Lipoprotein (a) and atherogenic indices in Sudanese patients with type 2 diabetes

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Abstract

Background & Aims: Type 2 diabetes is associated with significant cardiac morbidity and mortality with a more than threefold increased risk of coronary artery disease (CAD). We aimed to assess the serum concentrations of lipoprotein (a) and lipid profile indexes as predictors for CAD in Sudanese type 2 diabetics compared to healthy subjects.

Materials & Methods: This case-control study was conducted at Jabir Abu-Aliz Specialized Center, Khartoum State, Sudan. After overnight fasting, 4 ml blood samples were collected in plain vials through venous puncture from each subject. Lipid profile parameters were estimated by standard laboratory procedures. Serum lipoprotein (a) was measured by immunoturbidimetric method. Statistical analyses were performed using SPSS software (version 17).

Results: Compared to healthy controls, type 2 diabetics showed significantly higher serum lipids and lipoprotein fractions. The mean values of lipoprotein (a), TC: HDL-C (5.55±.8 vs. 3.89±.6) and LDL-C: HDL-C (3.80±0.7 vs. 2.32±0.5) were significantly increased in patients. Lipoprotein (a) was significantly correlated with TG (p=.025), HDL-C (inversely, p<.001), VLDL-C (p= .025), and TC: HDL-C ratio (p= 016). Moreover, LDL-C: HDL-C ratio and TC were the most common risk factors distributed among the study patients (80% and 71.4% respectively).

Conclusion: Diabetic patients characterized by higher ratios of TC: HDL –C and LDL: HDL and Lipoprotein (a) compared to healthy controls. The evaluation of these lipid indices in diabetics, beside the routine lipid profile analysis, may be a crucial helpful step in the prevention of coronary artery disease since these atherogenic factors can be reduced by lifestyle modifications.

Keywords: Sudan, type 2 diabetes, lipoprotein (a), TC: HDL-C ratio, LDL-C: HDL-C ratio, coronary artery disease.

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Introduction:

Diabetes mellitus (DM) is a chronic metabolic disorder that is often associated with unacceptably high disease burden especially in developing countries. [1] Expert Committee proposed two major classes of DM and named them, insulin-dependent diabetes mellitus (IDDM, type 1 diabetes) and non-insulin-dependent diabetes mellitus (NIDDM, type 2 diabetes). [2] About 14 million individuals in 2011 were estimated to have diabetes in Africa, and this is expected to rise to 28 million by 2030. [3] In Sudan, the prevalence of DM is increasing to epidemic proportions, affecting about 14% of a total population of 31 million. [4]

Type 2 diabetes mellitus (T2DM) is associated with significant cardiac morbidity and mortality with a more than threefold increased risk of coronary artery disease (CAD). [5] CAD represents a wide spectrum from angina pectoris, myocardial infarction and sudden death to silent myocardial ischemia. The latter has a reported prevalence of 10-20% in diabetic population as compared to 1-4% in non-diabetic population. [6] Risk factors for CAD have been divided into conventional and novel risk factors. Hypertension, diabetes, smoking, dyslipidemia, sedentary lifestyle, and abdominal obesity are considered to be traditional risk factors for CAD. [7]

Lipoprotein (a) "Lp (a)", firstly discovered by Berg in 1963, is a novel established risk factor for cardiovascular disease in nondiabetic individuals. [8, 9] Serum Lp (a) is strongly associated with coronary artery occlusion and carotid wall thickness. [10] Structurally, Lp (a) consists of a low-density lipoprotein (LDL)-like particle that is covalently linked to apolipoprotein (a) – the distinct protein component of Lp (a) that is mainly responsible for its signature structural and functional properties. [11]

This study aimed to analyze the serum concentrations of Lp (a) and lipid profile indexes (including lipoprotein ratios; Total cholesterol: high-density lipoprotein cholesterol and low-density lipoprotein: high-density lipoprotein cholesterol) in Sudanese non-insulin dependent diabetic patients compared with non-diabetic healthy subjects. In addition, we explored the frequencies of these modifiable cardiometabolic parameters as risk factors for CAD in these patients. In adults, lipoprotein ratios are recognized as being more useful than isolated lipid values for cardiovascular disease risk assessment because they better reflect the interactions between lipid fractions. [12]

Materials & methods

Study design and subjects.

