LDL SUBCLASS PATTERNS AND ATHEROGENICITY IN NON-INSULIN DEPENDENT DIABETES MELLITUS

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Abstract- Low density lipoprotein (LDL) subclass pattern B is characterized by predominance of small dense LDL. Subjects with pattern B tend to have higher triglyceride (TG) and lower levels of high density lipoprotein cholesterol (HDL-C). Small dense LDL is associated with increased risk of coronary disease. In this study, effect of non-insulin dependent diabetes mellitus (NIDDM) on LDL size, plasma TG and HDL-C were assessed in Iranian patients. LDL size was determined by non-denaturing gradient polyacrylamide gel electrophoresis in 81 NIDDM and 81 healthy subjects (age 50 to 70 yrs). TG and HDL-C were measured by analytical kits. Fifty nine percent of diabetics and 27% of controls showed LDL pattern B. LDL size was significantly lower in diabetics than controls (25.1± 1.5 vs 25.8± 2.8 nm, $P<0.001$). Diabetics had significantly higher triglyceride than controls (187.8 ± 90 vs 145.6 ± 69 mg/dl, $P<0.001$) and lower HDL-C (47.5± 12 vs 57.1± 14 mg/dl, $P<0.001$). LDL size correlated negatively with TG (r = -0.281, $P<0.05$). Diabetics showed 2-fold increase in frequency of pattern B which may explain increased risk of coronary disease in these patients.

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Key words: Small dense low density lipoprotein, pattern B, triglyceride, non-insulin dependent diabetes mellitus

INTRODUCTION

Low density lipoprotein (LDL) particles are non homogenous in terms of size, density and composition (1). Austin *et al.* described two distinct LDL subclasses denoted the A and B patterns (2). Type A pattern is characterized by predominance of large buoyant LDL particles with LDL peak diameter of > 25.5 nm; type B pattern is characterized by predominance of small dense LDL particles with LDL peak diameter of ≤ 25.5 nm (2,3). Pattern B usually occurs with moderately raised plasma triglyceride (TG) and reduced levels of high density lipoprotein cholesterol (HDL-C). This lipid profile is known as atherogenic lipoprotein phenotype. Atherogenic lipoprotein phenotype may be the product of dyslipidemia which could be related to insulin resistance (4,5).

Association between LDL particle size and coronary artery disease has been suggested in a number of investigations (6,7). Increased atherogenicity of small dense LDL is thought to be due to a number of characteristics including increased susceptibility to oxidation, enhanced endothelial penetration and decreased affinity for LDL receptors as a consequence of apo B conformational changes which result in longer period of residence in plasma and an increased affinity for arterial proteoglycans (8).

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Non-insulin dependent diabetes mellitus (NIDDM) is associated with 2 to 4 fold increase of coronary artery disease (9). Investigations indicate an increase in small dense LDL particles in NIDDM patients (10).

Regarding ethnic differences found in LDL size distribution (11-13), in this study LDL size phenotype in Iranian NIDDM patients was determined for the first time. Furthermore, association between LDL size and plasma levels of TG and HDL-C was assessed.

MATERIALS AND METHODS

Eighty one men and women diagnosed with NIDDM according to the criteria of the World Health Organization, who were under treatment with hypoglycemic agents or insulin and aged 50 to 70 years, were selected.

Eighty one healthy individuals of the same age range with fasting glucose levels less than 126 mg/dl were also selected as the control group. Volunteers were non-smokers with no evidence of renal, thyroid or liver disease. None of the subjects had taken medications that could affect lipid metabolism for at least 3 weeks before the initiation of the study (14). We obtained informed consent from all NIDDM patients and healthy individuals.

Body mass index (BMI, weight in kilograms divided by the square of height in meters) was calculated in all participants. After an over night 12 to 14 hour fast, blood was drawn from the subjects into tubes containing EDTA (final concentration 1 mg/ml). Plasma was isolated and stored as individual aliquots at –70 °C (15). The particle size of LDL was determined by 2-16% non-denaturing polyacrylamide gel electrophoresis. Gradient gels were cast on the basis of published protocols (16). Briefly, electrophoresis was carried out at 8-10°C in 2-16% polyacrylamide gradient gels using Tris base (90 mM), boric acid (80 mM) buffer, pH 8.3, containing 3 mM EDTA and 3 mM NaN3. The gels were prerun in Tris buffer at 120 V for 20 minutes. Seven µl plasma was subjected to electrophoresis after diluting 4:1 with 50% sucrose and 0.01% bromophenol blue tracking.

For particle size calibration two separate preparations were applied to two separate lanes in the gel. One calibration preparation consisted of standard protein mixture of thyroglobulin 17 nm, ferritin 12.2 nm, catalase 10.4 nm, lactate dehydrogenase 8.1 nm, albumin 7 nm (Pharmacia Biotech, St Albans ,UK) and the second preparation consisted of a solution of latex particles 50 nm (Alfa Aesar). Voltage was set to 15 V for 15 minutes followed by 70 V for 20 minutes and finally 125 V for 24 hours. For lipid staining Oil Red O and for protein staining Coomassie R-250 was used (Figures 1 and 2).

