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Assessment of small, dense low density lipoprotein cholesterol as a marker of cardiovascular risk in Indian patients with type 2 diabetes mellitus

KEYWORDS: sdLDL-C, Atherosclerosis, T2DM

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ABSTRACT *Aims*

the present study was conducted to asses sdLDL-C and Oxidized LDL (oxLDL-C) levels in patients with T2DM in comparison to hypertensive and healthy controls, and their correlation with Framingham risk score (FRS).

Study Population

The study population consisted of patients with T2DM (>5 years) along with hypertension (n=55), newly detected patients with T2DM (<2 years) without hypertension (n=28), patients with hypertension (n=31) and healthy controls (n=30).

Results

Serum levels of sdLDL-C and oxLDL-C were significantly higher in patients with T2DM compared to hypertensive and healthy controls. (All P<0.001).sdLDL-C also showed a strong correlation with FRS and HbA1c in the total study population. (P<0.001)

Conclusions

sdLDL-C levels were increased in patients with diabetes indicating high risk of developing subclinical atherosclerosis. In addition, a strong correlation between sdLDL-C and FRS suggests that sdLDL-C may contribute significantly to the excess risk of CVD in patients with T2DM.

Introduction

Cardiovascular diseases (CVD) are the main cause of death globally. According to WHOit was estimated that 17.5 million people died of CVD in 2008, demonstrating 30% of all global demises.[1] It was also observed that over 80% of CVD deaths took place in low- and middle-income countries and occur almost equally in men and women. [2]Most CVDs can be prevented by catering to risk factors such as hyperlipidaemia, diabetes mellitus, hypertension, obesity and physical inactivity. [3]Dyslipidaemia, frequently occurring in patients with type 2 diabetes mellitus (T2DM), might play a critical role in accelerated macro vascular atherosclerosis formation and contribute significantly to the excess risk of CVD in patients with T2DM. [4]

Depending upon nature of lipoprotein, various subtypes of cholesterol include very-low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C).[5]Amongst all,LDL is the main cholesterol carrying lipoprotein in the circulation. With density gradient centrifugation several sub fractions of LDL such as large, intermediate and small LDL are separated.[6]Out of these sub fractions, small, dense LDL (sdLDL-C) (≤ 25.5 nm) is a particularly atherogenic form of the lipoprotein. [7,8] sdLDL-C has enhanced susceptibility to oxidative modification resulting in formation of oxidized LDL cholesterol (oxLDL-C)[9]It is then taken up by scavenger receptors present on monocytes resulting in formation of foam cells, which is the beginning of atherosclerosis.[10]

Hence, in the present study we assessed levels of sdLDL-C and oxLDL-C in patients with T2DM in comparison to hypertensive and healthy controls. We also evaluated correlation between sdLDL-C and Framingham risk score (FRS) in patients with T2DM, to evaluate the exact risk of CVD in these patients.

Methods

Study design and participants

The patients for this study were recruited from Diabetes Clinic of Sir H.N.Hospital& Research Centre between November 2011 and April 2013. Healthy controls were selected from the hospital staff. The study population consisted of patients with T2DM for > 5 years along with hypertension (group A-I), newly diagnosed patients with T2DM (of < 2 years duration) without hypertension (group A-II),patients with hypertension only (group B-I), and healthy controls (group B-II).

All participants were above 40 years of age and of either gender. Also patients with long term systemic illness were excluded from the study. Medical history was taken from each participant before enrolment.

Ethics consideration

Informed consent was obtained from each participant. The study was approved by the Institutional Ethics Committee, and was carried out in accordance with the "Ethical Guidelines for Biomedical Research on Human Participants, 2006" by the Indian Council of Medical Research and the Declaration of Helsinki, 2008.

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Baseline Examination

Height measurements were performed using anthropometric rod with subject standing in erect position with the head in the ear-eye plane. The body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist and hip measurements were noted, and the waist-to-hip ratio was calculated. Blood pressure (BP) was measured in right arm after five minutes of rest by a clinical assistant using mercury sphygmomanometer in a sitting position with the right forearm placed horizontal on the desk as recommended by the American Society of Hypertension.

Laboratory Investigations

Participants from all the groups underwent a detailed history taking, physical examination, and the following laboratory investigations such as: urine collection for microalbuminuria and blood collection for glycatedhemoglobin (HbA1c), fasting blood sugar (FBS), triglyceride, total cholesterol, HDL-C and LDL-C. The analyses were done by using standard protocols. Samples were also collected for assessing small dense LDL-C by Elisa method using kits (Online Antibodies and Mybiosource, USA). Samples were collected from each individual at a single time point and kept at −80°C until analysis.

