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ORIGINAL ARTICLE

Estimation of Dysbetalipoproteinemia Incidence in Iraqi Patients with Diabetes Mellitus and Cardiovascular Disease

SAMAR T. HAMEED[1], QASIM S. AL-MAYAH[2], MAHMOOD S. KHUDHAIR[3], GHASSAN A. AL-SHAMMA[4]
[1]Department of Pathological Analysis Technologies, College of Health and Medical Techniques, Al-Bayan University, Baghdad, Iraq
[2]Medical Research Unit, College of Medicine, Al-Nahrain University, Baghdad, Iraq
[3]Department of Internal Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq
[4]Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq
Correspondence to: Samar T. Hameed, Email: samar.thamer@gmail.com

ABSTRACT

Background: The main obvious manifestations in dysbetalipiproteinemia (DBL) are Apo-B, non-high-density lipoprotein cholesterol (non-HDLc) and Apo-E gene polymorphism, with a remarkable controversy in the results among different workers.
The aim of the study was to find a suitable variable or formula for diagnosis of familial or secondary DBL in a sample of Iraqi patients with type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD)**.**
Material and methods: The study involved 50 patients with T2DM (mean age 46.48 ±9.3 years), 26 patients with CVD (mean age: 43.15±7.34 years) and 73 apparently healthy normal control individuals (mean age: 34.51±11.47 years) with almost equal male/female ratio.

Serum lipids (TC, TG, HDL-c, LDL-c, VLDL-c, Remnant like particles RLP, Apo-B, and Apo-E) were estimated in patients and controls. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of non-HDL-c and non-HDL-c /Apo-B in the context of discrimination between patients with- and without DBL.
Results: All measured serum lipids, were higher in patients than controls, except HDL-c. Using Sniderman algorithm, 13 patients (18.06%) among T2DM and CVD were considered to have DBL, while none of control group had this disorder.
Conclusion Based on Sniderman algorithm, ROC revealed a better specificity and sensitivity for non- HDLc to diagnose DBL,
Keywords: dysbetalipoproteinemia, Apo-B, Non- HDL cholesterol, Sniderman algorithm, Cardiovascular disease, Diabetes mellitus.

INTRODUCTION

Familial dysbetalipoproteinemia (DBL) is the most prevalent primary dyslipidemia. Its precise definition and best method for diagnosis are controversial. The most characteristic feature of the disease is the elevation in apoprotein-B (Apo-B) with different serum lipid profiles, presented with either mixed hyperlipidemia, isolated hypercholesterolemia, hypertriglyceridemia, or even as a normal serum lipid profile[1].

Genetically, this disorder is associated with a mutation in Apoprotein- E (Apo-E), namely Apo-E2, which decreases the ability of the encoded protein to convert very low-density lipoprotein (VLDL) and its remnants (intermediate density lipoprotein, IDL) to LDL particles in the blood with decreased clearance of chylomicron remnants[2]. This results in the appearance of high cholesterol-containing remnants which accumulate in the blood leading to premature coronary heart disease[3].

One of the common features of the DBL is the striking increase in total cholesterol (TC) and triglycerides (TG) reaching about 600 mg /dL for both, with elevated VLDL cholesterol to triglycerides ratio and low LDL-c, while high density lipoprotein cholesterol (HDL-c) could be low or normal[3]. The condition is usually associated with the presence of different types of xanthomas .[2,4,5] A genetic-based study on 367 hyper-lipidemic people showed the presence of the DBL in 4.9 %, their serum lipid profile demonstrated significantly higher levels of TC,TG, LDL-c and non-HDL-c as compared to the control group, while plasma HDL-c levels were significantly lower.[6]

Early works on the diagnosis of this disorder recommended TC/ Apo B, Apo B/Apo-E, TG /Apo-B, Apo AII / HDL-c and non-HDL-c /Apo-B ratio.[5, 7,8,9]

For the estimation of DBL, one recent report suggested the Sniderman algorithm, which is based on apo-B quantitation and approved by genetic study. The results obtained from a cohort study on 1,771 fasting individuals revealed that this disorder can be defined by combination of four estimates, namely: Apo-B < 120 mg/ dl, TG > 133 mg/dl, TG/Apo-B < 8.8, and TC/Apo-B >2.4.[8]

The reported complications of DBL included peripheral vascular disease, obesity, coronary artery disease and insulin resistance.[10,11,12] A group of workers reported coronary heart disease incidence to be 27.8 % among their hyperlipemia patients[6].

