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The association between small dense low density lipoprotein, apolipoprotein B, apolipoprotein B/apolipoprotein A1 ratio and coronary artery stenosis*

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Abstract

Background: Recently, small dense low density lipoprotein (sdLDL) has been highlighted as a new risk factor for the coronary artery disease (CAD).Small dense LDLs are believed to be atherogenic since these particles are taken up more easily by arterial wall. They are readily oxidized and have reduced affinity for low density lipoprotein (LDL) receptor and increased affinity for arterial proteoglicans. LDL cholesterol is only a measure of the cholesterol level in the LDL whereas apolipoprotein B(apo B) is a measure of the cholesterol levels of all the atherogenic particles, including very low density lipoprotein, intermediate density, and low density lipoproteins. Therefore, it might be a better marker than other traditional lipids. The aim of the present study was to evaluate the association between serum small dense LDL, apolipoprotein B, apolipoprotein A1 (apo A1) and apoB/apoA1 ratio and the coronary stenosis.

Methods: 86 patients with coronary stenosis , 35 patients without coronary stenosis identified by angiography who were referred to Rajaii Heart Center , and 30 healthy individuals were studied. SdLDL was measured by a direct homogenous LDL-C assay in the supernatant of serum which remained after heparin-magnesium precipitation. Serum apolipoprotein A1 and apolipoprotein B were measured by using immunoturbidimetric method.

Results: The results showed that the sdLDL levels were higher in patients with coronary stenosis than patients without coronary stenosis and healthy individuals (21.54±7.1, 16.88±4.4 and 15.45±5mg/dl, p=0.001, respectively). In addition the level of apoB (with stenosis: 113.71±21.8, without stenosis:100.88±18.7 and healthy: 102.30±9.6, p=0.003) and apoB/apoA1 ratio (with stenosis:1.100±0.24, without stenosis :0.589±0.26 and healthy:0.751±0.16, p=0.001) were significantly higher in patients with coronary stenosis. SdLDL levels were positively correlated with the level of apoB(r=0.589), apoB/apoA1 ratio(r=0.416), triglyceride(r=0.494), LDL-C (r=0.749), Total cholesterol(r=0.354) and were inversely correlated with the level of HDL-C (r = -0.586)(p<0.01).

Conclusion: The elevated levels of small dense LDL, apoB and apoB/apoA1 ratio were associated with coronary artery stenosis.

Keywords: small dense LDL, apolipoprotein, coronary artery disease

Introduction

Although increased serum cholesterol, especially LDL-C, is considered as a major risk factor for the development of coronary artery disease (CAD), many individuals with CAD have

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normal range of LDL-C levels [1]. Therefore, there might be some other risk factors which are responsible in the development of coronary artery disease.

LDL particles are heterogeneous with respect to their size, density and lipid composition. They have two phenotypes: phenotype A , consisting of large buoyant LDL and phenotype B which contains small dense LDL (sdLDL). Among LDL particles, the sdLDLs are believed to be atherogenic since these particles are taken up more easily by arterial wall. They are readily oxidized and have reduced affinity for LDL receptor and increased affinity for arterial proteoglicans [2,3]. Several studies have highlighted sdLDL as a new risk factor for coronary artery disease [4-6].

Apolipoprotein B (apoB) and apolipoprotein A1 (apoA1) are the major proteins of atherogenic lipoproteins and high density lipoproteins (HDL), respectively. LDL-C is a measure of the cholesterol level in the LDL particle but provides no information on other atherogenic lipoprotein particles. In contrast, apolipoprotein B is present in LDL, IDL and VLDL particles. Therefore, total apoB levels give information about total number of atherogenic particles [7,8]. ApoB levels also provide a good measure of the numbers of sdLDL particles [9]. ApoA1 levels are strongly correlated with the number of HDL particles [8] and it is more important than HDL-C for biochemical pathways that make HDL anti-atherogenic agent, due to apoA1-mediated cellular cholesterol efflux [10], maturation of HDL-C by lecithin cholesterol acyltransferase [11], and some antioxidative processes [12]. Several studies have reported that inceased apoB/apoA1 ratio is a potential risk factor for CAD [13,14].

