.533	1.25(1.08 - 1.44)	0.002		
	T1	1	-	1	-		
TO > 5	T2	1.83(0.58 - 5.79)	0.303	2.02(0.85-4.79)	0.110		
TC > 5  mmol/L	T3	4.41(1.53 - 12.73)	0.006	3.27(1.42 - 7.55)	0.005		
	All	1.27(1.12 - 1.45)	< 0.001	1.18(1.03 - 1.36)	0.021		
	T1	1	-	1	-		
I IIDI	T2	1.77(0.76 - 4.11)	0.186	0.24 (0.09 - 0.64)	0.005		
Low HDL	T3	0.86(0.34 - 2.19)	0.754	0.04(0.01-0.28)	0.001		
	All	0.95(0.83 - 1.08)	0.432	0.67(0.53 - 0.85)	0.001		
	T1	1	-	1	-		
IDI > 2	T2	1.98(0.84 - 4.69)	0.121	1.86(0.87 - 3.99)	0.111		
LDL > 3  mmol/L	T3	3.07(1.33 - 7.10)	0.009	3.33(1.58 - 7.04)	0.002		
	All	1.19(1.06 - 1.34)	0.003	1.21(1.06 - 1.38)	0.005		
	T1	1	-	1	-		
TG > 1.7 mmol/L	T2	1.18(0.53 - 2.52)	0.675	1.79(0.89 - 3.61)	0.105		
1G > 1./ mmol/L	T3	1.58(0.75 - 3.32)	0.323	2.07(1.03 - 4.18)	0.041		
	All	1.13(1.02-1.26)	0.024	1.14(1.00-1.29)	0.050		
	T1	1	-	1	-		
WC > 81 cm	T2	1.89(0.84 - 3.90)	0.125	3.80(1.73 - 8.37)	0.001		
	T3	2.54(1.20 - 5.40)	0.015	5.60 (2.54 – 12.31)	< 0.001		
	All	1.18(1.06 - 1.32)	0.003	1.43(1.23 - 1.66)	> 0.001		

T1 < 36.5 cm, T2 36.5 - 39 cm, T3 > 39 cm in men; T1 < 32 cm, T2 32 - 34 cm, T3 > 34 cm in women; Low HDL: < 1 mmol/L in men, < 1.3 mmol/L in women.

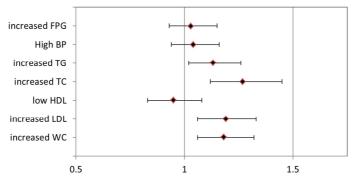


Figure 1. Odds ratio for increased FPG, high BP, raised TG, TC, LDL, WC and low HDL by neck circumference in men.

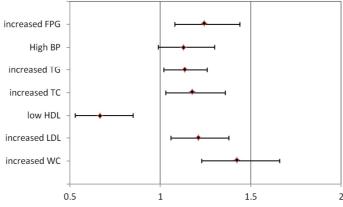


Figure 2. Odds ratio for increased FPG, high BP, raised TG, TC, LDL, WC and low HDL by neck circumference in women.

#### 3.4. NC Cut-off Values for Cardiometabolic Risk Factors

We determined the NC cut-off levels by relating them to risk factors. Tables 4 and 5 show the sensitivity and specificity for each NC level for the detection of raised blood

pressure, hyperglycemia and dyslipidemia in men and women. Cut-off points for NC where sensitivity approximates specificity for each risk factor are between 37.5 and 38 cm in men, between 32.5 and 33 cm in women.

Table 4. Sensitivity and specificity for neck circumference cut-off points for cardiometabolic risk factors in men.

NC cut-off (cm)	BP > 130/85	BP > 130/85 mmHg		FPG > 5.6 mmol/L		TG > 1.7 mmol/L		TC > 5.0 mmol/L	
	Se (%)	Sp (%)	Se (%)	Sp (%)	Se (%)	Sp (%)	Se (%)	Sp (%)	
36	88.6	13.8	83.6	6.9	88.5	13.7	96.9	14.9	
36.5	74.3	38.5	64.8	31.9	72.1	36.7	84.4	37.5	
37	71.4	42.3	61.7	36.1	68.9	40.3	81.3	41.1	
37.5	60.0	46.2	57.0	45.8	68.9	49.6	78.1	48.2	
37.7	58.6	51.5	54.4	52.8	65.6	54.0	78.1	53.8	
38	54.3	55.4	52.3	59.7	59.0	41.0	68.8	56.0	
38.5	38.6	58.5	43.8	66.7	47.5	63.3	62.5	64.3	
39	22.9	70.0	27.3	72.2	29.5	73.4	46.9	76.2	
39.5	20.0	76.9	23.4	80.6	27.9	80.6	43.8	82.1	
40	15.7	81.5	18.8	84.7	26.2	86.3	34.4	85.7	
41	14.3	90.8	12.5	91.7	18.0	92.1	21.9	91.1	
AUC	0.526		0.511		0.585		0.695		
(95% IC)	(0.443 - 0.610)		(0.429 - 0.3)	(0.429 - 0.592)		(0.497 - 0.674)		(0.600 - 0.791)	
p value	0.542		0.796		0.055		> 0.001		

Table 4. Continued.