To our knowledge, there is no report on the incidence of DBL in Iraqi patients, however there is only one recent report on APOE gene polymorphism in normal Iraqi Kurdish population.[13]

In this article we were trying to apply the Sniderman algorithm to estimate the incidence of DBL in groups of T2DM and CVD patients and the best variable for its diagnosis.

MATERIAL AND METHODS

The Study Population: A total of 76 patients participated in this study; of whom 50 patients T2DM and 26 had CVD (their mean age was 46.48 ± 9.3 and 43.15± 7.34 respectively).Seventy-three apparently healthy individuals served as control group were involved, (their mean age was 34.51±11.47).
Blood Sampling: Samples were obtained at Al-Imamain Al-Kadhimain Medical City and Ibn Al-Nafees Hospital for Cardiovascular Medicine and Surgery, during the period from September 2019 to May 2021.

Five milliliters of blood samples were obtained from all participants. Four milliliters of blood samples were left for 20 minutes in the gel tube at room temperature. Serum was obtained by centrifugation at 2000 rpm for 15 min. and used for measurement of lipid profile, renal function, blood sugar, lipoprotein electrophoresis, Apo-B, Apo-E, RLP Cholesterol and one mL of fresh blood was left in EDTA tube for HbA1c test by Atellica CH analyzer.

Ready commercial kits (Atellica IM, Germany) were used for measurement of lipid profile (TC, TG, HDL-c and LDL-c), glucose, urea creatinine and BUN.

Elisa kits (Melsin Medical) were used for Apo-B, Apo- E and RLP cholesterol.
Statistical Analysis: Statistical analyses were performed by using SPSS software version 25.0 (SPSS, Chicago). Continuous data were subjected to normality test (Shapiro Wilk test). Data with normally distribution were presented as mean and standard deviation and analyzed with analysis of variance (ANOVA). Data with non-normal distribution were presented as median and range and analyzed with Kruskal Wallis. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Receiver operating characteristic curve (ROC) was used to evaluate the diagnostic value of some markers in the context of discrimination between patients with and without DBL. A

p- value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Demographic Characteristics of the Study Population: The mean age of the patients with DM and CVD was similar to that of controls with no significant difference. Females were more frequent in DM group than either CVD group or controls with no significant differences. However, the mean weight and BMI in control group was significantly lower than that of DM or CVD groups, (table1).

Table 1: Demographic characteristics of the study population

Variables	Diabetes (n=50)	CVD (n=26)	Controls (n=73)	p- value
Age, years Mean ±SD Range	46.48±9.3 25-67	43.15±7.34 29-57	43.51±11.47 18-56	0.286
Gender Male Female	20(40%) 30(60%)	12(46.15%) 14(53.85%)	30(41.1%) 43(58.9%)	0.868
BMI Mean± SD Range	31.74±6.37[a] 21.33-49.05	32.1 ±2.83[a] 20.31-41.67	28.11 ±5.98[b] 18.69- 46.88	0.003

Different small letters indicate significant differences

Serum lipid Profile: Table 2 shows serum lipid profile in patients and controls. Data regarding the components of lipid profile were found to be non-normally distributed. Accordingly, data were mostly expressed as median and nonparametric Kruskal Wallis test was used to compare the medians of the groups. Median serum level of TC and TG in DM group did not differ significantly from that of CVD group, However, the two groups were significantly higher than controls. Similarly, patients in DM and CVD groups had significantly higher level of LDL-c, VLDL-c and non-HDL-c with highly significant differences.

Table 2: Serum lipid profile in different groups

Variables	Diabetes (n=50)	CVD (n=26)	Controls (n=73)	p-value
TC, mg/dL Mean± SD Median Range	254.77±50.65 245.59[a] 185.84-420	258.47±85.09 231.04[a] 201-639.64	151.45±30.01 150.54[b] 74.85-198	<0.001
TG, mg/dL Mean± SD Median Range	347.61±206.04 287[a] 150.45-1106.25	455.44±443.68 305.75[a] 58-2214	92.20±32.85 97.35[b] 38-150	<0.001
HDL-c, mg/dL Mean± SD Median Range	50.99±14.74 50.09 21.96-108	55.48±18.76 50.2 31.3-113.52	50.3±14.11 49.3 23.16-88.78	0.667
LDL-c, mg/dL Mean± SD Median Range	145.26±50.98 137.44[a] 33.51-274.4	140.76±85.04 124.1[a] 25.02-480.20	83.21±24.59 82.5[b] 34.89-135.29	<0.001
VLDL-c, mg/dL Mean± SD Median Range	69.47±41.21 57.4[a] 30.09-221.25	76.50±50.72 53.21[a] 11.70-180	18.28±6.48 19.47[b] 7.60-30.09	<0.001
Non-HDL-c mg/dL Mean± SD Median Range	204.98±50.74 196.79[a] 104-378.	202.99±85.53 182.32[a] 128-583.94	101.15±26.83 105.4[b] 44.1-161.84	<0.001