This is a case-control study with a total of sixty-six participants (35 diabetics and 31 age- and sex-matched healthy controls). To define diabetics, American Diabetes Association (ADA) criteria having fasting blood glucose level equal to or more than 126 mg/dl was chosen. Healthy non-diabetic subjects were recruited from the community; they neither had been diagnosed as diabetes nor use hypoglycaemic medication; or any history of known disease. The study was conducted at Jabir Abu-Aliz Specialized Center, Khartoum State, Sudan.

A pre-piloted questionnaire was used to obtain basic information from each participant, including age, educational level, current cigarette smoking status, and the occupation.

Anthropometric measures:

Measures of body weight (to the nearest 0.1 kilograms) and height (to the 0.01metre) were recorded for each subject. Weight was measured using calibrated electronic weighing scales and height was measured using a stadiometer. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared using BMI calculator in the website: http://www.nhlbi.nih.gov/guidelines/obesity/BMI/bmi-m.htm. The WHO classification for BMI was used to estimate the degree of obesity. Subjects were categorized as normal if BMI was less than 25 kg/m^2, overweight if BMI was between 25 - 29.9 kg/m^2, and obese if BMI was greater than or equal to 30 kg/m^2.

Waist circumference (to the nearest 0.5cm) was measured in standing position using an anthropometric tape at a level midway between the lower rib margin and iliac crest with the tape all around the body in a horizontal position. For males, waist circumference of less than 94 cm was considered low, while 94–102 cm was high and more than102 cm was very high. For females, waist circumference of less than 80 cm was considered low, 80–88

cm was high and more than 88 cm was very high.

Hip circumference (HC) (to the nearest 0.5cm) was measured at the widest point of the buttocks, and Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference

Measurement of blood pressure:

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured prior to drawing blood samples in the sitting position, using a standard mercury sphygmomanometer following standard procedure. [13] Two consecutive readings were recorded for each of SBP and DBP and the averages were used.Subjects were defined as hypertensive if their systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg

Blood sampling& serum preparation:

After overnight fasting, 4 ml blood samples were collected in plain vial through venous puncture from each subject. For the serum, blood allowed to clot for about 30 minutes at room temperature followed by centrifugation at 3000 RPM for 15 minutes to separate the serum. The serum was transferred with a pipette to a tube and stored at $-20C^{0}$ till analysis.

Measurement of lipid profile & lipoprotein (a):

Serum total cholesterol (TC), triglycerides (TG) were analyzed using standard enzymatic methods with commercial kits purchased from Human Diagnostics (Wiesbaden, Germany). High-density lipoprotein-cholesterol (HDL-C) was measured in the serum after a precipitation with phosphotungstic acid in presence of magnesium chloride. Low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald formula: [LDL-C = TC - HDL-C - TG/5 (mg/dl)].Very low-density lipoprotein cholesterol (VLDL-C) was calculated by formula [VLDL-C = TC – (HDL-C + LDL-C (mg/dl)].

Total to HDL cholesterol ratio (TC: HDL-C) was calculated as total cholesterol divided by HDL cholesterol while LDL to HDL cholesterol ratio (LDL-C: HDL-C) was calculated as LDL cholesterol divided by HDL cholesterol.

