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RESEARCH ARTICLE

Significance of low density lipoprotein cholesterol particles size in cardiovascular disease associated with metabolic disease

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Abstract:

Background: Increasing evidence suggests factors other than ordinary lipid profile as predictors of atherosclerosis. LDL size seems to be an important predictor of cardiovascular events and progression of coronary artery disease, and a predominance of small, dense LDL has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III. Increased plasma LDL-cholesterol (LDL-C) level is one of the most important risk factors for coronary artery disease. In particular, small dense LDL (sdLDL) has been demonstrated to be a new risk factor for the development of CAD.

Method: Cases were divided into three groups namely; Group 1 (n=50) normal, healthy adult as control group, Group 2 (n=56) CVD patients with metabolic syndrome and Group 3 (n=44) CVD patients without metabolic syndrome. Lipid profile, sd-LDL-C, LB-LDL-C and Non-HDL-C were measured by using standard procedures.

Results: Significant differences ($p<0.0001$) were found between all study groups with all measured variables except the HDL-C levels. The HDL-C was slightly lower in CVD with MetS than without MetS with no significant ($p=0.233$) values but significantly higher in control subjects than both patients groups.

Conclusion: The best indicator of response to lipid therapy is a reduction in the plasma concentration of atherogenic lipoproteins, as conventionally measured by LDL and triglycerides, but alternatively by non-HDL cholesterol also.

Keywords: *Lipid profile, sd-LDL-C, LB-LDL-C, N-HDL-C*

Introduction:

Cardiovascular diseases are still the primary cause of death in most industrialized countries. Effective prevention includes treatment of a series of risk factors: smoking, hypertension, diabetes, obesity, and dyslipidaemias, which includes elevated triglycerides, total and low-density-lipoprotein (LDL) cholesterol levels, as well as lowered high-density- lipoprotein (HDL) cholesterol.[1] Increased plasma LDL-cholesterol (LDL-C) level is one of the most important risk factors for coronary artery disease (CAD).[2] Plasma LDL comprises multiple discrete subclasses, differing in size and density. In particular, small dense LDL (sdLDL) has been demonstrated to be a new risk factor for the development of CAD.[3-4] LDL size correlates positively with plasma HDL levels and negatively with plasma triglyceride concentrations, and the combination of small, dense LDL, decreased HDL cholesterol and increased triglycerides has been called the 'atherogenic lipoprotein phenotype'.[5] This partly heritable trait is a feature of the metabolic syndrome, and is associated with increased cardiovascular risk. Evaluation of the metabolic triad can be used to predict cardiovascular diseases more efficiently.LDL particles are known as heterogeneous particles due to their size, density, and lipid composition.[3] Two evident phenotypes were recognized for LDL particles using gradient gel electrophoresis. Phenotype A consists of large, buoyant LDL particles with a size of more than 25.5 nm, and phenotype B is comprised of small dense LDL (sdLDL) and is 25.5 nm or less.[3-6] Small dense LDLs are considered to be atherogenic because they readily penetrate the arterial wall and have a low affinity for LDL receptor. They are also susceptible to oxidation. In many studies, it was shown that coronary artery disease (CAD) risk was increased 2 to 3 fold in patients with sdLDL.[7-9] Therefore, sdLDL is considered as a new marker for coronary artery stenosis.[10]

Material and Methods:

A total of 100 volunteer patients with known history of cardiovascular disease were selected for the study. The study was carried out in the Department of Clinical Biochemistry in Mahatma Gandhi Memorial Medical College, Indore Madhya Pradesh. The study was involved administration of any drug/medication or any surgical procedure to the patients. Only blood samples collected in the Clinical Biochemistry laboratory were used for in vitro biochemical analysis. The samples were collected by standard procedures under aseptic conditions. Standard procedures were followed for the preservation and storage of samples before analysis. Cases were divided into three groups namely; Group 1 (n=50) normal, healthy adult as control group, Group 2 (n=56) CVD patients with metabolic syndrome and Group 3 (n=44) CVD patients without metabolic syndrome. On the day of the study, each participant underwent a structured examination, which included an interview. Height, weight, waist circumference (WC) and blood pressure (BP) measurements, a fasting venipuncture were done. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The lipid profile was measured with enzymatic and using colorimetric methods. Small dense LDL cholesterol (C) was quantified by using heparin-magnesium precipitation method.[11] Large buoyant LDL (LB-LDL-C) particles were calculated by subtracting the sd-LDL-C from LDL-C concentration and Non-HDL-C was calculated by subtracting the HDL-C from the total cholesterol.