Gels were then scanned with a computer assisted densitometer, using UVI photo MW software adapted to UVI Tech densitometer. Peak particle diameter referred to as LDL size was calculated from calibration curve using above mentioned high molecular weight standards. Plasma TG and HDL-C concentrations were determined by commercially available enzyme based assays (Ziest Chimie, Iran).

The dextran sulfate–Mg^{2+} precipitation procedure was used to precipitate VLDL and LDL before measuring HDL-C (17).

Statistical analysis

Statistical analyses were all performed with SPSS statistical package. Data are presented as mean±SD.

The following tests were performed: independent *t* test, Pearson correlation, Z-test for comparison of correlation coefficients. Two tailed *P* value < 0.05 was considered significant.

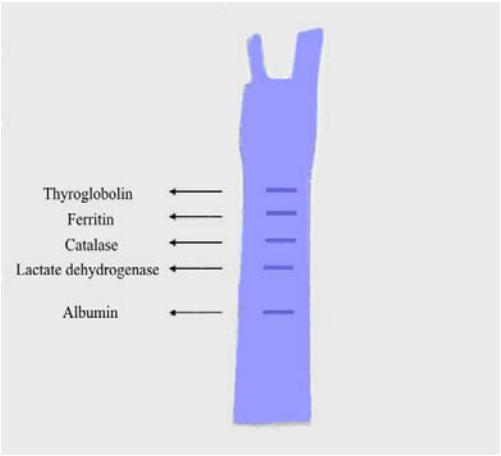

Fig. 1. Protein standard mixture on 2-16 % PAGE.

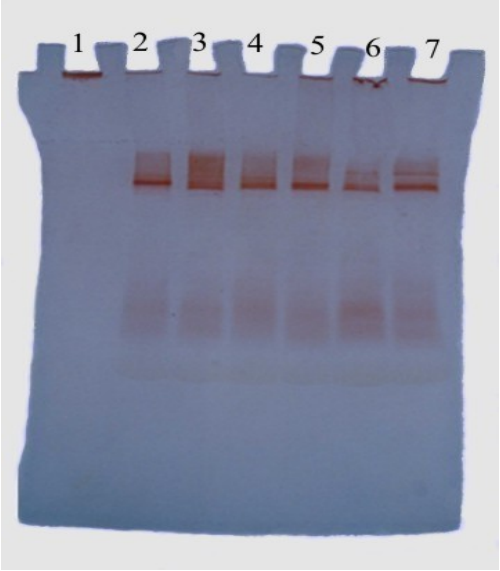

Fig. 2. Well no. 1 containing standard latex particles and wells no. 2, 3, 4, 5, 6 and 7 containing plasma samples.

RESULTS

As shown in table 1, compared to controls diabetics had significantly higher triglyceride levels (187.86±90 vs 145.6±69 mg/dl, *P*< 0.001) and BMI (27.2±1.5 vs 25.2±1.4 kg/m2, *P*<0.001), and lower HDL-C (47.5±12 vs 57.1±14 mg/dl, *P*<0.001).

Table 1. Clinical and metabolic characteristics of diabetics and controls*

Characteristic	Diabetics (n= 81)	Controls (n= 81)	*P* value
Age (year)	56±6.4	53±4.6	
BMI (kg/m2)	27.2±1.5	25.2±14	< 0001
TG (mg/dl)	187.86+90	145.6±69	< 0.001
HDL-C (mg/dl)	47.5+1.2	57.1±14.6	< 0.001
LDL size (nm)	25.1±1.5	25.8± 2	< 0.05
LDL Phenotype			
A(%)	40.7†	73†	
B(%)	59.3†	27†	

Abbreviations: BMI, body mass index; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL, low density lipoprotein.

*Data are presented as mean±SD unless otherwise specified.

† Mean.

Measurement of LDL predominant particle diameter in both diabetic and control group by use of non-denaturing polyacrylamide gel electrophoresis revealed that 59% of diabetics and 27% of control subjects show LDL pattern B (Table 1).

As expected, LDL size was significantly lower in diabetic subjects than controls (25.1±1.5 vs 25.8±2.1 nm, *P*<0.05) (Table 1). As shown in table 2, after stratification for sex, diabetic men have significantly smaller LDL size, higher triglyceride and lower BMI compared to women. HDL-C levels in men were lower than women although this was not statistically significant.

Table 3 shows correlation between LDL size and a number of clinical and biochemical parameters, which reveals that in diabetics TG is negatively correlated with LDL size (r = -0.281, *P*<0.05). Relationship between HDL-C and LDL size however had only a borderline significance (r = 0.215, *P* = 0.054). We found no relationship between LDL size and BMI in diabetics. As shown in table 3 only HDL-C was positively correlated with LDL size in control group (r = 0.364, *P*<0.001).

