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STUDY OF SERUM SMALL DENSE LDL IN TYPE 2 DIABETES MELLITUS PATIENTS AND ITS ASSOCIATION WITH CORONARY ARTERY DISEASE

Krunali D. Gaidhane *	Assistant Professor, dept. of Biochemistry, Govt. Medical College & Super-specialty Hospital, Nagpur. *Corresponding Author.
S.B.Asegaonkar	Associate professor, Dept. of Biochemistry, Govt. Medical College, Aurangabad.
Minal S. Sonune	Junior resident, Seth G S Medical College, Mumbai.
R.J.Shendye	Professor & Head, Dept. of Biochemistry, Govt. Medical College, Aurangabad.

ABSTRACT

AIM: Coronary heart disease is one of the most leading causes of death in this modern era. It has been found that diabetic patients are more prone for coronary heart disease and diabetic dyslipidemia is the main culprit. We aimed this study to find the association between lipid profile, sdLDL in patients of T2DM with and without CAD.

MATERIAL AND METHOD: It was a case-control study conducted on 180 patients who were divided into three groups as randomly selected controls, patients with DM and CAD, and patients having DM without CAD. Blood samples were compared for lipid profile and sdLDL was calculated based on Friedwald's equation. level of sdLDL particles among diabetic patients associated with (50.73 ± 15.56, $p < 0.001$) and without (50.12 ± 17.01, $p<0.001$) CAD and found that there is significant rise of sdLDL in DM patients compared with controls (24.72 ± 5.49). on comparing the sdLDL-C among diabetic patients associated with and without CAD (Pearson chi square test, p=0.00 with Odds ratio 11.81 with 95% CI 3.3-41.0) which gives 11 times increased risk of raised sdLDL value in DM with CAD patients.

CONCLUSION: sdLDL is proved to be the better index for the early detection and monitoring of cardiac health in diabetic individuals.

KEYWORDS :

INTRODUCTION:

Coronary heart disease (CHD) is the leading cause of death in patients with Type 2 Diabetes Mellitus (T2DM). More than 70% of patients with T2DM die of cardiovascular causes 1. Previous studies have shown that individuals with diabetes have 2-4 fold increased risk of CHD compared with that of non-diabetic individuals 2. The similar level of risk of DM and previous CHD has led to the suggestion that individuals with DM should be treated as CHD risk equivalents 3.

The most common cause for this atherogenicity is the underlying dyslipidemia in T2DM. Dyslipidemia in Diabetes is the combination of high Triglycerides, low High Density Lipoproteins (HDL) and elevated Low density lipoprotein cholesterol (LDL-C). Each of these dyslipidemic features is associated with an increased risk of cardiovascular disease 4. Elevated level of low density lipoprotein-cholesterol (LDL-C) has long been established as one of the strongest risk factors for coronary artery disease (CAD). Depending on the size, chemical composition and density, there are different subclasses of LDL that can be separated by advanced techniques. Two phenotypes of LDL based on particle size have been identified – pattern A with LDL diameter > 25.5nm (large buoyant LDL or lbLDL) and pattern B with LDL diameter ≤ 25.5nm (small, dense LDL or sdLDL).

DM itself increases the level of sdLDL but not that of LDL. The sdLDL found in T2DM are more atherogenic and associated with >3 fold increase in the risk of CHD. A number of mechanisms have been proposed to explain the enhanced atherogenicity of sdLDL 5-9, including a) a lower affinity for the LDL receptors, b) facilitated entry into the arterial wall, c) greater arterial retention because of increased binding to proteoglycans, and d) greater susceptibility to oxidation. The NCEP and recently updated guidelines of the National Academy of Clinical Biochemistry expert panel accept predominance of sdLDL as an emerging cardiovascular risk factor 3,10 .

Several epidemiological studies have demonstrated that many patients with CVD had LDL-C levels in the same range compared with healthy subjects, whereas the distribution of LDL particle size shifted towards smaller 11-14. To improve risk assessment, measurements of lipid and non-lipid biomarkers have been suggested.

We can estimate the sdLDL-C concentration from the classic lipid measures by Friedwald formula sdLDL (mg/dl) = 0.580 (non-HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 12.05 ; which will provide a cost-effective method for screening patients for the risk of CVD15.

Hence, this study is proposed to find the association between lipid profile, sdLDL in patients of T2DM with and without CAD.

MATERIALS AND METHODS:

It was a hospital based Case Control study. Cases of T2DM who met the criteria of T2DM defined by Expert Committee on the diagnosis and classification of DM (2013 revision) with and without CAD attending medicine OPD in GMCH, Aurangabad were taken as study population.

Written informed consent was obtained from all subjects. The study was approved by the Institutional Ethical Committee.
Total 180 individuals were studied.

The study population was divided into 3 groups. Cases were divided into 2 groups and controls were considered as third group.

Each group contained age and sex matched individuals between 25 to 60 yrs.