TG: triglycerides, HDL-c: high density lipoprotein cholesterol, LDL-c: low density lipoprotein cholesterol, VLDL-c : very low-density lipoprotein cholesterol. Different small letters indicate significant differences

The median level of α-LP in DM and CVD was significantly lower than that of controls. In contrast, the serum level of preβ-LP, β-LP, Apo-B, Apo-E and RLP were higher in DM and CVD patients than controls with highly significant differences.

For derived ratios, each of non-HDL/Apo-B, TC/Apo-B and TG/Apo-B ration was higher in DM group and CVD group than their controls with highly significant differences (Table 3).

Table 3: Serum lipoprotein and Derived Lipid profile ratios.

Variables	Diabetes (n=50)	CVD (n= 26)	Controls (n=73)	p-value
α-LP, % Mean±SD Median Range	25.20±8.27 26.67[a] 2.78-38.22	27.19±5.52 26.59[a] 13.87-37.12	32.20±9.39 30.35[b] 11.82-50.25	<0.001
Preβ-LP, % Mean ±SD Median Range	51.75±9.75 53.67 26.81-67.55	50.446±8.37 50.18 34.74-68.82	51.44±8.30 53 38.12-53.78	0.481
β-LP, % Mean± SD Median Range	21.95±11.12 18.67[a] 9.64-53.78	50.46±8.37 20.08[a] 5.27-38.93	51.44±8.31 15.64[b] 4.82-51.25	0.002
APO-B, µg/ml Mean± SD Median Range	1115.7±200.54 1137.2[a] 710.6-1677.59	1282.35±135.11 1254.79[a] 1061.83-1677.59	941.88±99.72 914.76[b] 710.63-1240.7	<0.001
APO-E, µg/ml Mean± SD Range	4.06±0.393 3.16-5.22[a]	4.06±0.49 3.25-5.35[a]	3.09±0.55 2.37-4.10[b]	<0.001
Remnant LP, µmol/L Mean ± SD Median Range	664.20±662.62 552.84 218.28-50006.3	584.35±203.44 556.37 208.81-1125.82	342.79±523.97 171.75 23.46-3131.58	<0.001
Non-HDL-c/Apo-B Mean ± SD Median Range	0.16±0.04 0.150[a] 0.08-0.27	0.16±0.07 0.150[a] 0.08-13.52	0.11±0.03 0.11[b] 0.05-0.19	<0.001
Remnant/TG Mean ± SD Median Range	2.38±2.39 1.82 0.43-16.69	2.34±2.18 1.62 0.16-10.81	4.73±10.31 2.22 0.18-63.91	0.201
VLDL/TG Mean ± SD Median Range	0.188±0.01 0.2 0.19-0.20	0.194-0.36 0.2 0.02-0.22	0.197±0.23 0.2 0.00-0.20	0.385
TC/Apo-B Mean ± SD Range	0.18±0.05[a] 0.15-15.37	0.20±0.07[a] 0.13-0.53	0.16±0.03[b] 0.09-0.23	<0.001
TG/Apo-B Mean ± SD Median Range	0.27±0.15 0.22[a] 0.12-17.42	0.37±0.4 0.21[a] 0.04-2.02	0.1±0.04 0.09[b] 0.04-0.20	<0.001

Different small letters indicate significant differences

The Proportion of Dysbetalipoproteinemia: According to the Sniderman algorithm, out of the total 76 patients (with DM or CVD), 13 (18.06%) patients have had DBL disorder and 59 (81.94%) patients have no DBL (Figure 1). On the other hand, none of control group had this disorder. Therefore, it was excluded from further analysis.