Serum lipoprotein Lp (a) was measured by Latex enhanced immunoturbidimetric method using commercially available kit supplied by Human Gesellschaftfür Biochemica und Diagnostica mbH, Germany. Briefly, this method was based on the agglutination of Lp (a) present in the sample with the latex particles coated with antibodies against LP (a). The agglutination was proportional to the Lp (a) concentration in the sample. The concentrations of LP (a) in the samples were determined by comparing the optical density of the samples to the standard calibration curve

Dyslipidemia was defined as per the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines. [14, 15]: TC ≥200 mg/dL; LDL-C ≥100 mg/dL; TG level ≥150 mg/dL; HDL-C ≤40 mg/dL; VLDL-C ≥32 mg/dL

Statistical analysis:

All statistical analyses were performed using the statistical package for social sciences (SPSS) software (version 17). The clinical and biochemical data of the study subjects were expressed as mean ±SD (for continuous data) or number (percent) (for categorical data). The differences between type 2 diabetics and healthy controls were examined by an unpaired t-test for the continuous variables and by chi-square test for the categorical variables. The Pearson correlation was used to examine the relation between Lp (a) and BMI, WC, FBS, TC, TG, LDL-C, HDL-C, TC;HDL-C, LDL-C:HDL-C. A *P*-value less than 0.05 was considered significant

Results

1. Baseline demographic and clinical characteristics of the study diabetics and controls:

The characteristics of the study participants are shown in Table 1. As displayed in the table, there were 35 patients with type 2 diabetes and 31 healthy non-diabetic subjects in this study. Type 2 diabetes patients had significantly greater BMI, waist circumference, systolic and diastolic blood pressure than controls. More than half of the patients were on hypoglycemic agent medication with mean diabetes duration equal to 11.30±7.6 years. Almost all of the patients reported that they had a family history of diabetes mellitus. There was no significant

difference in waist: hip ratio between subjects in the two groups.

1. Fasting Blood glucose , lipoprotein (a) and Lipid profile indices in type 2 diabetics and healthy controls:

As in table 2 when compared to subjects in the control group, type 2 diabetes patients showed significantly higher fasting blood glucose as well as serum lipids and lipoprotein fractions (TC, TG, LDL-C, and VLDL-C) but lower HDL-C). Moreover, regarding Lp (a) and lipid ratios the same pattern of dyslipidemia was observed in type 2 diabetic, that is, the means values of Lp (a), TC:HDL-C ratio, and LDL-C: HDL-C ratio were significantly increased in patients compared to healthy controls.

2. Relationship between Lipoprotein (a) and selected variables:

Pearson correlations for Lp (a) with BMI, WC, and lipid Profile are shown in table 3. Lp (a) was significantly correlated with WC (p=.012), FBS (p=.001), TG (p=.025), HDL-C (inversely, p<.001), VLDL-C (p= .025), and TC: HDL-C ratio (p= 016). Data did not show any correlation between Lp (a) with BMI, TC, LDL-C, and LDL-C: HDL-C ratio. Results of Pearson coefficients and P-values using two-tailed bivariate correlations analyses are given in Table 3.

3. Prevalence of the modifiable cardio-metabolic risk factors in type 2 diabetics

Figure 1 illustrates the distribution of the cardiometabolic factors in the study population with type 2 diabetes mellitus. LDL-C: HDL-C ratio and TC were the most common risk factors distributed among the study patients (80% and 71.4% at ≥ 200mg/dl and > 6.0 respectively). Less than half of the patients were hypertensive i.e., 45.7% had systolic blood pressure exceed 140 mmHg while 34.3% had diastolic blood pressure exceed 90 mmHg. TC: HDL-C ratio was the least risk factor seen among the type 2 diabetics in the study.

Table 1.Baseline demographic and clinical characteristics of type 2 diabetics and controls

Variable		Type 2 diabetics (n=35)	Non-diabetic controls (n=31)	p-value
Gender (M/F)		17/18	18/13	.441
Age (years)		57.06±9.6	56.13±6.9	.659
Weight (Kg)		79.79±9.2	81.90±6.4	.287
Height (meter)		1.63±.10	1.70±.06	.002[*]
BMI (kg/m^2)		30.14±4.1	28.31±2.5	.035[*]
WC (cm)	male	106.47±9.7	101.89±4.13	<.001[*]
	Female	98.78±8.25	91.08±6.81	
WHR		0.89±.04	0.86±.07	.092
Systolic BP		137.10±24.7	116.29±5.5	<.001[*]
Diastolic BP		81.94±8.3	72.97±5.2	<.001[*]
Treatment of DM				
	Diet alone	02(5.7%)	--	<.001[*]
	insulin	02(5.7%)	--	
	OHA	21(60%)	--	
	Insulin+ OHA	10(28.6%)	--	
Duration DM (years)		11.30±7.6	--	
Family history of DM				
	+ ve	33(94.3%)	4(12.9%)	<.001[*]
	- ve	02(5.7%)	27 (87.1%)	