Inclusion Criteria:

Group 1: Cases of TYPE 2 Diabetes Mellitus who met the revised criteria published by Expert Committee on the Diagnosis & Classification of Diabetes Mellitus (2013 revision) as follows
•Fasting plasma glucose(FPG) ≥126mg/dl (7.0 mmol/l) OR symptoms (such as polyuria, polydipsia, unexplained weight loss)
•Random plasma glucose ≥ 200mg/dl (11.1 mmol/l) OR
•plasma glucose ≥ 200 mg/dl911.1 mmol/l) 2 hours after a 75gm glucose load OR
•A1C ≥ 6.5%
•Ready to give informed written consent
•with CAD diagnosis of which is based on a past history of documented myocardial infarction and / or medical therapy or revascularization for CAD and/ or electrocardiographic (ECG) changes suggestive of Q wave changes (Minnesota codes 1-1-1to 1-1-7) and/ or ST segment depression (Minnesota codes 4-1to 4-2).

Group 2: Patients of Type 2 DM with above mentioned criteria without CAD.

Group 3: Age & Sex matched healthy individuals not having TYPE 2 DM and CAD as Controls

Exclusion Criteria:

1. Presence of infectious disease at the time of evaluation or

during the 15 days prior to enrollment
2.Subjects with smoking, alcoholism, taking anti-inflammatory, antihistaminic, antifolate, anticonvulsant drugs during the same time period will be excluded from the study group.
3.Patients with medical history of severe renal disease, severe hepatic disease, infectious disease or malignancy.
4.Pregnancy
5.Patients with HIV
6. Not willing to participate in the study

Anthropometric Measurements like Height (Ht), Weight (Wt), Body mass index (BMI), Waist circumference and Blood Pressure were noted down.

Biochemical Investigations:
After written informed consent, 12 hour fasting venous blood samples were collected from all participants in fluoride, plain and EDTA bulbs; again 2 hours postprandial blood sample was collected in fluoride bulb for blood sugar estimation. Serum was separated after 1 hour by centrifugation at 3000 rpm for 10 minutes, and was tested for biochemical parameters.

OBSERVATIONS AND RESULTS:
A total of 180 participants were enrolled in the study 60 in each of three groups
Group 1: Healthy Controls
Group 2: Patients of T2DM without CAD
Group 3: Patients of T2DM with CAD

Demographic and biochemical characteristics of all the participants were analyzed as mean + Standard Deviation (S.D.).

One way ANOVA Variance test for comparing all three groups & unpaired-t test was applied to analyze the differences of studied characters in between two study groups. P value was obtained from the unpaired t test as > 0.05 not significant, < 0.05 significant and < 0.01 highly significant.

Correlation coefficients (r) were calculated among various parameters in group 1, group 2 and group 3 Positive and negative r values were interpreted as follows:

r: 0 (no correlation), r: 0- 0.3 (poor correlation), r: 0.3- 0.7 (considerable correlation) and r: 0.8 or more (strong correlation).
The results of demographic and biochemical data are as follows:

Table 1: Baseline demographic data of study population

characteristics	Group I Healthy Controls	Group II T2DM patients without CAD	Group III T2DM patients with CAD
No. of patients	60	60	60
Age (years) (Mean ± SD)	54.86 ± 14.1	48.24 ± 8.29	50.68 ± 17.4
Sex(male/female)	30/30	31/29	31/29
Body mass index(kg/m^2) (mean ± SD)	25.39 ± 3.77	27.28 ± 5.73	28.82 ± 6.08
Waist circumference (cm) (mean ± SD)	93.04 ± 9.2	94.4 ± 10.9	94.7 ± 9.3
Systolic BP (mmHg) (mean ± SD)	119 ± 12.03	128 ± 15.6	166 ± 22.01
Diastolic BP (mmHg) (mean ± SD)	78.67 ± 8.33	80.93 ± 8.28	96.67 ± 8.16

Table 2 : Lipid parameters DM without CAD and controls

Lipid parameters	Controls		DM without CAD		P value[b]
	Mean	SD	Mean	SD	
TC	156.75	18.30	209.52	52.30	<0.001
TG	101.50	31.04	218.80	100.95	<0.001
HDL	46.46	6.42	42.45	7.71	<0.05
LDL-C	90.07	15.51	120.47	45.05	<0.05
LDL-D	92.52	14.11	118.93	42.06	<0.05
VLDL	20.30	6.20	43.54	20.24	<0.001
sdLDL	24.72	5.49	50.12	17.01	<0.001

b- un paired t test
Table is showing the values of HDL and LDL are significantly different (<0.05) among patients of DM without CAD and controls, while other lipid parameters showing highly significant (<0.001) difference.

Table 3: Lipid parameters DM with CAD and controls

Lipid parameters	Controls		DM with CAD		P value[b]
	Mean	SD	Mean	SD	
TC	156.75	18.30	227.38	48.98	<0.001
TG	101.50	31.04	205.88	87.29	<0.001
HDL	46.46	6.42	38.32	6.00	<0.001
LDL-C	90.07	15.51	147.89	43.75	<0.001
LDL-D	92.52	14.11	146.08	39.99	<0.001
VLDL	20.30	6.20	41.18	17.46	<0.001
sdLDL	24.72	5.49	50.73	15.56	<0.001

b- un paired t test

Table is showing highly significant difference (<0.001) of all lipid parameters among patients of DM with CAD and controls.

