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Original Article

Phenotypes of High-Density Lipoprotein Cholesterol and their Relationship with Glycemic Status in Type 2 Diabetic Patients

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Abstract

Background: There are varying reports on the association of high-density lipoprotein cholesterol (HDL-C) phenotypes and glycemia in type 2 diabetes mellitus (T2D) patients. Such information is lacking in Nigerian data. **Aim:** The aim of this study was to determine the relationship between HDL-C phenotypes and glycemic control in T2D patients. **Materials and Methods:** Clinical and laboratory data were collected from consenting T2D patients. Information was managed and analyzed with appropriate software. **Results:** Four hundred T2D patients consisting of 235 and 165 persons with and without poor glycemic control, respectively, were recruited for this study. There was a significant negative correlation between glycosylated hemoglobin (HbA1c) and the HDL_2-C phenotype ($r_s = -0.12$, $P = 0.01$). The correlation between HbA1c and the HDL_3-C phenotype was not statistically significant ($r_s = -0.06$, $P = 0.21$). **Conclusion:** The HDL_2 phenotype has a stronger correlation with glycemic status than the HDL_3 phenotype in T2D patients.

Keywords: Glycemic control, high-density lipoprotein, phenotype

Introduction

Diabetic dyslipidemia, consisting of any or a combination of hypertriglyceridemia, reduction in high-density lipoprotein cholesterol (HDL-C), and elevated levels of small dense low-density lipoprotein cholesterol, contributes to cardiovascular burden in type 2 diabetes mellitus (T2D) patients.[1] Triglycerides (TGs) in diet are digested by gastric and pancreatic lipases into monoglycerides and free fatty acids. Cholesterol esters are de-esterified by pancreatic esterase into cholesterol. TGs are transported by chylomicrons from within enterocytes through lymphatics into the bloodstream. Lipoprotein lipase converts chylomicron TG into fatty acids and glycerol for energy use by adipocytes and skeletal muscle. HDLs are produced in the enterocytes and the liver. The role of HDL is to transport cholesterol from tissues and other lipoproteins to other tissues and lipoproteins using cholesteryl ester transfer protein.

There are two phenotypes of HDL: HDL_2 and HDL_3, with densities of 1.063–1.125 kg/L and 1.125–1.210 kg/L, respectively. HDL_2 contains apo A-I whereas HDL_3 contains both apo A-I and A-II.[2] HDL_2 particles are partially depleted of cholesteryl esters and enriched in TGs. Hepatic lipase hydrolyzes the TG-enriched HDL_2 particles and regenerates HDL_3, yielding particles that are again suited to accept cholesterol from peripheral cells. The apo A-I HDL particles are associated with the cholesterol efflux-promoting effects of HDL; particles containing both apolipoproteins (e.g., HDL_3) are less effective at mobilizing cholesterol from peripheral stores and appear to have various other functions. The clinical significance of the different HDL subfractions is not fully understood, but studies done outside Africa have demonstrated that HDL_2 cholesterol is a better predictor of coronary atherosclerosis and glycemia than HDL_3 or total HDL

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cholesterol in persons with T2D.[3,4] Abnormalities in HDL-C are common in African patients with a significant impact on glycemic status.[5-10]

Information of such association between HDL-C phenotypes and glycemia is lacking in Nigerians with T2D. This study, therefore, targeted to describe this gap in scientific knowledge. The aim of this study was to determine the relationship between the two phenotypes of HDL-C and glycemia in Nigerian T2D patients.

