

Correlation of Small Dense LDL Cholesterol and Apolipoprotein B with LDL Cholesterol and its Clinical Significance in Overweight, Type 2 Diabetes Mellitus and Coronary Artery Disease

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ABSTRACT

Introduction: Atherosclerotic Coronary Artery Disease (CAD) is fundamentally related to disorders of lipid metabolism. Health problems like obesity, glucose intolerance and metabolic syndrome increase atherosclerotic CAD risk. A fraction of Low density lipoprotein cholesterol (LDL) is called small dense low density lipoprotein cholesterol (sdLDL). These particles are more atherogenic because they are taken up more easily by arterial wall, readily oxidized and not easily cleared from plasma. Every LDL particle contain an Apo B molecule. **Methods:** In this cross sectional study we recruited 100 known cases each of CAD, type 2 diabetes, overweight and 100 age and sex matched healthy controls. We took a detailed case summary along with anthropometric measurements. We measured sdLDL by heparin magnesium precipitation method followed by direct estimation of the LDL in the supernatant. **Result:** Linear regressive analysis showed positive correlation between sdLDL and Apolipoprotein B (Apo B) with LDL cholesterol ($r=0.61$, $p=0.004$), ($r=0.754$, $p=0.0034$) respectively. Multiple Comparisons after Kruskalwallis test of sdLDL and Apo B levels of type 2 diabetes, CAD and overweight with controls were significant ($p<0.001$). **Conclusion:** Our findings suggest that the estimation of sdLDL and Apo B provide a complimentary benefit in assessment of cases with CAD, type 2 diabetes and overweight.

Keywords: small dense LDL, Apo B, CAD, type 2 diabetes.

INTRODUCTION

It has been found that individuals with a central deposition of adipose tissue have increased cardiovascular morbidity and mortality, including stroke, congestive heart failure, myocardial infarction and cardiovascular death¹. South Asian population

have central deposition of body, thus increasing the risk for type 2 diabetes and

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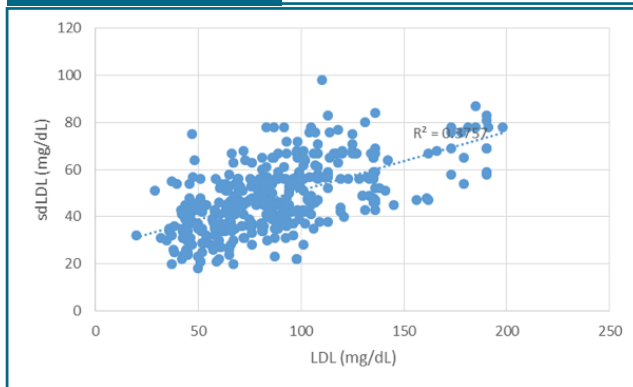


Fig. 1 Linear regressive analysis of sdLDL with LDL

cardiovascular disease at BMI that is lower than the existing BMI cutoff point for overweight².

Type 2 diabetics and overweight patients have increased triglyceride and HDL levels, increased triglyceride content of LDL and HDL, glycation of apolipoprotein and increased susceptibility of LDL to oxidation.³

In cases of type 2 diabetes and CAD, Krauss et al.⁴ has confirmed that many patients with normal LDL level have abnormal cardiac events leading to death. These findings suggest that smaller particles like sdLDL may have a predictive value for type 2 diabetes patients with CAD. The determination of LDL subclasses including sdLDL and large buoyant LDL (lbLDL) are more important in maximizing the effectiveness of CAD risk assessment in metabolic syndrome⁵.

sdLDL particles have increased affinity for arterial proteoglycans, which results in a prolonged residence time in the subendothelial space. This greater affinity of sdLDL to proteoglycans may be related to the sialic acid content of these particles. Although presence of sdLDL is in part genetically influenced, it can also be significantly modulated by

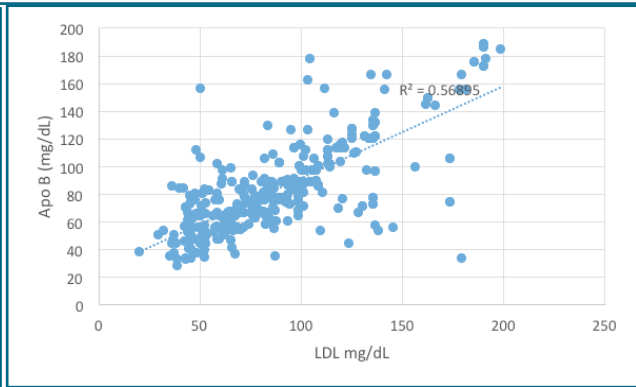


Fig. 2 Linear regressive analysis of Apo B with LDL

environmental factors, including dietary modifications and physical activity⁶.

Apolipoprotein B (Apo B) is the major protein moiety of LDL particles. Unlike cholesterol, which concentration can vary substantially within one LDL particle, there is only one Apo B molecule per LDL particle. Thus, assessing Apo B levels within the LDL sub fraction provide a direct measure of LDL particle number in the circulation⁶. Apo B is found to be more closely associated than non HDL cholesterol with central obesity, insulin resistance and inflammation. Apo B is thus a better risk parameter than non HDL-cholesterol for identifying a subgroup of individuals with or without metabolic syndrome with elevated cardiovascular risk⁷.

METHODS

In this cross-sectional descriptive study we recruited 100 known cases each of CAD, type 2 diabetes, overweight and 100 age and sex matched healthy controls. We took a detailed case summary along with anthropometric measurement. Cases with BMI > 25 kg/m² were considered overweight. Clinically significant CAD was defined as >50% stenosis of one or more branches of the coronary arteries on angiographic findings. Fasting venous blood

samples were collected in plain tubes after minimum fast of 12 hours.