Cardiovascular risk calculations

The Framingham risk score (FRS) was used for calculating 30 yrs cardiovascular risk of all the study participants. The predictors used by FRS were participant's age, systolic BP, use of anti-hypertensive treatment (yes/no), smoking, diabetes mellitus, total cholesterol, HDL cholesterol and BMI.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science software version 21.0 (SPSS Inc, Chicago, IL). Continuous variables were presented as mean ± standard deviation. Categorical variables were expressed as percentages. After testing for normality using the Kolmogorov–Smirnov test, between groups comparison was done using either one-way analysis of variance- ANOVA (if normally distributed) or Kruskal-Wallis test (if not normally distributed) with post-hoc tests. Correlation between 2 numerical variables was assessed using Spearman's rho correlation coefficient. Statistical analysis was considered significant at $p < 0.05$.

Results

Out of 144 participants recruited in this study, 55 patients had T2DM for more than 5 years along with hypertension (group A-I), 28 were newly diagnosed patients with T2DM for < 2 years without hypertension (group A-II), 31 patients with essential hypertension only (group B-I) and 30 healthy controls (group B-II)

Baseline characteristics

All the groups were comparable with respect to the baseline characteristics and anthropometric measurements except for age and blood pressure as shown in Table 1. Systolic as well as diastolic BP were significantly higher in patients with T2DM (>5 years) with hypertension compared to newly detected patients with T2DM.

Table 1: Anthropometric characteristics across 4 study groups (Mean ±SD)

Parameter	Group A-I (n= 55)	Group A-II (n=28)	Group B-I (n= 31)	Group B-II (n=30)	Overall P value(Post-hoc P value after Bonferroni's correction)
Age in years	59.3 ± 9.6	52.1 ± 10.4	55.1 ± 10.7	51.7 ± 9.5	0.002* (a:0.001, c:0.007)
Male: Female	30:25	13:15	16:15	16:14	0.92
BMI in kg/m^2	26.69 ± 4.37	24.99 ± 4.1	26.5 ± 4.8	26.0 ± 6.0	0.41
Waist to hip ratio	0.89 ± 0.07	0.88 ± 0.06	0.87 ± 0.06	0.87 ± 0.05	0.10
Systolic blood pressure in mmHg	145.7 ± 18.8	134.8 ± 8.9	138.7 ± 18.1	129.0 ± 13.3	0.001*(a:0.003,c:<0.001)
Diastolic blood pressure in mmHg	83.9 ± 7.0	78.6 ± 4.9	82.7 ± 8.0	80.0 ± 6.6	0.03* (a: 0.003)

a: A-I vs A-II; b: A-I vs B-I; c: A-I vs B-II; d: A-II vs B-I; e:A-II vs B-II; f: B-I vs B-II
*p<0.02

Biochemical parameters

Table 2 shows that blood glucose (fasting and post-prandial) and HbA1c were significantly higher in patients with T2DM compared to hypertensive controls and healthy controls (all $p < 0.001$). We observed a significant increase in serum total cholesterol and LDL cholesterol in patients with newly detected T2DM compared to patients with long term T2DM, and hypertensive controls. Micro albumin levels were significantly higher in patients with long term T2DM with hypertension compared to newly detected patients with T2DM, hypertensive controls and healthy controls (all $p < 0.001$).

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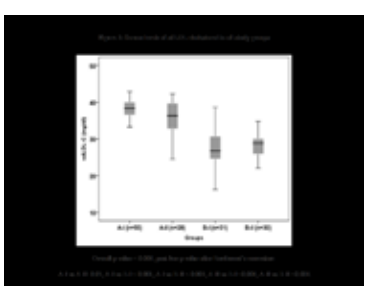
Table 2: Biochemical parameters across 4 study groups (expressed as mean ± standard deviation)