The aim of the present study was to evaluate the association between serum small dense LDL, apoB, apoA1 and apoB/apoA1 ratio and the coronary artery stenosis.

Methods

This is a comparative cross-sectional and case-control study. 86 patients with coronary stenosis (28 single vessel disease, 23 two vessel disease and 35 three vessel disease), 35 patients without coronary stenosis (who had the symptoms of atherosclerosis such as chest pain and shortness of breath but they did not have stenosis) identified by angiography, and 30 healthy individuals without the history of diabetes and hypertension were studied.

Small dense LDL assay was performed by using a precipitation method described previously [15]. Briefly, the precipitation reagent (0.1 mL) containing 150 U/mL of heparin sodium salt and 90 mmol/L MgCl2 was added to a serum sample (0.1 mL) and the sample was incubated for 10 minutes at 37°C.Then each sample was placed in an ice bath for 15 minutes. After centrifugation at 15000 rpm for 15 minutes at 4°C, the precipitate was packed at the bottom of the tube and the clear supernatant consisted of sdLDL and HDL. The supernatant was removed for the measurement of LDL-C by a direct and selective homogenous method (Pars Azmoon).

Serum apolipoprotein A1 and apolipoprotein B were measured by using immunoturbidimetric method (Pars Azmoon). The levels of plasma cholesterol and triglyceride were determined using enzymatic method.HDL and LDL cholesterol were measured directly with HDL-C and LDL-C diagnostic kits (Pars Azmoon) .

All values were expressed as the mean±SD, and one-way analysis of variance (ANOVA) were used to compare mean values among groups.A statistically significant difference was defined as $p<0.05$. The linear regression analysis and Pearson correlation coefficient were used to assess correlation between two parameters.

Results

As shown in Table 1, there was not any significant difference in LDL-C and cholesterol

Variables	Healthy n=30	without stenosis n=35	with stenosis n=86	P
Small dense LDL(mg/dL)	15.45±5	16.88±4.4	21.54±7.1	0.000
SdLDL/LDL(mg/dL)	0.128±0.034	0.143±0.021	0.177±0.036	0.000
ApoA1(mg/dL)	141.97±31	123.1±29.4	105.25±18.4	0.000
ApoB(mg/dL)	102.30±9.6	100.88±18.7	113.71±23.8	0.003
ApoB/ApoA1(mg/dL)	0.751±0.16	0.589±0.26	1.10±0.24	0.000

Values are mean±SD

Table 2. Small dense LDL and apolipoprotein in subjects.

levels among the three groups. Serum triglyceride level was higher and HDL-C lower in patients with coronary stenosis.

The small dense LDL levels and sdLDL /LDL ratio were significantly higher in patients with coronary artery stenosis ($p<0.05$) (Table 2). In addition, patients with coronary artery stenosis had higher apoB and apoB/ apoA1 ratio than two other groups. However, they had lower value of apoA1.

The results show that the level of sdLDL was higher in females than males (23.15±6.48 versus 20.2±6.67 mg/dL, $p<0.05$) but they also had higher triglyceride. SdLDL levels were positively correlated with the level of apoB ($r= 0.598$, $p=0.001$), triglyceride ($r=0.494$, $p= 0.001$), LDL-C ($r=0.749$, $p=0.001$) and total cholesterol ($r= 0.354$, $p=0.001$) and were inversely correlated with the level of HDL-C ($r= - 0.585$, $p=0.001$), but not with apoA1 ($r=0.073$, $p=0.369$) (Fig.1).

It was also found out that small dense LDL had a positive correlation with apoB/apoA1 ratio ($r=0.416$, $p= 0.001$).