Materials and Methods

This was a cross-sectional study done at the side laboratory of the medical ward complex of the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, South-East Nigeria, involving T2D patients aged 30–69 years who attended the diabetes mellitus (DM) clinic of the NAUTH, Nnewi. Study participants were consecutively recruited from the NAUTH DM clinic for this study after informed consent was sought and details of the study were explained to them. History taking and physical examination were done on each study participant, and data were entered in a researcher-administered study protocol. Weight, height, and waist circumference were determined using the WHO STEPS anthropometric tool.[11] Body mass index was calculated as weight in kilograms divided by the square of the height in meters. Blood pressure was measured using a mercury sphygmomanometer (Accoson, England), and hypertension was defined as systolic blood pressure of 140 mmHg and above, or diastolic blood pressure of 90 mmHg and above, or use of antihypertensive medications.[12]

Ten milliliters of venous blood was collected from the intercubital vein of each participant between 8 and 10 am after an overnight fast of 12–14 h. Two milliliters of the sample was put in a fluoride oxalate bottle for plasma glucose estimation using the Trinder glucose oxidase technique whereas 1 mL of blood was put in an ethylenediaminetetraacetic acid bottle for glycosylated hemoglobin (HbA1c) estimation using the boronate affinity chromatography method.[13,14] The remaining 7 mL of blood was placed in a plain bottle for plasma HDL-C phenotype estimation (precipitation method), serum creatinine estimation (alkaline picrate method), serum alanine transferase estimation (dinitrophenylhydrazine method), hemoglobin estimation, and hemoglobin electrophoresis in the NAUTH chemical pathology laboratory.[15-19] Blood samples were stored in a freezer (Haier Thermocool Ltd., UK) at a temperature of −20°C before laboratory analysis using a spectrophometer (Spectronic ZOD, Milton Roy Company, England). Unknown blood levels of biochemical indices were determined by interpolation of absorbances on the calibration curves. Within-run and between-run assay coefficients of variation were within the acceptable limit of <10%.[20]

Poor glycemic control was defined as HbA1c >6.5%.[21] Exclusion criteria included presence of type 1 DM; use of medications such as statins, fibrates, nicotinic acid, glucocorticoids, androgens, hormonal contraception, and beta-blockers; pregnancy; renal disease (defined as estimated glomerular filtration rate of <60 mL/1.73 m^2/min); necro-inflammatory liver disease (defined as serum alanine transferase >45 IU/L which was the upper reference limit for alanine transferase in the NAUTH chemical pathology laboratory); anemia (defined as hemoglobin <12 g/dL); familial forms of dyslipidemia; and presence of sickle cell disease.[22,23] Individuals with presence of atherosclerotic cardiovascular diseases such as cerebrovascular disease, coronary artery disease, or peripheral artery disease were also excluded from this study. Details of medication use, history of familial dyslipidemias, and presence of atherosclerotic cardiovascular disease were obtained from the participants' clinic case files.

Collected data were transferred to Microsoft Office Excel® 2010 software for data management before transfer to the Statistical Package for the Social Sciences version 26 (IBM corporation, California, USA) for statistical analyses. Kolmogorov–Smirnov test was used to determine the normality of data distribution. Continuous variables were presented as median interquartile range (IQR) whereas categorical variables were presented as proportions, *n* (%). IQR was presented as (25^{th} percentile, 75^{th} percentile). Logarithmic transformation of skewed data was done before correlation of quantitative variables. Quantitative variables were compared among two groups using Mann–Whitney *U*-test whereas strength of association between continuous variables was done using Spearman rank correlation coefficient, r_s. Statistical significance was defined as $P < 0.05$. Tables, figures, and text were used to summarize results.

Results

Four hundred participants with T2D were recruited for this study. Table 1 shows the baseline characteristics of study participants. The study participants consisted of 174 (43.5%) males and 226 (56.5%) females with a median age of 60 years.

Table 2 shows the comparison of demographic, clinical, and biochemical indices in participants with and without good glycemic control. Two hundred and thirty-five (58.8%) and 165 (41.2%) study participants had poor and good glycemic control, respectively. There were significantly lower total HDL-C, HDL_2-C phenotype, and HDL_2-C/HDL_3-C ratio in participants with poor glycemic control compared to those with good glycemic control. The difference in HDL_3-C among patients with and without good glycemic control was not statistically significant ($P = 0.21$). The median total HDL-C values in individuals with poor and good glycemic control were 1.20 mmol/L and 1.27 mmol/L, respectively.