Abbreviations: M, male; F, female; BMI, body mass index; WC, waist circumferences; WHR, waist: hip ratio; BP, blood pressure; DM, diabetes mellitus; OHA, oral hypoglycemic agent;
Variables are presented as mean± SD or number (percent).
* P-value<0.05; compared type 2 diabetic patients to non-diabetic healthy controls.

Table 2: Fasting blood glucose, lipid profile, and lipoprotein (a) in type 2 diabetics compared to healthy controls

Variable	Type 2 diabetics (n=35)	Non-diabetic controls (n=31)	p-value
FBG (mg/dl)	210.72±66.4	84.26±5.5	<.001[*]
TC (mg/dl)	214.26±35.2	179.60±14.2	<.001[*]
TG (mg/dl)	143.43±28.1	132.10±8.1	.034[*]
HDL-C (mg/dl)	38.66±4.2	46.86±5.3	<.001[*]
LDL-C (mg/dl)	146.91±33.7	106.32±16.4	<.001[*]
VLDL-C (mg/dl)	28.69±5.6	26.42±1.6	.034[*]
TC:HDL-C ratio	5.55±.8	3.89±.6	<.001[*]
LDL-C:HDL-C ratio	3.80±0.7	2.32±0.5	<.001[*]
Lp (a) (mg/dl)	28.49±5.5	23.06±4.2	<.001[*]

Abbreviations: FBG, fasting blood glucose; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C,very low density lipoprotein cholesterol; Lp (a), lipoprotein (a)
Variables are presented as mean ± SD
* P-value<0.05; compared type 2 diabetic patients to non-diabetic healthy subjects.

Table 3.Correlation between lp (a) and biochemical parameters in type 2 diabetics

Variable	r	P
BMI	.009	.943
WC	.307	.012[*]
FBG	.439	.001[*]
TC	.046	.715
TG	.275	.025[*]
HDL-C	-.473	<.001
VLDL-C	.275	.025[*]
TC:HDL-C ratio	.294	.016[*]
LDL-C: HDL-C ratio	.241	.051
LDL-C	.096	.444

BMI, body mass index; WC, waist circumferences; FBG, fasting blood glucose; TC, total cholesterol; TG , triglyceride; HD-CL, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; Lp (a), lipoprotein (a)
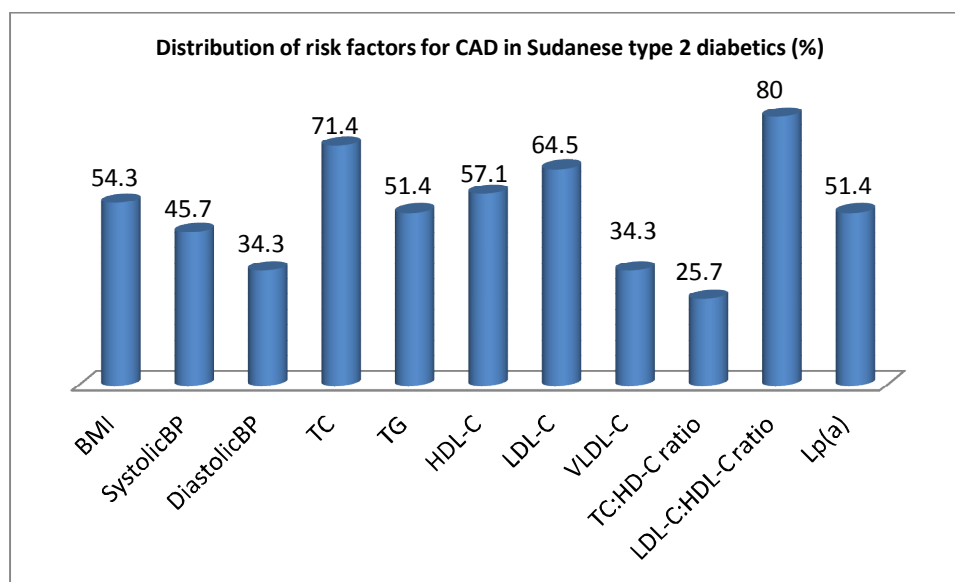. *p<0.05