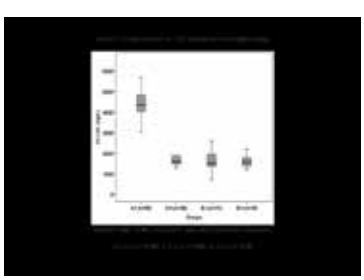
Parameters	Group A-I (n= 55)	Group A-II (n=28)	Group B-I (n= 31)	Group B-II (n=30)	Overall p value (Post-hoc p value after Bonferroni's correction)
Glucose Profile					
Fasting blood sugar (mg/dl)	149.8 ± 50.3	142.2 ± 56.6	102.2 ± 29.9	99.7 ± 20.5	0.001 (b,c,d,e:<0.001)
Post-prandial blood sugar (mg/dl)	217.9 ± 74.4	178.8± 79.2	120± 44.7	107.2 ± 24.5	0.001 (a:0.01, b:<0.001, c:<0.001, d:0.002, e:<0.001)
HbA1c (%)	7.90 ± 1.22	6..21 ± 1.43	5.0 ± 0.7	5.04 ± 0.5	0.001 (a:0.01, b,c,d: < 0.001, e: 0.001)
Lipid Profile					
Total cholesterol (TC) (mg/dl)	171.2 ± 59.1	195.9 ± 53.8	160.4 ± 45.2	180.7 ± 43.5	0.02 (a:0.04, d: 0.01, f: 0.04)
HDL cholesterol (mg/dl)	47.8 ± 13.3	52.4 ± 17.7	50.4 ± 23.1	48.5 ± 12.61	0.3
LDL cholesterol (mg/dl)	95.3 ± 49.5	110.8 ± 32.2	87.7± 30.1	116.8 ± 25.6	0.001(a: 0.02, c: 0.003, d:0.007, f: 0.003)
VLDL cholesterol (mg/dl)	28.6 ± 8.6	27.1 ± 13.2	21.8 ± 8.51	22.4 ± 8.79	0.3
Triglycerides (mg/dl)	143 ± 82.3	136.3 ± 54.6	107.4 ± 41.7	106.4 ± 47.4	0.1
Urine Albumin					
Microalbumin (mg/dl)	91.0 ± 15.61	28.7 ± 22.7	37.7 ± 16.25	38.5 ± 22.6	0.001 (a,b,c: < 0.001)

a: A-I vs A-II; b: A-I vs B-I; c: A-I vs B-II; d: A-II vs B-I; e: A-II vs B-II; f: B-I vs B-II

Serum levels of sdLDL cholesterol and oxLDL cholesterol across the four study groups

Serum sdLDL cholesterol and oxLDL cholesterol were significantly elevated in patients with long term T2DM (> 5 years) with hypertension as compared to patients with newly detected T2DM, hypertensive controls and healthy controls. (Figure 1 and 2)





Relationship between HbA1c, microalbumin, sdLDL cholesterol and oxLDL cholesterol in all study groups

Serum sdLDL cholesterol levels showed a strong correlation with glycosylated haemoglobin (HbA1c) and moderate correlation with oxLDL cholesterol and microalbumin across the four study groups as shown in table 3

Table 3: Correlation between HbA1c, microalbumin, sdLDL and oxLDL in all the four study groups

Parameters		Spearman's correlation coefficient
sd-LDL cholesterol (mg/dl)	HbA1c (%)	0.6*
	Microalbumin (mg/dl)	0.5*
ox-LDL cholesterol (ng/L)	HbA1c (%)	0.54*
	Microalbumin (mg/dl)	0.2

*p = 0.001

Influence of HbA1c on sdLDL cholesterol and oxLDL cholesterol in patients with long term T2DM with hypertension (Group A-I)

The levels of sdLDL cholesterol and oxLDL cholesterol were significantly increased in patients with long term T2DM with HbA1c > 6.5% compared to those with HbA1c ≤ 6.5% as shown in Table 4.

Table 4: Influence of HbA1c on sdLDL-C and oxLDL-C in patients with long term diabetes with hypertension

Parameters	HbA1c ≤ 6.5% (n= 25)	HbA1c > 6.5% (n= 30)	P value
sdLDL cholesterol (mg/dl)	32.26 ± 2.69	37.1 ± 1.85	0.02
oxLDL cholesterol (ng/L)	4293.9 ± 523.1	5207.6 ± 232.1	0.01

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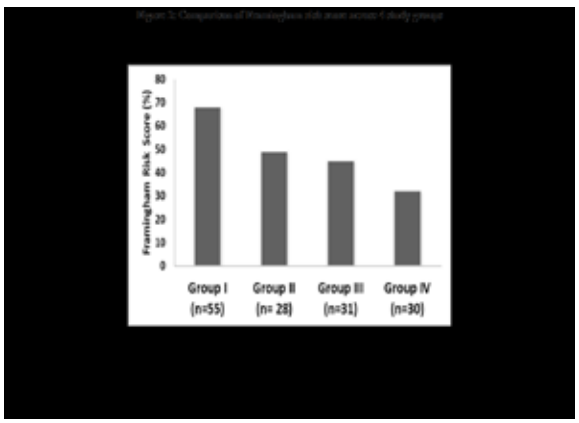
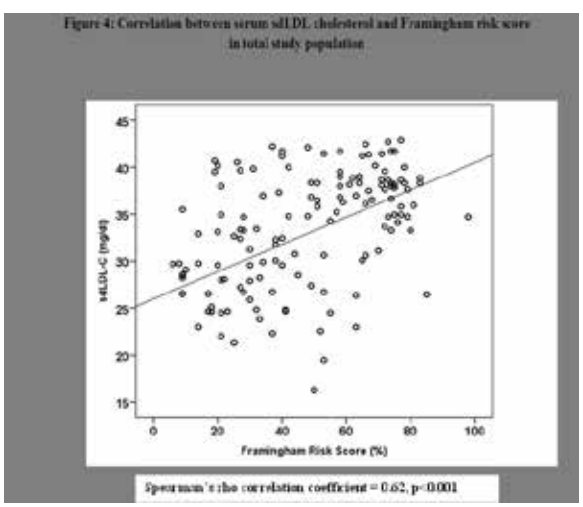
Framingham risk score (FRS)