Statistical Analysis:

Data analysis was performed using the XLSTAT 2014 program with a value of $p<0.05$ considered significant. One-way analysis of variance (ANOVA) for repeated measures within a group was measured. Comparison of two groups was done by the Student's paired t-test. Results are expressed as MEAN SD or as proportion (%). Spearman rank correlation coefficient was used to assess the correlation between measured variables.

Results:

Table 1& figure 1 shows the comparison between physical characteristics of CVD patients with (n=56), without (n=44) metabolic syndrome and control (n=50). The physical measurements like age, BMI, WC, and blood pressure category were significantly elevated in patients with MetS ($p<0.05$) than without MetS patients of CVD and significantly higher ($p<0.001$) in both patients groups than control subjects.

Both patients group demonstrate the higher lipid profile as TC, TG, LDL-C, VLDL-C and their sub-fractions as sd-LDL-C and N-HDL-C were also found increased in CVD with MetS than without MetS group while LB-LDL-C lower in CVD with MetS to without MetS. All were statistically ($p<0.001$) significant. The HDL-C was slightly lower in CVD with MetS than without MetS with no significant ($p=0.233$) values but significantly higher in control subjects than both patients groups (table 1 & figure 2, 3). Table 2 showing the rank correlation between all measured variables and found significant relationship to star marked variables. The minus (-) sign showing the inverse relationship between the variables.

Table: 1 Comparison of anthropometric measurements, fasting lipid & their subtractions between CVD with and without MetS & control group

Variables	CVD with MetS N=56	CVD without MetS N=44	Control N=50
Age (Yrs)	56.80±8.64	51.84±9.43	45.87±11.28
BMI (Kg/M$^{2)}$	27.62±2.22	25.58±2.22	22.02±2.77
Waist (cm)	37.44±2.27	35.56±2.36	82.65±6.62
BPS mm/Hg	142.76±21.03	129.02±17.68	118.11±2.83
BPD mm/Hg	88.10±7.96	83.56±7.34	78.97 ±2.53
TC (mmol/L)	5.50±0.82	4.97±0.88	3.61±0.44
TG (mmol/L)	2.22±0.38	2.02±0.47	1.24±0.17
HDL-C (mmol/L)	0.77±0.11	0.80±0.12	1.21±0.18
LDL-C (mmol/L)	3.71±0.83	3.24±0.90	1.85± 0.45
VLDL-C (mmol/L)	1.09±0.17	0.92±0.21	0.57±0.07
sd-LDL-C (mmol/L)	2.66±0.74	2.33±0.85	0.80±0.19
LB-LDL-C (mmol/L)	0.90±0.19	1.51±0.37	1.04±0.19
N-HDL-C (mmol/L)	4.73±0.85	4.17±0.88	2.42±0.44

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Table: 2 Spearman rank correlation of sd-LDL-C levels with other covariates by fasting status

	HDL-C	LDL-C	Sd-LDL-C	LB-LDL-C	N-HDL-C
Age (Yrs)	0.065	-0.069	-0.010	-0.115	-0.035
BMI (Kg/M$^{2)}$	**-0.388***	0.057	0.057	0.107	0.089
Waist (cm)	**-0.356***	0.066	0.069	0.120	0.099
BPS mm/Hg	-0.107	-0.013	-0.034	0.115	0.023
BPD mm/Hg	-0.064	0.007	0.019	0.056	0.077
TC (mmol/L)	0.029	**0.948***	**0.871***	**0.431***	**0.977***
TG (mmol/L)	-0.111	0.016	0.007	0.169	**0.224****
HDL-C (mmol/L)	**1***	-0.087	-0.076	-0.144	-0.124
LDL-C (mmol/L)	-0.087	**1***	**0.922***	0.439	**0.958***
sd-LDL-C (mmol/L)	-0.076	**0.922***	**1***	0.125	**0.884***
LB-LDL-C (mmol/L)	-0.144	**0.439***	0.125	**1***	**0.428***
N-HDL-C (mmol/L)	-0.124	**0.958***	**0.884***	0.428	**1***

All variables found the highly significant p<0.0001

BMI=body mass index, WC=waist circumferences, BPS=systolic blood pressure, BPD=diastolic blood pressure, TC=total cholesterol, TG=triglycerides, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein, VLDL-C=very low density lipoprotein cholesterol, sd-LDL-C=small dense low density lipoprotein cholesterol, LB-LDL-C=large buoyant low density lipoprotein, N-HDL-C=non high density lipoprotein cholesterol)