Fig (1): frequencies of cardio-metabolic risk factors among Sudanese patients with type 2 diabetes (data represented as %)

Cut-off values for the cardiometabolic risk factors as the following: BMI≥ 30 kg/m^2, systolic BP≥140mmHg, diastolic BP≥90 mmHg, TC≥200mg/dl, TG≥150mg/dl, HDL-C ≤40mg/dl, LDL-C≥100mg/dl, VLDL-C≥32mg/dl, TC: HDL-C>3.5, LDL-C: HDL-C>6, Lp (a) >6

Abbreviations: BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; TC, total cholesterol; TG , triglyceride; HD-CL, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; Lp (a), lipoprotein (a)

Discussion:

Cardiovascular disease is responsible for a large proportion of morbidity and mortality in patients with type 2 diabetes who have an age-adjusted prevalence of coronary heart disease (CHD) that is twice that of people without diabetes and corresponds to a risk of death from cardiovascular causes that is two to six times greater. [16-18] Moreover, three-fourths of global deaths due to CHD occurred in the low and middle-income countries. The rapid rise in CHD burden in most of the low and middle and income countries is due to socio-economic changes, increase in life span and acquisition of lifestyle related risk factor like elevated levels of blood pressure, cholesterol, tobacco, obesity, and physical inactivity. [19]

This study was conducted in a hospital based population (Jabir Abu-Aliz Specialized center for diabetes) to assess the serum concentrations of lipoprotein (a) and lipid profile indexes as predictors for CAD in Sudanese insulin non-dependent diabetic patients compared with non-diabetic healthy subjects. Consistent with several reports from developing countries, [20-23] Sudanese type 2 diabetics in this study showed dyslipidemia based on the NCEP-ATPIII guidelines which focuse on the role of the clinical approach to prevention of CHD.

The alteration in blood lipids and lipoproteins in patients with type 2 diabetes mellitus (represented by significantly higher TC, TG, LDL-C, VLDL-C and Lower HDL) can be as a consequence to defects in both insulin action and hyperglycemia. [24] The insulin deficiency in diabetes reduces suppression of hormone sensitive lipase activity thereby increasing intra-cellular hydrolysis of triglycerides in the adipose tissue, result in releasing free fatty acids (FFA) in the portal circulation. [25] The excess FFA from adipose

tissue are further accumulated in the liver and converted to glycerides which are packaged in ApoB-containing very low-density lipoproteins (VLDLs). The presence of increased VLDL increases the available substrate for cholesterol ester transfer protein which enhances the exchange of triglycerides for cholesterol from the HDL to the LDL particles increasing the triglyceride content of both particles which causes increased activity of the enzyme hepatic lipase that results in an increased production of both small dense LDL and small dense HDL particles. [26, 27] In addition, insulin resistance might be associated with diminished level of LDL receptors which in turn might be causing increase in LDL cholesterol. [28]

Type 2 diabetes mellitus and dyslipidemia are important risk factors for cardiovascular disease. [29] Elevated serum levels of LDL-C and TG and low levels of HDL-C are strongly associated with increased risk for macrovascular events (e.g., myocardial infarction, ischemic stroke, and coronary mortality) among patients with T2DM. [30] Small dense LDL particles are more atherogenic because these particles, especially when glycated, are more easily oxidized and 'picked up' by the scavenger receptor on the macrophage which has a much greater affinity for oxidized LDL than for non-oxidized LDL. Macrophages therefore facilitate the transportation of these particles through the intima to the subintimal space and media of the artery where the process of atherogenesis is initiated and accelerated by these highly atherogenic particles. [26]