NC cut-off (cm)	HDL > 1 mmo	HDL > 1 mmol/L		LDL > 3 mmol/L		
	Se (%)	Sp (%)	Se (%)	Sp (%)	Se (%)	Sp (%)
36	84.5	2.6	96.2	16.2	95.6	17.4
36.5	65.2	30.8	80.8	39.2	77.9	40.2
37	62.1	35.9	78.8	43.2	76.5	44.7
37.5	57.8	51.3	75.0	50.7	72.1	52.3
37.7	55.3	61.5	73.1	55.4	67.6	56.1
38	51.6	66.7	67.3	58.8	63.2	59.8
38.5	44.1	74.4	55.8	64.9	51.5	65.2
39	28.0	74.4	38.5	76.4	36.8	77.3
39.5	23.6	84.6	32.7	81.8	30.9	82.6
40	18.6	87.2	25.0	85.1	27.9	87.9
41	12.4	94.9	13.5	89.9	16.2	90.9
AUC	0.529		0.652		0.635	
(95% IC)	(0.440 - 0.617)		(0.569 - 0.734)	(0.569 - 0.734)		5)
p value	0.577		0.001		0.002	

 Table 5. Sensitivity and specificity for neck circumference cut-off points for cardiometabolic risk factors in women.

NC cut-off (cm)	BP > 130/85 m	BP > 130/85 mmHg		FPG > 5.6 mmol/L		ol/L
	Se (%)	Sp (%)	Se (%)	Sp (%)	Se (%)	Sp (%)
31	93.9	6.1	94.3	7.8	95.5	8.1
31.5	86.4	20.9	87.0	28.6	87.6	24.3
32	78.8	33.6	78.0	41.6	77.5	35.1
32.5	69.7	42.5	69.1	50.6	71.9	47.7
33	59.1	47.8	61.8	57.1	66.3	55.0
33.5	45.5	61.2	48.8	71.4	49.4	66.7
34	40.9	69.4	40.7	76.6	39.3	70.3
34.5	33.3	76.1	32.5	81.8	32.6	77.5
35	30.3	82.1	26.8	85.7	25.8	81.1
36	19.7	88.1	17.9	89.6	15.7	86.5
AUC	0.572		0.636	0.636		
(95% IC)	(0.488 - 0.657)		(0.557 - 0.71)	(0.557 - 0.715)		9)
p value	0.097		0.001	0.001		

WC > 81 cm TC > 5.0 mmol/LHDL > 1.3 mmol/L LDL > 3 mmol/LNC cut-off (cm) Se (%) Sp (%) Se (%) Sp (%) Se (%) Sp (%) Se (%) Sp (%) 31 98.1 98.6 8.2 93.1 3.8 9.4 98.8 11.8 31.5 96.2 24.0 82.8 30.8 93.2 25.2 93.8 27.7 84.9 34.9 32 75.9 65.4 82.2 37.0 86.4 40.3 32.5 79.2 45.2 67.2 80.8 78.1 48.0 82.7 52.9 33 73.6 52.7 60.3 84.6 69.9 54.3 74.1 58.8 33.5 54.7 65.1 46.0 92.3 54.8 66.9 54.3 68 1 71.2 47.2 38 5 96.2 73.2 469 748 34 46.6 34.5 34.0 75.3 31.0 100 34.2 77.2 39.5 81.5 79.5 27.4 87.4 35 26.4 25.3 100 81.1 35.8 36 15.1 85.6 100 85.8 24.7 91.6 16.7 15.1 **AUC** 0.639 0.732 0.644 0.706 (95% IC) (0.556 - 0.716)(0.672 - 0.827)(0.568 - 0.721)(0.634 - 0.777)0.003 > 0.001 0.001 > 0.001 p value

Table 5. Continued.

#### 4. Discussion

Our study sought the optimal cut-off point of NC as indicator of CMRF in a large, representative sample of the Kinshasa adult population. The optimal cut-off point for WC was found to be 38 cm in men and 33 cm in women for identifying CMFR, and we suggest that these values could be used as the appropriate detection of individuals at high cardiometabolic risk in Kinshasa until long-term mortality data become available.

Similar to previously published data, we observed associations between traditional CV risk factors and increasing NC [10, 13-15]. Participants with largest NC had significantly higher waist circumference. Therefore, NC may be an important part of the routine risk assessment as it is associated with cardiometabolic risk factors and less intrusive to measure than waist circumference.

The limitation of our study is that the data are based on a cross-sectional survey that did not address the issue of NC and morbidity/mortality. Until long-term prospective studies are undertaken in various ethnic groups, cut-off points will have to be judged on studies such as ours.

The strength of the study lies in the fact that it is a large population-based study and representative of Kinshasa's adults. We describe, for the first time in Sub-Saharan region, the cross-sectional relationship of NC with cardiometabolic risk factors.

#### 5. Conclusion

Our study suggests that a NC of 38 cm in men and 33 cm in women represents more appropriate cut-off points may potentially be beneficial in correctly identifying individuals at high cardiometabolic risk. Further research, particularly long-term, prospective, mortality studies, is urgently needed in Central Africa region to validate our findings.

## 6. State of Current Knowledge on the Subject

- 1. Cardiometabolic syndrome is a disease that is difficult to treat and diagnose;
- 2. Cardiometabolic syndrome is the basis of high morbidity and mortality;
- 3. Cardiometabolic syndrome is diagnosed at a late stage with a complication (obesity, hypertension, diabete millitus).

## 7. Contribution of Our Study to Knowledge

- 1. Poor knowledge of Cardiometabolic syndrome by healthcare providers in the DRC;
- 2. Poor diagnosis of disease to know its extent in the Congolese environment;
- 3. The cardiometabolic syndrome and the factors associated, previously unknown, with this work, currently available in DRC.
- 4. These results allow, in the clinical practice of healthcare providers, to improve the management of patients with Cardiometabolic syndrome. They also help to improve the monitoring of patients with Cardiometabolic syndrome.

#### **Conflict of Interest**

The authors declare no conflict of interest.

#### **Author's Contributions**

DMM and ANN designed and analyzed the statistical data for the study. M, VN and GLL contributed to the data collection. BLM, JBKL, EM supervised the study. All authors have read and approved the final and revised version of the manuscript.

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