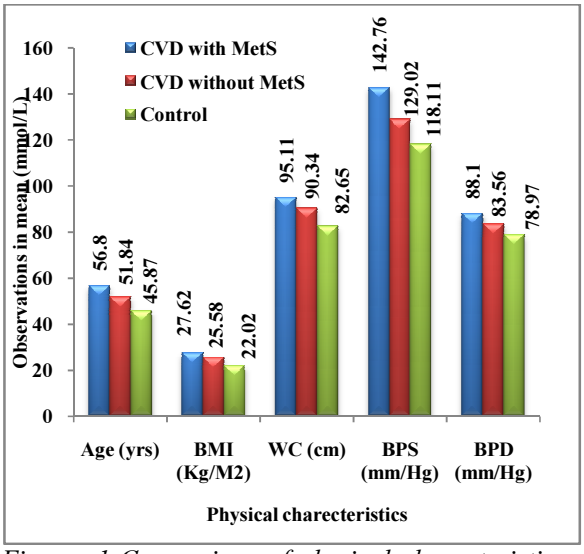

Figure: 1 Comparison of physical characteristics in all three study group

(BMI-body mass index, WC-waist circumferences, BPS-systolic blood pressure, BPD-diastolic blood pressure) (TC-total cholesterol, TG -triglycerides, HDL-C - high density (sd-LDL-C -small dense low density lipoprotein cholesterol, lipoprotein cholesterol, LDL-C -low density lipoprotein, LB-LDL-C-large buoyant low density lipoprotein VLDL-C -very low density lipoprotein cholesterol) N-HDL-C-non high density lipoprotein cholesterol)

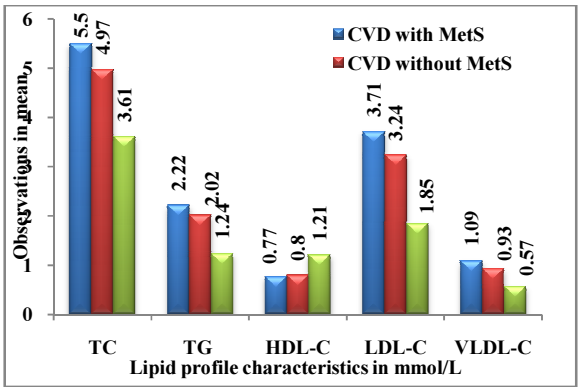

Figure: 2 Comparison of lipid profile in all three study group

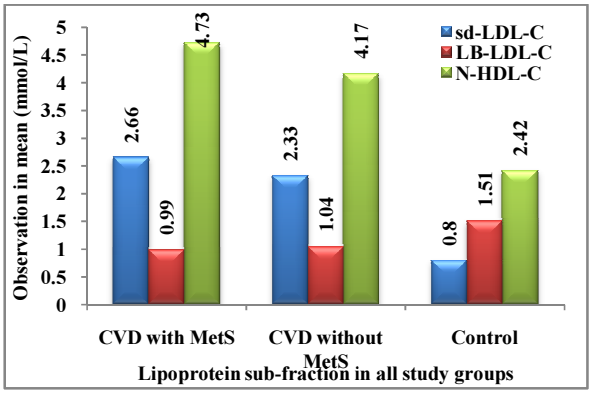

Figure: 3 Comparison of lipid profile in all three study group

Discussion:

In present study fasting lipid profile showed that patients with and without MetS had slightly elevated mean TC, LDL cholesterol and TG levels and lower HDL concentrations than the control subjects (table 1& figure 2) and significantly so. This is agreement with the author Ntyintyane in elevation of lipids in with MetS than without MetS

but not in significant. The study support to our results in sd-LDL-C levels which was found high in patients with MetS and without MetS also had significantly smaller, dense LDL particles than control subjects.[12]

Nakano S et al., [13] studied reported the significantly higher fasting total-cholesterol (C), LDL-C, triglyceride, sLDL-C levels than non-metabolic and non-diabetic subjects. HDL-C levels were significantly decreased in the former compared to the latter. Among the metabolic syndrome subjects, those with type 2 diabetes had significantly higher fasting systolic blood pressure than those without diabetes. SLDL-C, LDL-C were the highest and HDL-C was lowest in the metabolic syndrome with diabetes group. Metabolic syndrome is a significant determinant of the plasma sLDL-C level. Therefore, type 2 diabetes may further increase the risk of coronary artery disease in the metabolic syndrome subjects through cardiovascular inflammation. In reference with this finding our study showed the similarities in some extent. Significantly higher lipids with sd-LDL-C and HDL-C slightly lower in CVD with MetS patients in our finding.