Discussion

In the current study we report the association between serum small dense LDL concentration and coronary artery stenosis. We also report the association between apoB and apoB/apoA1 levels and coronary stenosis. In this study, patients with coronary stenosis had increased levels of triglyceride and reduced levels of HDL-C. There was not any significant difference in LDL-C levels among three groups. Griffen et al indicated that the elevated level of LDL up to a triglyceride value of 1.5 mmol/l was because of an increase in the bouyant subclass. Above 1.5 mmol/l, small dense LDL was shown to increase significantly, while the concentration of the large and buoyant LDL dropped, resulting in decrease of total LDL [16].

We showed that small dense LDL was significantly higher in patients with coronary stenosis. In addition patients had higher ratios of sdLDL/LDL.Our findings are in agreement with Hiranos [17] and Nozues [18] findings. Perviously,it was demonstrated that patients with CAD had smaller LDL particles [19]. In this study LDL cholesterol had a positive association and HDL cholesterol had a negative association with sdLDL. Since LDL and HDL

Variables	Healthy n=30	Without stenosis n=35	With stenosis n=86	P
Sex(female/male)	(15/15)	(21/14)	(31/55)	0.067
Age(y)	50.69±10.2	54.66±10.8	56.28±9.4	0.038
LDL-C(mg/dL)	118.83±13.3	116.11±17	119.47±16.(	0.583
HDL-C(mg/dL)	47.57±10.4	43.17±6.8	37.05±6.4	0.000
Triglyceride(mg/dL)	153.3±28.4	141/21±39.4	177.7±62.6	0.002
Total Cholesterol (mg/dL	178.13±28.6	168.91±35.7	177.52±44.(	0.522

Values are mean±SD

Table1. Baseline Characteristics of the subjects.

