Original paper

Inter-relationship between low-density lipoprotein phenotype and carotid intima-media thickness in North Indian type 2 diabetic subjects

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ARTICLE INFO

ABSTRACT

Aims: Diabetic dyslipidemia is characterized by a preponderance of small dense LDL which is highly atherogenic. The aim of this study was to examine the interrelationship between LDL Phenotype and atherosclerosis; to determine the factors determining LDL phenotype; and evaluate LDLc:apo-B ratio as a surrogate for the assessment of LDL phenotype in a group of North Indian Type 2 diabetic subjects.
Methods: 285 diabetic subjects attending the outpatient Endocrine Clinic were subjected to detailed anthropometry and fasting serum lipid and apo-B was measured. The carotid intima-media thickness (IMT) was determined using a high resolution B-mode Ultrasonography. LDLc:apo-B ratio was taken as a surrogate index for LDL size.
Results: 29.5% patients with normal triglyceride levels and 52.1% patients with normal LDLc levels showed the presence of small dense LDL or Phenotype B as estimated by the LDL cholesterol/apo-B ratio. The mean IMT in Phenotype B group was higher (0.88 mm vs. 0.68 mm). Triglycerides was the most important predictor variable predicting carotid IMT ($R^2 = 0.15$, $\beta = 0.376$) as well as LDL phenotype B ($R^2 = 0.28$, $\beta = 0.561$).
Conclusions: Triglycerides and HDLc contribute independently to the variability in LDL particle size, and LDL particle size was associated with preclinical atherosclerosis as determined by carotid IMT in North Indian Type 2 diabetic subjects. LDL cholesterol/apo-B ratio serves as an easy clinical tool to determine the elevated small dense LDL.

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1. Introduction

Diabetic dyslipidemia is characterized by elevated triglyceride, low HDL cholesterol (HDLc), and a preponderance of small dense LDL phenotype or Phenotype B [1,2]. LDL cholesterol (LDLc) lowering is the primary lipid target in current guidelines but it does not fully account for the cardiovascular risk associated with diabetes, either alone or in combination with triglycerides and HDLc [3,4]. More than 50% of the subjects with CAD have normal LDLc levels, thus the calculated LDLc fails to be an adequate index of lipid associated risk. Measurements beyond traditional lipids, such as measurements of the presence of small dense LDL in patients with diabetes, may help to identify cardiovascular risk subgroups and individualize hypolipidemic treatments [5].

In several cross-sectional studies subjects with coronary artery disease (CAD) have had smaller and denser LDL particles than controls [6–8]. The determination of LDL size with density gradient ultracentrifugation and gradient gel electrophoresis [9], is difficult, time consuming and requires specialized instruments. Electrophoresis quantifies just the size of the predominant LDL species or the average size of LDL. NMR which is another rapid and convenient method for determining LDL size and subfractions concentration is limited by lack of published data on detailed procedures, calibration, and validation. LDLc:apo-B ratio can be used as a surrogate for the assessment of LDL phenotype as shown in the AMORIS study [10]. Several reports have shown that a higher LDLc:apo-B ratio identifies subjects with predominantly large buoyant LDL particles, whereas a low value indicates the presence of predominantly small dense LDL particles [11–13]. It has been suggested that the intima-media thickness (IMT) of the common carotid artery may be the most sensitive marker for the earliest stages of atherosclerosis [14].

Very few studies have examined the interrelationship between LDL Phenotype and atherosclerosis and the factors determining of LDL phenotype in North Indian Type 2 diabetic subjects. The aim of this study was to evaluate the interrelationship between LDL particle size, insulin resistance and atherosclerosis thus identifying diabetic subjects with high cardiovascular risk.

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2. Methods

2.1. Study population

The study population consisted of 285 diabetic subjects (153 men and 132 women) attending the outpatient Endocrine Clinic at the Centre for Diabetes and Endocrinology. The mean age of diabetic subjects was 50.6 ± 9.6 years (Range 40–70 years). Patients with recent myocardial infarction or acute coronary syndrome; cerebrovascular accidents; inherited or acquired disorders of lipid/lipoprotein metabolism and/or family history of such diseases; deranged liver function; or medications known to affect lipid metabolism (other than a sulfonylurea) were excluded from the study. Informed consent was obtained from all subjects. The local medical ethics committee approved the study, which was carried out in accordance with the Declaration of Helsinki.

2.2. Anthropometric measurements

Physical examination included height and weight measurements and the body mass index (BMI) were calculated. Waist measurement was done in the standing position [15]. Blood pressure (BP) was recorded to the nearest 2 mmHg in the sitting position in the right arm with a mercury sphygmomanometer.