We measured lipid profile using reagents in ERBA autoanalyser EM-360. sdLDL was estimated by Heparin Magnesium Precipitation method by Hirano et al.⁸. Apo B was estimated using immunoturbidimetric assay in EM-360.

The inclusion criteria included hundred each of known cases of type 2 diabetes, coronary artery disease and overweight. Patients receiving insulin therapy were excluded from the study. Our exclusion criteria includes; a) Cases with acute and chronic liver and kidney disease, b) Cases with clinically overt autoimmune disease like SLE, Rheumatoid arthritis, c) Cases with acute infections, d) Hypo or hyperthyroidism, e) Type 1 diabetes

Multiple comparison after Krushkalwallis test was used to derive the significance of small dense LDL and Apo B in cases compared to controls with p value of <0.05 considered significant. The sample size was calculated by taking the normal prevalence for Apo B of 110 mg/dL with 0.27 SD, for normal patients and 130 mg/dL with 0.33 SD for CAD cases⁹. Taking 95% confidence interval and 10% error of margin, sample size is 70 for each group, here we have taken 100 cases each.

RESULTS

We included 100 cases of CAD, type 2 diabetes and overweight and 100 age and sex matched healthy controls in our study after the inclusion and exclusion criteria were met. All our cases were male patients due to the nature of patients visiting the tertiary care center. The distribution of sdLDL according to severity of CAD showed that 25 (62.5%) cases with triple

vessel disease (TVD) had sdLDL>50 mg/dL, whereas only 4 (11.5%) cases having sdLDL<30 mg/dL had single vessel disease. Similarly 20 (50%) cases with TVD and 11 (46%) cases with DVD had Apo B >100 mg/dL.

Multiple Comparison after Krushkalwallis test for small dense LDL and Apo B showed good significance ($p<0.001$) between cases and controls. Linear regressive analysis showed positive correlation between sdLDL and Apo B with LDL cholesterol levels ($r=0.61$, $p=0.004$), ($r=0.754$, $p=0.0034$) respectively.

DISCUSSION

Our study was aimed at correlation of sdLDL and Apo B in serum with LDL cholesterol and its clinical significance in overweight, CAD, type 2 diabetes. We found a positive correlation of sdLDL and Apo B with LDL cholesterol levels. We found that values of small dense LDL and Apo B are significant ($p<0.001$) in cases with CAD, type 2 diabetes and obesity as compared to controls by multiple comparisons after Krushkalwallis test.

Our results show that the increase in triglycerides is maximum in type 2 diabetics then CAD and least increase was found in overweight, ref table 1. sdLDL is associated with increased serum triglyceride level because TG moves from VLDL to LDL and HDL in exchange for cholesteryl ester and is ultimately removed from these particles rendering them smaller (atherogenic lipoprotein phenotype B), Witztumet al¹⁰. The increase in sdLDL in cases has followed the same pattern as triglyceride levels.

Our results are comparable to study by Ronald M Krauss in patients with myocardial Infarction and angiographically documented coronary artery disease (CAD) demonstrated that 40-50% of patients have the small dense LDL phenotype which includes predominance of sdLDL, serum triglycerides and Apo B particles have 2- to 3-fold increase in disease risk¹¹.

One of the largest study on Apo B, which is having a similar results as ours is the survey by National Health and Nutrition Examination Survey III (NHANES III). In this survey Apo B was more closely associated than non HDL cholesterol with central obesity, insulin resistance and inflammation. Apo B is thus a better risk parameter than non HDL-cholesterol for identifying a subgroup of individuals with or without metabolic syndrome with elevated cardiovascular risk⁷.

Our results of positive correlation of sdLDL and Apo B with LDL cholesterol support the hypothesis put forward by Tornvall et al.¹²

Since a larger number of smaller particles is needed to carry a certain amount of cholesterol than if they are larger, LDL particle number, in addition to composition, may be important in cases with CAD. They have also suggested that prevention and treatment of IHD should be focused on reducing the number of atherogenic particles.

CONCLUSION

We have found a positive correlation of sdLDL and Apo B with LDL cholesterol levels in overweight, CAD and type 2 diabetes cases. The levels of sdLDL and Apo B are markedly high in cases as compared to controls and also are clinically significant in relation to severity of the Coronary Artery Disease.

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Table 1. Distribution of anthropometric and biochemical parameters

	Diabetic (mean± SD)	CAD (mean± SD)	Overweight (mean± SD)	Controls (mean± SD)
Age (years)	49.52±10.69	56.66±9.17	49.73±11.44	53.16±11.7
BMI (kg/m ²)	26.2±3.5	25.925±3.197	26.9±3.41	24.34±2.56
Waist (cm)	78±5.23	96.63±8.45	88.82±6.56	71.58±6.61
Triglycerides (mg/dL)	273±121.4	165.61±108.24	150.86±42.39	127.23±51.02
Cholesterol (mg/dL)	174.43±41.88	140±39.85	152.75±33.21	134.2±23.73
HDL (mg/dL)	38.63±13.03	38.43±10.24	42.114±10.94	41.02±8.73
LDL (mg/dL)	109.9±38.46	87.92±37.50	80.5±28.0	67.79±19.9
Apo B (mg/dL)	95.08±36.08	86.18±34.55	82.29±26.6	65.99±16.45
sdLDL (mg/dL)	56.26±12.9	53.22±13.48	48.04±11.27	34.28±8.06

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