We performed Z-test to compare the correlation coefficients in diabetics and controls. Based on our results only the correlation coefficient between TG and LDL size among diabetics and controls showed significant difference (*P*< 0.005).

Table 2. Clinical and metabolic characteristics of diabetics by sex*

Characteristic	Men (n=35)	Women (n= 46)	*P* value
Age (years)	55.9± 5.6	57.7±6.8	
BMI (kg/m^2)	26.6±1.6	27.7±1.3	<0.05
TG (mg/dl)	217.8±102	165±74	<0.05
HDL-C (mg/dl)	46.9±9.5	47.9±15	0.7
LDL size (nm)	24.6± 1.7	25.5±1.3	<0.05
LDL Phenotype			
A (%)	25.2†	52.2†	
B (%)	74.8†	47.8†	

Abbreviations: BMI, body mass index; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL, low density lipoprotein.

*Data are presented as mean±SD unless otherwise specified.

† Mean.

Table 3. The correlation between LDL size and parameters listed in diabetic and control

	Diabetics (n=81)		Controls (n=81)	
Parameter	**r**	***P* value**	**r**	***P* value**
BMI	- 0.092	0.414	0.119	0298
HDL-C	0.215	0.054	0.364	<0.001
TG	-0.281	<0.05	-0.059	0.598

Abbreviations: BMI, body mass index; HDL-C, high density lipoprotein cholesterol; TG, triglyceride.

DISCUSSION

Investigations indicate an increase in small dense LDL particles in NIDDM patients. In this study, effect of NIDDM on LDL size, plasma TG and HDL-C were assessed in Iranian patients. Diabetics showed higher TG and BMI and lower HDL-C compared with non-diabetic subjects.

Numerous studies have described lipid and lipoprotein abnormalities associated with diabetes mellitus (18-20). Due to insulin resistance, the suppression of hepatic VLDL secretion by insulin is impaired in NIDDM patients; furthermore, lipoprotein lipase activity in these patients is decreased which together results in increased concentration of VLDL in circulation. These VLDL particles are rich in TG and therefore help to promote hypertriglyceridemia.

Hypertriglyceridemia changes the composition of all lipoproteins, enriches them with TG and makes them a better substrate for hepatic lipase. This leads to decreased levels of HDL-C (21).

Based on our results diabetic men show higher TG ($P< 0.05$) and lower HDL-C levels (not significant). In a biethnic population of Mexican American and non-Hispanic whites, Haffner *et al.* also reported that diabetic men have smaller LDL size and higher percentage of pattern B than women. They also had lower BMI, lower HDL-C and higher TG than did women (22). In the contrary, Walden *et al.* demonstrated that diabetic women have consistently higher VLDL-TG and LDL-C levels and lower HDL-C levels than control subjects or diabetic men (23). Siegel *et al.* showed that diabetes in women leads to more profound lipoprotein abnormalities than in men, with significant hypertriglyceridemia, elevated apo B and lower HDL-C and apo A_1 being noted (24). These inconsistencies may be due to differences in the classification of diabetics, assessment methods, populations under study and differences in subject characteristics.

As many groups have reported previously (25,26), we also found a negative correlation between LDL size and TG in diabetics, who showed moderately high concentration of TG. We found no relationship between TG and LDL size in controls. This study supports previous findings that in even moderate hypertriglyceridemia increased concentration of small dense LDL has to be expected (27). Probably hyperglyceridemia increases the rate of cholesterol and TG transfer between VLDL-TG rich particles and LDL, resulting in TG enriched cholesterol-ester depleted LDL particles. These particles are susceptible to action of hepatic lipase which hydrolysis LDL triglyceride, resulting in formation of small dense LDL particles (28).

In this study smaller LDL size in diabetic men compared to diabetic women may be also due to higher TG levels in men compared to women.

We observed a 2 fold increase in the prevalence of LDL pattern B in diabetic subjects compared to control group, which is consistent with previous findings (29). Furthermore, we observed significant difference in LDL size between diabetic patients and control groups.

In accordance with present study, smaller LDL size in diabetics has been reported previously (10,23,30). Factors determining higher prevalence of small dense LDL in diabetics are not fully understood. However, it has been suggested that hypertriglyceridemia (27), decreased activity of lipoprotein lipase and increased activity of hepatic lipase in NIDDM subjects (31) facilitate formation of small dense LDL particles.

In conclusion, we found that Iranian NIDDM patients show a two fold increase in the prevalence of LDL pattern B. LDL size in diabetics is significantly lower than controls. In addition, diabetics have lower plasma HDL-C and higher TG levels compared to control group. This all may partly explain an increased risk of coronary artery disease in these patients.

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