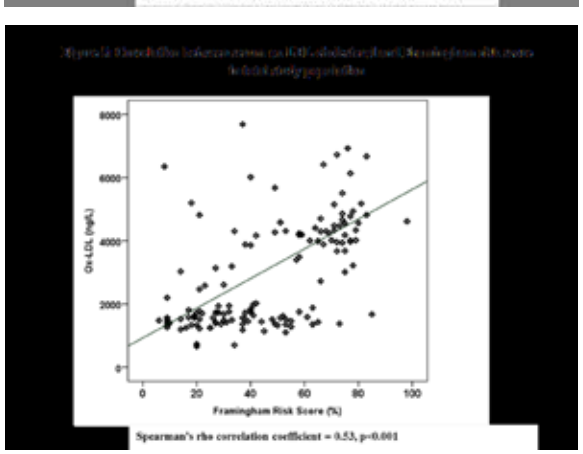
The risk of CVD was significantly higher (p = 0.001) when compared with FRS in patients with diabetes mellitus (with and without hypertension) (68 ± 13.4 and 49 ± 17.5, respectively) and hypertensive controls (45 ± 17.7) compared to healthy controls (32 ± 12.6). (Figure 3)



vii. Association between serum sdLDL cholesterol and ox-LDL cholesterol with Framingham risk score in the total study population

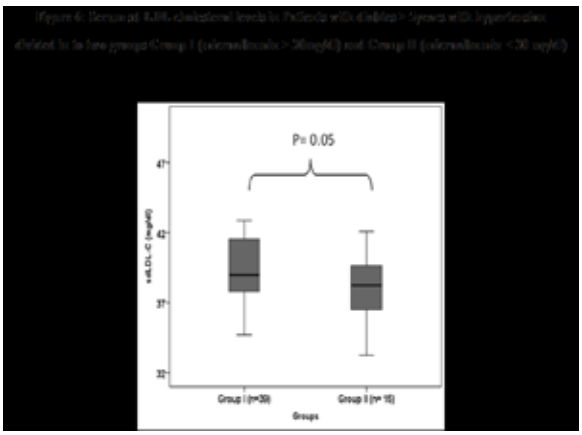
The Framingham risk score showed a strong (Spearman's rho, σ=0.62) and a moderate correlation (σ=0.53) (both p < 0.001) with serum sdLDL-C and oxLDL-C, respectively in the total study population as shown in Figures 4 and 5.





Effect of microalbumin on serum sdLDL cholesterol levels in patients with T2DM for > 5 years with hypertension

Serum sdLDL cholesterol levels were significantly elevated in patients with long term diabetes with microalbumin> 30mg/dl as compared to those with microalbumin< 30mg/dl (39.24 ± 2.6 and 39.24 ± 2.6 respectively) as shown in Figure 6.



Discussion

In the current study, serum levels of sdLDL-C and oxLDL-C were significantly higher in patients with T2DM (long term as well as newly detected) compared to hypertensive and healthy controls. Furthermore, HbA1c as well as the Framingham risk score showed strong and moderate correlation with serum sdLDL-C and oxLDL-C levels, respectively in the study population.