Figure 1 shows the relationship between HbA1c and HDL_2-C in study participants. There was a significant negative correlation between HbA1c and HDL_2-C ($r_s = −0.12$, $P = 0.01$). Figure 2 shows the relationship between HbA1c and HDL_3-C in the participants. There was a negative correlation between HbA1c and HDL_3-C, but this association was not statistically

significant (r_s = −0.06, P = 0.21). Figure 3 shows the relationship between HbA1c and HDL_2-C/HDL_3-C ratio in study participants. There was a significant negative correlation between HbA1c and HDL_2-C/HDL_3-C (r_s = −0.11, P = 0.03).

Discussion

This study found significantly lower HDL_2-C and HDL_2-C/HDL_3-C ratio in T2D patients with poor glycemic control compared to those with good glycemic control. There were low HDL_3-C levels in persons with poor glycemic control compared to those with good glycemic control, though the difference was not statistically significant. There was a significant inverse relationship between HbA1c and each of HDL_2-C and HDL_2-C/HDL_3-C with HDL_2-C having a stronger association with HbA1c. There was no significant association between HDL_3-C and HbA1c. The median values of total HDL-C in individuals with poor and good glycemic control were 1.20 mmol/L and 1.27 mmol/L, respectively. These values may be explained by the nonparametric nature of the population distribution and the fact that these patients were on treatment for DM. These have been compared with findings from previous research works.

Xian *et al*. reported a significant inverse relationship between glycemic control and HDL_2-C in Chinese T2D patients.[24] This is similar to the finding from the present study which also found a similar relationship between the HDL_2 phenotype and glycemic control. Even though the study by Xian *et al*. was carried out in 38 persons compared to the present study that involved 400 T2D patients and with different methodologies for HDL-C phenotype measurement in both studies, the findings were similar. Maeda *et al*. reported a stronger negative correlation between FPG and HDL_2-C compared to that between FPG and HDL_3-C in 418 Japanese-Americans studied.[25] The present study also found a stronger negative correlation of HbA1c with HDL_2-C than with HDL_3-cholesterol.

Some studies differed in their findings regarding the association of glycemic control and HDL-C phenotypes in diabetic patients when compared to findings from the present study. Paniagua *et al*. found a significant relationship between glycemia and the HDL_3-C phenotype in T2D patients although this study recruited only twenty participants compared to the present study that involved 400 T2D patients.[26] Fukui *et al*. found a significant association between HDL_3-C and HbA1c in 69 T2D patients studied although some of these individuals

Table 1: Baseline characteristics of study participants

Variables	Study participants (n=400), median (IQR)/n (%)
Age (years)	60 (48-65)
Sex	
Males	174 (43.5)
Females	226 (56.5)
BMI (kg/m^2)	28.5 (25.6-32.0)
WC (cm)	99 (90-107)
SBP (mmHg)	140 (120-150)
DBP (mmHg)	80 (70-100)

BMI: Body mass index, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, IQR: Interquartile range, WC: Waist circumference

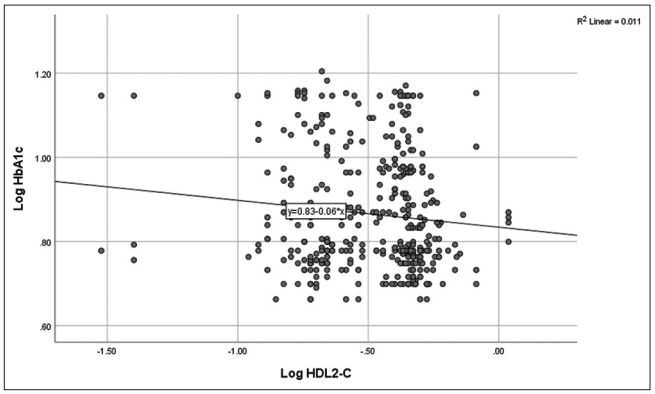

Figure 1: Relationship between glycosylated hemoglobin and high-density lipoprotein 2 cholesterol phenotype. This relationship is described by the linear regression equation: Log glycosylated hemoglobin = 0.83–0.06 log high-density lipoprotein 2 cholesterol (r_s = −0.12, P = 0.01)