Although the third Adult Treatment Panel guidelines of the US National Cholesterol Education Program (ATP III) only provide for evaluation of individual lipid fractions, the use of ratios such as TC/HDL-C or LDL-C/HDL-C may provide refined risk assessment for cardiovascular outcomes by simultaneously taking into account both atherogenic and cardioprotective lipid fractions. [31] The ratio of TC/HDL-C is regarded as a predictor of CHD risk, especially with values >6.0, [32] while a cut-off value for a high LDL-C/HDL-C ratio was defined as 3.5. [33] Therefore, in the current study we were interested to evaluated cholesterol ratios mainly TC/HDL-C or LDL-C/HDL-C. Data pointed that the diabetic patients characterized by higher ratios TC:

HDL –C (5.55±.8 vs. 3.89±.6) and LDL-C: HDL-C (3.80±0.7 vs. 2.32±0.5) compared to healthy controls. About a quarter of diabetics had TC:HDL-C ratio greater than 6 while eighty percent had LDL-C:HDL-C ratio exceeded 3.5 as shown in figure 1. In the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk prospective population study, individuals with a TC/HDL-C ratio greater than 5 had a hazard ratio for future CHD of 2.19 (95% CI: 1.22 to 3.93) when compared with those with a TC/HDL-C ratio less than 5. The authors concluded that subjects with high non–HDL-C levels, high TG levels, or with an elevated TC/HDL-C ratio, independently of their plasma LDL-C levels, were at increased CHD risk. [34] Recently, Enomoto M et al [35] from a prospective large-scale epidemiological study reported that elevated LDL/HDL ratio is not just a marker of atherosclerosis but may play a causal role in the pathogenesis of human carotid intima-media thickness (IMT) progression than LDL-C or HDL-C alone. The author reported that changes in IMT after eight years follow up were significantly related to HDL-C (inversely, P < 0.05) and to LDL-C/HDL-C ratio (P < 0.05)

In addition to lipoprotein ratios, Lp (a) have been established as a novel risk factors for CVD. [36] However, studies regarding association of the mean Lp (a) levels with diabetes is contradictory. [37] In this report the mean concentration of Lp (a) in Sudanese type 2 diabetics was significantly higher than healthy controls (28.49±5.5 vs. 23.06±4.2 mg/dl, p< .001). However, as shown in Figure (1) nearly half of the patients (51.4%) had Lp (a) greater than 30 mg/dl which is considered as a cut-off value of a higher risk for CVD. [38] In agreement with our finding, Singla et al [37] reported that the Lp(a) levels was significantly increased in in the patients of type 2 diabetes mellitus as compared to the control group (*P*< 0.001). Moreover, Singla et al reported that Lp(a) levels in the patients of type 2 diabetes mellitus were associated with the LDL:HDL ratio and HbA1c which conflicted with our finding that no association between Lp (a) and LDL-C:HDL-C ratio was noticed as shown in table 3. In contrast to these findings, Daghash et al [39] reported non-significant difference (p=0.148) in Lp (a) level in Qatari patients with T2DM compared to non-diabetic controls. The authors concluded that Lp(a) may not be an

independent risk factor for CAD in Qatari nationals patients with DM. The mechanism of Lp (a) atherothrombotic role has been elusive. In vitro studies demonstrate that Lp(a) can inhibit fibrinolysis by interfering with plasminogen binding and activity, and both apo(a) mouse and rabbit transgenic models have reduced clot lysis. [40] Moreover, Lp(a) inhibits the activation of transforming growth factor and contributes to the growth of arterial atherosclerotic lesions by promoting the proliferation of vascular smooth muscle cells and the migration of smooth muscle cells to endothelial cells. [41]

Conclusion:

In addition to dyslipidemia, this study shows the alteration of lipoprotein (a) and lipoprotein ratios in Sudanese patients with type 2 diabetes. The evaluation of lipid indices such as Lp(a) and lipid ratios in diabetics, beside the routine analysis of lipid profile parameters) may be a crucial helpful step in the prevention of coronary artery disease since these atherogenic factors can be modified by lifestyle changes.

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