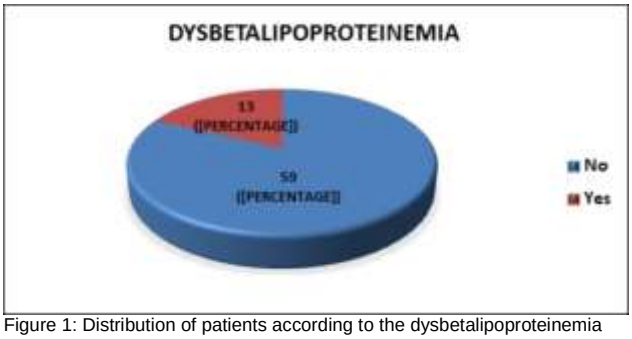

Figure 1: Distribution of patients according to the dysbetalipoproteinemia

Association of Demographic Characteristics with Dysbetalipoproteinemia: Table 4 presents the demographic data of the study population. No significant difference was demonstrated regarding the age, gender, and the underlying diseases between those with- and without DBL. Although the majority of patients with DBL were with DM group (11 patients) compared with 2 patients from CVD group, however the difference was not significant.

Table 4. Demographic data of the study population

Variable	With DBL (n=13)	Without DBL (n=63)	p-value
Age (years) Mean ±SD	42.08±7.47	46.02±8.2	0.782
Gender (male/female)	3/10	29/34	0.127
Underlining disease DM/CVD	11/2	39/24	0.116

DM = diabetes mellitus; CVD = cardiovascular disease

Association of diabetic, renal function tests and lipid profile with dysbetalipoproteinemia: To study the possible association between DM related-factors and the presence of DBL, table 5 illustrates no significant difference in the HbA1c and glucose level between those with- and without DBL.

Table 5: Association of diabetic related-factors and dysbetalipoproteinemia

Variable	With DBL (n=13)	Without DBL (n=63)	p-value
HbA1c (%)	7.05±1.89	7.07±2.07	0.235
Glucose (mg/dL)	145.65±62.97	158.61±84.12	0.085

Similarly, no association was noticed between renal function tests (blood urea, creatinine, and blood urea nitrogen levels) and the presence or absence of DBL (Table 8).

Table 6: Renal function test-related factors and dysbetalipoproteinemia

Variable median / range	With DBL (n=13)	Without DBL (n=63)	p-value
Blood urea (mg/dL)	26.00 14.34-40.0	27.00 12.93-64.0	0.370
BUN (mg/dL)	12.15 6.70-18.69	12.62 6.04-29.91	0.385
Creatinine (mg/dL)	0.74 0.47-1.13	0.89 0.42-1.43	0.310

BUN = blood urea nitrogen

For serum lipid profile, the TC and LDL levels were significantly higher in patients with DBL as compared with those without DBL. Likewise, non-HDL-c and non-HDL-c/Apo-B were significantly higher in patients with DBL as compared with those without DBL Furthermore, TC/Apo-B was significantly higher in those with DBL, as compared with those without DBL (2.59 vs. 1.83). On the contrary, the HDL-c, VLDL-c, TG, α-Lp, preβ-Lp, β-LP, Apo-B, Apo-E, remnant LP levels, remnant LP/TG, VLDL/TG, and TG/Apo-B were not significantly different between the two groups (Table 3-14).

Table 7: Lipid profile test-related factors and dysbetalipoproteinemia

Variable median / range	With DBL (n=13)	Without DBL (n=63)	p-value
TC (mg/dL)	312.0 / 281-639.64	228.0 /185.84-311	<0.001
HDL-c (mg/dL)	54.0 /35-84.92	50.0 / 21.96-244	0.279
LDL-c (mg/dL)	200.3 / 33.51-480.2	131.1 / 25.02-244	0.002
VLDL-c (mg/dL)	65.60 / 28.5-221.25	55.60 / 11.7-180	0.282
TG (mg/dL)	238.00 / 143-1106.25	286.00 /58-2214	0.338
α-Lipoprotein, %	24.24 / 2.78-33.02	26.81 / 2.9-38.22	0.174
Pre β-Lipoprotein, %	53.56 / 27.4-67.12	52.61 / 26.81-68.82	0.994
β-Lipoprotein, %	18.83 / 8.64-53.78	18.85 / 5.27-53.38	0.777
Apoprotein-B, µg/mL	124.01/ 111.8-145.28	127.86 / 106.18-167.76	0.071
Apoprotein-E, µg/mL	3.85 / 3.37-4.51	4.12 /3.16-5.35	0.373
Remnant LP, µmol/L	512.49 / 308.81-978.38	562.06 / 218.28-5006.29	0.327
Non-HDL-c, mg/dL	254.76 / 221.4-583.94	181.9 / 104-274.12	<0.001
Non-HDL-c/Apo- B	0.21/ 0.18-0.48	0.14/ 0.08-0.20	<0.001
Remnant/TG	1.58 / 0.55-3.36	1.80 / 0.16-16.69	0.188
VLDL/TG	0.20 / 0.02-0.02	0.20 / 0.02-0.22	1.00
TC/Apo-B	2.59 / 2.4-5.31	1.83 / 1.26-2.39	<0.001
TG/Apo-B	2.93/ 1.19-8.47	2.17 / 0.44-20.21	0.227

TC = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ApoB = apoprotein-B; VLDL = very low-density lipoprotein; TG = triglyceride.