Table 2: Demographic, clinical, and biochemical indices in study participants with and without good glycemic control

Variables	Median (IQR)/n (%) (n=400)		Mann-Whitney U/χ^2	P
	T2D with poor glycemic control (n=235)	T2D with good glycemic control (n=165)		
Age (years)	63 (58-67)	61 (57-65)	15,390*	0.57
Sex				
Females	124 (52.8)	102 (61.8)	3.82†	0.37
Males	111 (7.2)	63 (38.2)		
Female/male ratio	1.12	1.62		
BMI (kg/m^2)	29.4 (26.1-36.1)	27.9 (25.0-33.6)	15,380*	0.09
HbA1c	10.5 (8.2-13.4)	6.0 (5.4-6.3)	22,743*	0.01‡
Total HDL-C (mmol/L)	1.20 (0.82-1.30)	1.27 (0.88-1.32)	23,720*	0.01‡
HDL_2-C (mmol/L)	0.38 (0.23-0.45)	0.44 (0.22-0.48)	24,549*	0.01‡
HDL_3-C (mmol/L)	0.80 (0.60-0.84)	0.81 (0.70-0.84)	25,338*	0.21
HDL_2-C/HDL_3-C ratio	0.47 (0.32-0.55)	0.52 (0.43-0.64)	24,425*	0.03‡

*Mann-Whitney U, †χ^2, ‡Statistically significant. BMI: Body mass index, HbA1c: Glycosylated hemoglobin, HDL-C: High-density lipoprotein cholesterol, HDL_2-C: HDL-2 cholesterol, HDL_3-C: HDL-3 cholesterol, IQR: Interquartile range, T2D: Type 2 diabetes

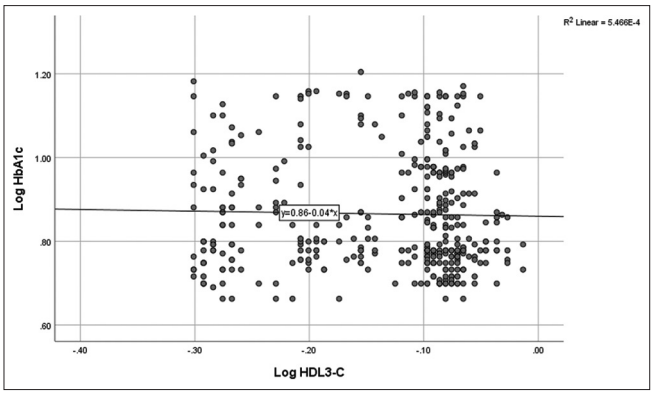

Figure 2: Relationship between glycosylated hemoglobin and high-density lipoprotein 3 cholesterol phenotype. This relationship is described by the linear regression equation: Log glycosylated hemoglobin = 0.86–0.04 log high-density lipoprotein 3 cholesterol ($r_s = -0.06$, $P = 0.21$)

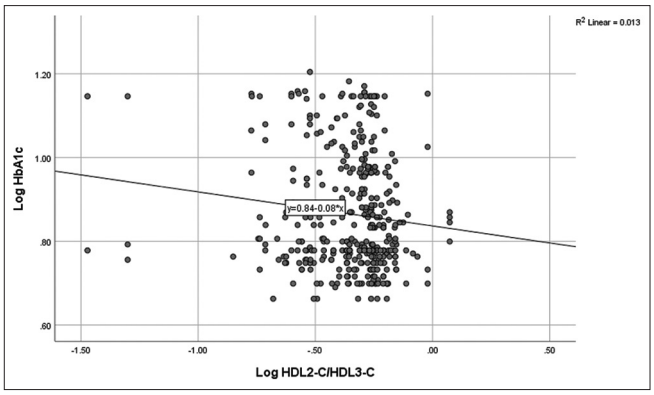

Figure 3: Relationship between glycosylated hemoglobin and ratio of high-density lipoprotein 2 cholesterol to high-density lipoprotein 3 cholesterol. This relationship is described by the linear regression equation: Log glycosylated hemoglobin = 0.84–0.08 log high-density lipoprotein 2 cholesterol/high-density lipoprotein 3 cholesterol ($r_s = -0.11$, $P = 0.03$)

were on statin therapy.[27] Individuals on lipid-lowering agents (statins inclusive) were excluded in the present study.