In recent study reported the similar finding with present study as the small dense low-density lipoprotein (sd-LDL) has been highlighted as a new risk factor for CHD. Sd-LDL is also closely associated with hypertriglyceridemia, suggesting a high prevalence of these atherogenic particles in metabolic syndrome. Sd-LDL-C levels were positively correlated with TG and LDL-C and were inversely with HDL-C. Sd-LDL-C levels were also correlated positively with waist circumference, blood pressure. Patients with type 2 diabetes and metabolic syndrome had substantially increased sd-LDL-C level.[14]. In contrast we found no correlation between sd-LDL-C and TG, blood pressure and waist circumferences (table 2).

The mechanism for Small dense LDLs is considered to be atherogenic because they readily penetrate the arterial wall and have a low affinity for LDL receptor. They are also susceptible to oxidation. In many studies, it was shown that coronary artery disease (CAD) risk was increased 2 to 3 fold in patients with sdLDL.[5] Therefore, sdLDL is considered as a new marker for coronary artery stenosis.[15] Several studies have revealed that sdLDL particles can intensify the atherosclerotic process due to their penetration of the arterial wall, less affinity for LDL receptor, and susceptibility to oxidation.[16] The previous study showed that patients with coronary artery stenosis have higher levels of sdLDL than patients without coronary artery stenosis and healthy individuals.[17] According to previous study, LDL particle size is negatively correlated with sdLDL levels. Therefore, patients with higher levels of sdLDL have a smaller LDL size. Hirano and colleagues have shown that sdLDL levels correlated with LDL size.[18] Their results indicated that patients with coronary artery stenosis have elevated serum triglyceride levels and reduced HDL cholesterol levels.

The mechanistic support for small LDL having a special atherogenicity depends on atherogenic actions being greater for small than for intermediate or large LDL. This case has not been proven, however. Both large and small LDL compared with intermediate size LDL has reduced affinity for the LDL receptor which clears LDL from plasma.[19] Decreased clearance of these forms of LDL by the liver and steroid genic tissues is thought to lead to increased uptake by the arterial wall. In vivo, small LDL has a longer residence time in plasma than large LDL.[20] This may be caused by reduced exposure on small LDL of the region of apo B that binds to the LDL receptor, an interaction that is necessary to clear LDL from the circulation. The long residence time in plasma for small LDL could foster atherosclerosis if small LDL entered the arterial intima more readily than other LDL. This finding suggested that for every unit of time, large LDL is just as likely as small LDL to enter the arterial intima. Because large LDL has more cholesterol ester than small LDL, a large LDL particle would deposit more cholesterol into plaque than small LDL. Small LDL binds to arterial proteoglycan in the arterial wall, but so does large cholesterol-rich LDL.[21]

The present findings confirm that non–HDL-C is a better predictor of CAD than LDL-C.[21-23] Previous studies have also found that the numbers of most atherogenic lipoprotein particles, as measured by apoB, were more strongly related to CAD risk than was non–HDL-C.[24-25] In the present study, we show that the association of LDL particles with CAD is almost equal to that of non–HDL-C. The fact that apoB captures all atherogenicapoB particles (including very low-density lipoprotein and LDL), whereas LDL-particles only measures LDL particles, may have contributed to this distinction. In the present study, we observed that both LDL-particles as well as non–HDL-C conferred predictive value for the CVD. Since the association between LDL particles and CAD was equal to that of non–HDL-C, the present findings do not advocate routine use of LDL particles in CVD risk assessment. In addition, the size of LDL particles may also contribute to the atherogenicity of LDL-C. Thus, at a given level of LDL-C, individuals with small LDL particles have greater

atherosclerotic risk than those with large-size LDL.

Conclusion:

In conclusion, we demonstrate that patients with cardiovascular disease with metabolic syndrome have higher smaller LDL particles, so sdLDL-C can increase the risk of heart disease. LDL size also seems to be an important predictor of cardiovascular events, and progression of coronary artery disease and a predominance of small, dense LDL as an emerging cardiovascular risk factor. The study demonstrates that large and small LDL subtypes are atherogenic. In as much as any type of LDL is contained in the plasma total LDL concentration, the standard clinical measurement of risk, all LDL types should be viewed as harmful. The best indicator of response to lipid therapy is a reduction in the plasma concentration of atherogenic lipoproteins, as conventionally measured by LDL and triglycerides, but alternatively by non-HDL cholesterol also.

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Conflict of Interests: The author declares that there is no conflict of interests regarding the publication of this paper**.**

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