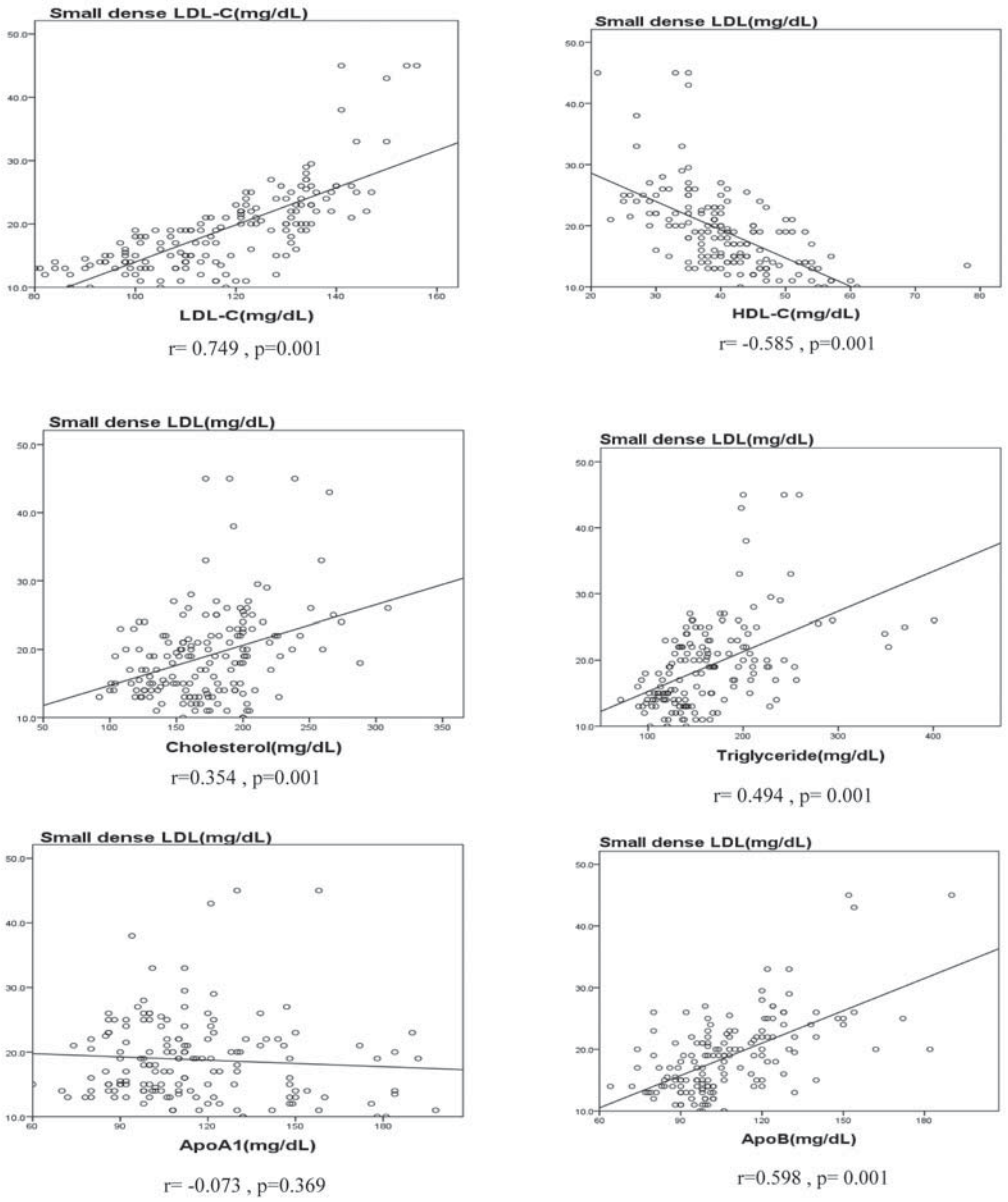

Fig. 1.Correlation between small dense LDL and serum LDL-C , HDL-C , cholesterol , triglyceride, apoA1 and apoB in all samples(P<0.001).

cholesterol are considered as major risk factors for CAD, sdLDL could be considered as a marker for coronary stenosis. Although females are supposed to have lower levels of sdLDL due to lower levels of hepatic lipase activity in females [20], our results showed that sdLDL value was higher in females than males .Females also had higher levels of triglyceride ,a significant determinant of sdLDL. Therefore, the gender difference of sdLDL disappeared after adjustment for triglyceride.

Data on metabolic and functional properties of lipoproteins suggest that apoB and apoA1 may be a better predictors of CAD than LDL and HDL cholesterols. ApoB is found in VLDL, LDL, IDL and LPa (lipoprotein (a)) and apoA1 is found in HDL [21].

We showed that patients with coronary

stenosis had elevated levels of apoB and decreased levels of apoA1. Surely, they had higher value of apoB/apoA1 ratio. Several studies have shown that apoB/apoA1 ratio is a suitable risk factor for CAD [13,14]. Furthermore Dunder ,et al showed that apoB/apoA1 ratio is a good predictor for the risk of myocardial infraction [22].

In this study small dense LDL had a positive correlation with serum triglyceride. The presence of this correlation was reported in some case-control studies [15,17]. In this study serum triglyceride levels were high in patients with coronary stenosis. The key abnormality leading to the generation of sdLDL is the development of mild to moderate hypertrigliceridemia. Overproduction of VLDL1 in the liver and its defective clearance from the circulation is leading to accumulation of VLDL1. Low lipoprotein lipase activity or an excess of apoCIII (an inhibitor of lipoprotein lipase) can reduce the efficient of VLDL1. Lipolysis of VLDL1 increases the level of sdLDL [2].

The result of the current study showed that small dense LDL values were positively correlated with serum apoB levels. Additionally, sdLDL had a positive association with apoB/apoA1 ratio. Hirano,et al found a very strong correlation between sdLDL-C and sdLDL-apoB and also a significant association between sdLDL-C and serum apoB (15).Therefore, the presence of hyper apoB may identify patients with coronary stenosis with high small dense LDL-C levels.

In summary, increased levels of triglyceride is a major reason for the small dense LDL production. Therefore, we should pay more attention to triglyceride than cholesterol. In addition, since LDL cholesterol is often have normal range in CAD patients [6], the sdLDL could be a better risk factor for CAD.

In conclusion, this study confirmed that patients with coronary stenosis had increased values of small dense LDL. Furthermore they had significant increased levels of apoB and apoB/apoA1 ratio.

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