Diagnostic value of non-HDL-c and non-HDL-c/apo B: The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of non-HDL-c and non-HDL-c/Apo-B in the context of discrimination between patients with- and without DBL. The result is shown in Figure 2.

For the non-HDL-c, the area under the curve (AUC) was 0.947, 95%CI=0.900-0.995, p=0000. The sensitivity and specificity of the test at the cutoff value of non-HDL= 227.1 was 85 % and 89 %, respectively.

For non-HDL-c /Apo-B, the AUC was 0.980, 95%CI=0.954-1.000, p=0000. The sensitivity and specificity of the test at the cutoff value of non-HDL/Apo-B= 0.16 was 77% and 52%, respectively.

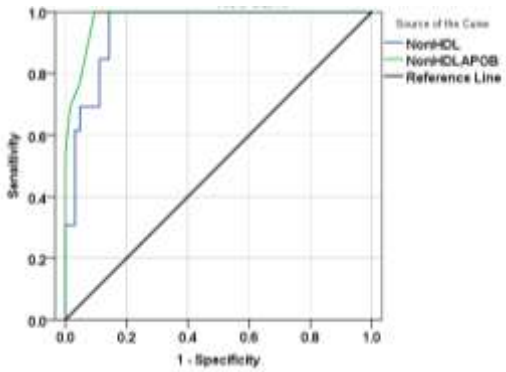

Figure 2: ROC curve for the non-HDL-c and non-HDL-c /Apo-B to diagnose cases with dysbetalipoproteinemia**.**

DISCUSSION

The results of this study showed that none of the variables suggested for estimation of DBL can be used alone. Until now the Sniderman formula used could satisfy the aim of this study.

As concerning the present results the age of patients and controls was over 20 years which is the age after which DBL symptoms were reported to appear. The male /female ration was almost the same in each group, however the more predominant affected individuals with DBL were females; a finding which agrees with recent report.[8]

The base of Sniderman algorithm includes Apo-B, TC/Apo-B and TG/Apo-B[8]. All have been recommended by many authors to be good markers for DBL. Apo-B100 (Apo-B) is the structural protein of atherogenic lipoproteins including VLDL, intermediate-density lipoprotein and LDL and these constitute the non-HDL part of serum lipids[5].

The lipoprotein gel electrophoresis did not give conclusive results in the present study. Previous reports noted that the results of serum agarose gel electrophoresis, had no good agreement with the results of Apo-B gene polymorphism, and was attributed, possibly, to an error in the visual interpretation of the electrophoresis results.[14]

A fluctuation in serum lipid concentrations in DBL from mixed or isolated hyperlipidemia, or normal lipid profile with high apo-B was found in pervious study[1].

Diabetes mellitus and obesity are considered factors which precipitate DBL symptoms[10], while DBL was claimed to be a major cause of premature coronary heart disease.[3,15] A recent report attributed the variation in serum lipoprotein and the risk of atherosclerosis to the common three variants of Apo-E .[16]

None of the patients (in either group) showed signs of renal failure, despite the higher renal function parameters than those of the control group, and apart from the diabetic patients no CVD patient showed high HbA1c.

The ROC analysis to assess the diagnostic value of non-HDL-c and non-HDL-c/Apo-B reveals higher specificity and sensitivity for the former, however the non-HDL-c / Apo-B ration was reported to be good variable for screening DBL[5].

This piolet study is, for our knowledge, the first report on DBL in Iraq, and it shows that the incidence of the heterozygous type of this disorder is 18.06 % among diabetic and cardiovascular patients. Unfortunately, the number of CVD patients was low relative to that of the DM patients which makes the comparison between the two groups invalid.

CONCLUSION

considering Sniderman algorithm, we may say that the non-HDL-c has a good specificity and sensitivity to diagnose DBL, and that the incidence of heterozygous DBL in Iraqi patients with T2DM and CVD is 18.06 %. However, larger screening survey, on the country level, is needed to detect Apo-E gene polymorphism in a large number of Iraqi populations and to be a base for future studies on this subject.

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