2.3. Laboratory measurements

Measurements of total cholesterol, triglycerides, HDLc, and LDLc were performed on serum collected after the subject's fasted overnight, using enzymatic methods (Pointe Scientific Inc., MI, USA). LDLc was calculated using the Friedewald formula [16]. Hemoglobin A_{1C} (HbA$_{1C}$) was measured by cation exchange resin as per directives of the supplier of the kit, Pointe Scientific Inc., MI, USA with normal values ranging from 4.6% to 5.8%. Apolipoprotein B (apo-B) was estimated by immune-turbidometry as per the directives of SPINREACT diagnostic systems; SPAIN.

2.4. Low-density lipoprotein subtypes

LDLc:apo-B ratio was taken as a surrogate index for LDL size as in the updated analysis of the AMORIS study [10]. LDLc:apo-B ratio <1.20 and ≥1.20 constituted Phenotype B/small dense LDL particle and Phenotype A/large buoyant LDL particle, respectively [17].

2.5. Ultrasonographic evaluation of carotid arteries

The intima-media thickness of the carotid arteries was determined using a high resolution B-mode Ultrasonography system (Logic 500 Proseries; Wipro GE) having an electrical linear transducer (multi-frequency probe of 5–9 MHz). An IMT more than 0.8 mm represents early changes of atherosclerosis [18–20]. Undulation and thickening of the internal line indicate plaque deposition which is echogenic. Focal depression, break in plaque surface or anechoic area within the plaque or slow moving eddies of colour within an anechoic region suggest plaque ulceration.

2.6. Statistical analysis

Statistical analysis was performed using SPSS version 11.0 statistical package for Windows (SPSS, Chicago, IL). Continuous variables are expressed as mean ± S.D. (Gaussian distribution) or range, and qualitative data is expressed in percentages. For continuous variables, and depending on normality distribution, unpaired t tests were used if comparing two groups. The association between continuous variables was tested by linear correlation. All tests were two-tailed, and a P value of ≤0.05 was considered significant. Stepwise multiple linear regression models were performed with LDL phenotype and carotid IMT as the dependent variable.

3. Results

3.1. Characteristics of the study subjects

The clinical and biological features of the 285 type 2 diabetic subjects (153 men and 132 women) included in the study are shown in Table 1. 183 (64.2%) patients were found to be hypertensive. Hypercholesterolemia, hypertriglyceridemia and low HDLc was present in 69 (24.2%), 102 (35.5%) and 96 (33%) subjects, respectively. High IMT (0.8 mm and above) was seen in 102 (35.8) subjects whereas LDLc:apo-B ratio less than 1.20 (Phenotype B) was found in 126 (44.2%) subjects. The mean IMT in Phenotype B group was higher (0.88 mm vs. 0.68 mm). LDL cholesterol/apo-B ratio was significantly lower in men than in women. The LDL$_c$:apo-B ratio in pattern B group was 1.16 ± 0.25.

Table 1
Patient characteristics according to LDLc phenotype.

Variables	Whole cohort n = 285	Phenotype A n = 159	Phenotype B n = 126
Age (years)	50.6 ± 9.6	50.5 ± 9.2	50.7 ± 10.1
Systolic BP (mmHg)	137.8 ± 19.8	137.4 ± 18.6	138.4 ± 21.3
Diastolic BP (mmHg)	86.9 ± 11.3	85.5 ± 12.2	88.6 ± 9.8
Body mass index (kg/m²)	26.1 ± 4.4	25.8 ± 5.0	26.3 ± 3.5
Waist:hip ratio	0.94 ± 0.10	0.94 ± 0.06	0.94 ± 0.14
Fasting PG (mg%)	130.3 ± 43.4	132.7 ± 48.2	127.4 ± 36.6
Post-prandial PG (mg%)	186.4 ± 59.4	186.3 ± 58.8	186.5 ± 60.2
HBA$_{1C}$ (%)	8.7 ± 0.6	8.7 ± 0.6	8.6 ± 0.6
Urinary albumin (mg/l)	31.4 ± 82.7	31.2 ± 96.0	31.6 ± 62.4
VLDL cholesterol (mg/dl)	32.5 ± 12.1	25.9 ± 8.5	40.9 ± 10.7
HDL cholesterol (mg/dl)	42.6 ± 6.6	43.18 ± 5.24	41.9 ± 8.0
Trigycerides (mg/dl)	153.3 ± 56.6	117.86 ± 26.80	197.9 ± 52.6
LDL cholesterol (mg/dl)	108.6 ± 25.5	103.37 ± 21.14	115.2 ± 28.8
Total cholesterol (mg/dl)	182.2 ± 32.0	171.1 ± 26.5	196.3 ± 32.9
Non-HDL Cholesterol (mg/dl)	139.6 ± 30.4	127.1 ± 20.8	155.3 ± 33.2
apo-B (mg/dl)	110.6 ± 37.9	91.2 ± 27.9	135.1 ± 34.6
LDL:apo-B ratio	1.24 ± .28	1.31 ± 0.23	1.16 ± 0.25
Mean IMT (mm)	0.78 ± .13	0.68 ± 0.12	0.88 ± 0.14

PG = plasma glucose; IMT = intima-medial thickness.