We found significantly higher levels of total cholesterol and LDL cholesterol in the patients with newly detected diabetes as compared to the patients with long-term diabetes with hypertension and hypertensive controls. Various studies have shown that lipid abnormalities are frequently seen in patients with T2DM.[11]And are predictors of coronary heart disease in this population.[12, 13]

Glycosylated haemoglobin (HbA1c) provides a measure of the glycaemic control of diabetes patients during the previous 2–3 months. [14]We found significantly higher HbA1c in patients with T2DM compared to hypertensive and healthy controls, substantiating the findings by Currie et al.[15]The present study showed that patients with long-term T2DM with hypertension had significant increase in micro-albumin compared to patients with newly detected T2DM, hypertensive controls and healthy controls. Moreover studies have also shown that any degree of albuminuria is a risk factor for cardiovascular events in individuals with or without DM and screening for albuminuria identifies people at high risk for cardiovascular events [16]

In this study Framingham equation was used for calculating 30-year cardiovascular risk as it is an independent predictor with a better odds ratio than metabolic syndrome alone. [17]our study predicts, that cardiovascular risk was significantly higher in patients with diabetes mellitus (with or without hypertension) and hypertensive controls compared to healthy controls. This is in concordance with findings by Selvinet al.[18]

OxLDL-C is more atherogenic than native LDL-C; it is taken up by scavenger receptor system ultimately leading to

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generation of foam cells and development of early atherosclerotic lesions, and is a chemo attractant for monocytes and T lymphocytes, and also inhibits macrophage motility, thereby promoting retention of macrophages in the arterial wall.[19]Furthermore, oxLDL promotes platelet adhesion, triggers DNA strand to break, and promotes apoptosis; all of which contribute to the development of atherosclerosis[20]We found increased levels of oxLDL-C in patients with long term T2DM as compared to hypertensive and healthy controls. The similar findings were quoted by Hayashidaet al and Nakhjavani et al. [21,22]Increased oxLDL levels could be a good residual lipid marker and oxidation of LDL is significantly influenced by the impairments in glucose control[23] This explains the increased serum levels of oxLDL-C in patients with T2DM. High levels of circulating oxLDL can serve as an independent and significant predictor for future cardiac events in type 2 diabetic patients. [24] We also found that serum oxLDL-C levels were significantly influenced by elevated HbA1c in patients with long term diabetes. Initially there were studies showing negative correlation between the two[25,26] but a study conducted by Holvoet et al showed correlation between serum oxLDL-C and HbA1c levels in vivo. It is documented that there is increased tendency of LDL to undergo oxidation due to elevated levels of HbA1c in patients with T2DM. [27]

sdLDL is highly atherogenic as it displays higher penetration into the arterial wall, and has low binding affinity for the LDL receptor, a prolonged half-life and a reduced resistance to oxidative stress [28,29] Our study showed increased serum sdLDL-C levels in patients with long term T2DM with hypertension as compared to hypertensive and healthy controls. This finding was in concordance with Suhet al who stated that patients with diabetes had a smaller mean-LDL particle size and higher proportion of sdLDL-C compared to those of subjects without diabetes. [30] sdLDL-C has also been shown to be associated with both coronary and non-coronary forms of atherosclerosis and is a risk factor for peripheral arterial disease [31] An epidemiological study conducted in urban Indians stated that sdLDL-C is associated with both diabetes and coronary artery disease (CAD). [32]Studies have shown a 2- to 3-fold increase in risk of coronary heart disease (CHD) among individuals with higher sdLDL-C[9]

We also found a good correlation between serum sdLDL-C, oxLDL-C and 30 year cardiovascular risk in the study population. Rabbani et al stated that sdLDL-C has increased arthrogenecity and may explain the escalation of cardiovascular events. [33] As we found strong correlation between the FRS and sdLDL-C we can conclude that sdLDL cholesterol can serve as an early marker of CVD in patients with T2DM. Although a study [34]has not shown correlation between oxLDL and FRS, our study on the contrary showed moderate correlation between the two.

A strong correlation was observed between serum sdLDL-C levels and HbA1c in the study population. Elevated levels of HbA1c also showed a significant impact on increased values of sdLDL-C in patients with long term diabetes in the present study. Similarly, Lee et al stated that sdLDL cholesterol levels were significantly elevated in diabetics than controls and it is independently associated with HbA1c values.[35]

A strong positive correlation was observed between serum sdLDL-C levels and microalbumin in the present study substantiating findings by[30]Allhaet al suggested that sdLDL can be used in conjunction with other biochemical markers for early diagnosis and assessment of diabetic nephropathy. [36]Thus patients with increased microalbumin and high serum sdLDL-C levels should be frequently monitored to prevent development of diabetic nephropathy.

Conclusion

sdLDL-C is a well-established marker of carotid atherosclerosis. This study highlights the fact that sdLDL-C correlated with other established risk markers of CVD like Framingham risk score, and its levels were elevated in patients with long term diabetes. Thus sdLDL-C has a potential to serve as a new and early marker for assessment of subclinical atherosclerosis and resultant cardiovascular risk. This might aid in better management of patients with diabetes, if levels of sdLDL-C are measured timely

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