The main strength of this study was the characterization of HDL-C phenotypes and their relationship with glycemic control in Nigerian T2D patients which has not been reported in previous Nigerian data. Individuals on lipid-lowering medications were also excluded from the present study because these agents play a role in affecting HDL-C levels. The main limitation of this study was the use of the precipitation technique in measuring HDL-C phenotypes and not the gold standard ultracentrifugation method. The ultracentrifugation method is expensive, cumbersome, and requires large volumes of blood for HDL-C phenotype analysis compared to the simple precipitation method. The precipitation method has gained widespread use because of its high accuracy in measuring HDL-C phenotypes when compared to the ultracentrifugation method.[28]

Conclusion

The present study showed a stronger association between HDL_2-C phenotype and glycemic status compared to that between each of total HDL-C and HDL_3-C and glycemia in Nigerian T2D patients. The clinical implication of this study finding is that the HDL_2-C phenotype should be measured in T2D patients because of its strong association with glycemia in these individuals. Total HDL-C is routinely measured because of its proven role in the pathogenesis of the atherosclerotic process in T2D patients and its strong association with glycemic status in these persons. The stronger association of HDL_2-C with glycemic control implies that this molecule should be a better variable to measure in T2D patients than total HDL-C.

Authors contribution:

All authors contributed substantially to the conception, conduct and reporting of the study. They all reviewed the manuscript for intellectual content and approved its final version.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

This study was approved by the NAUTH research ethics committee, Nnewi, Nigeria, before the study was commenced. All participants provided a written informed consent.

Data availability

Data are available by reasonable requests to the corresponding author.

References

1. Valabhji J, Elkeles RS. Dyslipidaemia in type 2 diabetes: Epidemiology and biochemistry. Br J Diabetes Vascular Dis 2003;3:184-9.
2. Link JJ, Rohatgi A, de Lemos JA. HDL cholesterol: Physiology, pathophysiology, and management. Curr Probl Cardiol 2007;32:268-314.
3. Syvanne M, Ahola M, Lahdenpera S, Kahri J, Kuusi T, Virtanen KS, *et al*. High density lipoprotein subfractions in non-insulin dependent diabetes mellitus and coronary disease. J Lipid Res 1995;36:573-82.
4. Gowri MS, van der Westhuyzen DR, Bridges SR, Anderson JW. Decreased protection by HDL from poorly controlled type 2 diabetic subjects against LDL oxidation may be due to the abnormal composition of HDL. Arterioscler Thromb Vasc Biol 1999;19:2226-33.
5. El-Nasri HA, Ahmed AM. Patterns of lipid changes among type 2 diabetes patients in Sudan. East Med Health J 2008;14:316-24.
6. Agboola-Abu CF, Ohwovoriole AE, Akinlade KS. The effect of glycaemic control on the prevalence and pattern of dyslipidaemia in Nigerian patients with newly diagnosed non-insulin dependent diabetes mellitus. WAJM 2000;19:27-33.
7. Okafor CI, Fasanmade OA, Oke DA. Pattern of dyslipidaemia among Nigerians with type 2 diabetes mellitus. Niger J Clin Pract 2008;11:25-31.
8. Jisieike-Onuigbo NN, Unuigbe EI, Oguejiofor CO. Dyslipidemias in type 2 diabetes mellitus patients in Nnewi South-East Nigeria. Ann Afr Med 2011;10:285-9.
9. Jimoh AK, Adediran OS, Agboola SM, Busari OA, Idowu A, Adeoye TA, *et al*. Lipid profile of type 2 diabetic patients at a rural tertiary hospital in Nigeria. J Diabetes Endocrinol 2010;1:46-51.
10. Edo A, Adediran OS. Dyslipidaemia among Nigerian oil workers with

type 2 diabetes mellitus. West Afr J Med 2011;30:206-9.
11. WHO STEPS Instruments. Available from: http//www.who.int/chp/steps. [Last accessed on 2021 Feb 05].
12. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr., *et al*. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The JNC 7 report. JAMA 2003;289:2560-72.
13. Marks V. An improved glucose oxidase method for determining blood, CSF, urine glucose levels. Clin Chim Acta 1996;251:19-24.
14. Flückiger R, Woodtli T, Berger W. Quantitation of glycosylated hemoglobin by boronate affinity chromatography. Diabetes 1984;33:73-6.
15. Hirano T, Nohtomi K, Koba S, Muroi A, Ito Y. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. J Lipid Res 2008;49:1130-6.
16. Husdan H, Rapoport A. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin Chem 1968;14:222-38.
17. Bergmeyer HU, Hørder M. International federation of clinical chemistry. Scientific committee. Expert panel on enzymes. IFCC document stage 2, draft 1; 1979-11-19 with a view to an IFCC recommendation. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1980;18:521-34.
18. Balasubramaniam P, Malathi A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. J Postgrad Med 1992;38:8-9.
19. Kumar AA, Patton MR, Hennek JW, Lee SY, D'Alesio-Spina G, Yang X, *et al*. Density-based separation in multiphase systems provides a simple method to identify sickle cell disease. Proc Natl Acad Sci U S A 2014;111:14864-9.
20. Logan JE, Bayse DD, Koedam JC, Mather A, Wilding P. International federation of clinical chemistry (IFCC). Scientific Committee, Analytical Section. IFCC/WHO principles and recommendations on evaluation of diagnostic reagent sets used in health laboratories with limited resources. Part 3. Selection and evaluation using reference materials. General considerations. J Clin Chem Clin Biochem 1984;22:573-82.
21. International Diabetes Federation. Global Guideline for Type 2 Diabetes. International Diabetes Federation Guideline Development Group. Diabetes Res Clin Pract 2014;104:1-52.
22. Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: Accuracy in good health and in chronic kidney disease. Ann Intern Med 2004;141:929-37.
23. World Health Organization. Worldwide Prevalence of Anemia 1993–2005. A Report of the Geneva: World Health Organization; 2008.
24. Xian X, Ma Y, Yang DD, Huang W, Wang Y, Mueller O, *et al*. Reduced high-density lipoprotein 2b in non-obese type 2 diabetic patients analysed by a microfluidic chip method in a case-control study. Biomarkers 2009;14:619-23.
25. Maeda S, Nakanishi S, Yoneda M, Awaya T, Yamane K, Hirano T, *et al*. Associations between small dense LDL, HDL subfractions (HDL2, HDL3) and risk of atherosclerosis in Japanese-Americans. J Atheroscler Thromb 2012;19:444-52.
26. Paniagua JA, López-Miranda J, Jansen S, Zambrana JL, López Segura F, Jiménez Perepérez JA, *et al*. Increased high-density lipoprotein-3 binding to leukocytes following weight loss and improved glycemic control in type 2 diabetic patients. Metabolism 2000;49:692-7.
27. Fukui T, Hirano T. High-density lipoprotein subspecies between patients with type 1 diabetes and type 2 diabetes without/with intensive insulin therapy. Endocr J 2012;59:561-9.
28. Dong J, Guo H, Yang R, Li H, Wang S, Zhang J, *et al*. A novel and precise method for simultaneous measurement of serum HDL and LDL subfractions and lipoprotein (a) cholesterol by ultracentrifugation and high-performance liquid chromatography. Clin Chim Acta 2012;413:1071-6.