Table 4 : Correlation of lipid parameters with sdLDL

Parameters	Controls		DM with CAD		DM without CAD	
	r	P value	r	P value	r	P value
TC	0.768	<0.001	0.866	<0.001	0.897	<0.001
TG	0.677	<0.001	0.804	<0.001	0.871	<0.001
HDL	-0.138	0.322	-0.513	<0.001	-0.241	0.008
LDL-C	0.664	<0.001	0.790	<0.001	0.792	<0.001
LDL-D	0.725	<0.001	0.879	<0.001	0.822	<0.001
VLDL	0.681	<0.001	0.804	<0.001	0.858	<0.001

r- Pearson's correlation coefficient

Table is showing positive correlation (P value <0.001) of sdLDL with the other lipid parameters.

Table 5: Pearson Chi-square test shows p= 0.00 (Highly significant) Odds ratio: 11.81 with 95% CI (3.3 – 41.0)

sdLDL in DM without CAD	sdLDL in DM with CAD		
	Normal	increased	total
Normal	31	6	37
increased	7	16	23
Total	38	22	60

The difference in the values of sdLDL in DM patients and DM with CAD patients is highly significant. Odds ratio 11.8 (95% CI 3.3-41.0) suggests 11 times increased risk of raised sdLDL value in DM with CAD patients.

DISCUSSION:
Diabetes mellitus is rising to an alarming level. India has the largest number of diabetic subjects in the world [16]. Epidemiological studies and clinical trials have shown that diabetic individuals are two-fold increased risk of cardiovascular events, which is the leading cause of death worldwide [2,17,18].Diabetic dyslipidemia, particularly LDL-C levels are linked to CAD. Furthermore, small dense fraction of LDL-C which has easy access to the endothelial layer of the blood vessels are found to be the main culprit for the etiology of CAD.

A very common metabolic abnormality associated with diabetes is dyslipidemia. Hypertriglyceridemia is considered the dominant lipid abnormality due to increased production and decreased clearance of TG-rich lipoproteins and plays a pivotal role in determining the characteristic lipid profile of diabetic dyslipidemia. In diabetes, glycation causes impaired recognition of LDL by its receptors on hepatocytes, thereby increasing its concentration; likewise advanced glycosylation end products (AGE) within the atherosclerotic plaques will lead to formation of lipid-rich atheroma.

In agreement with our study, multiple epidemiological studies have evaluated the correlation between dyslipidemia and CAD.

In the study done by **Abbott et al (1993)[19] , Sapna Smith et al. (2008)[20], Indumati et al. (2011)[21], Rakesh et al [22], Vicky JA Moor et al [23] , Dr. Musa Khan et al [24] , Li Qi et al[25]**

observed that any disorder in carbohydrate metabolism due to diabetes leads to disorder in lipid metabolism and affect blood lipid levels. So there is high concentration of cholesterol and TG and due to this there was reduction in HDL-C levels among diabetics as compared to controls.

Depending on the size, chemical composition and density, there are different subclasses of LDL-C that can be separated by ultracentrifugation as small dense and large buoyant particles. Out of which, sdLDL is said to be increased in diabetic individuals and is associated with coronary heart disease. sdLDL have easier access to the sub endothelial spaces in arterial walls and exhibit increased susceptibility to oxidation and uptake of macrophages, thereby facilitating the formation of foam cells. sdLDL have increased anchorage to arterial wall and also associated with elevated fibrinogen levels, thereby increasing the formation of atheromatous plaque.

Several methods have been developed for the assessment of sdLDL particles, such as density gradient ultracentrifugation, gradient gel electrophoresis, tube gel electrophoresis, and nuclear magnetic resonance .The methods can be expensive, time-consuming, and technically demanding, making them too laborious for routine clinical practice or screening a large population. Recently, Denka Seiken developed a simple method to measure sdLDL-cholesterol (sdLDL-C) by a novel homogeneous enzymatic assay (Denka Seiken, Tokyo,Japan) [26]. Although this method is easier to implement and has the potential for daily clinical use, the reagent cost may be prohibitively expensive for general or screening use.

In this study, we have used Friedwald's equation for the measurement of sdLDL-cholesterol. With the Friedwald equation, cLDL-C (in mg/dl) = TC – (HDL-C) – (TG/5); a value of TG/5 represents VLDL-C concentration. An overproduction of the TG-enriched large VLDL causing high generation of sdLDL might lead to overestimation of VLDL-C and underestimation of the cLDL-C concentrations. The difference between the cLDL-C and dLDL-C has been ascribed to variation in TG, HDL-C, and, potentially, the presence of sdLDL. So, we can estimate the sdLDL-C concentration from the classic lipid measures by Friedwald formula sdLDL (mg/dl) = 0.580 (non-HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 12.05 ; which will provide a cost-effective method for screening patients for the risk of CVD.

In this study, we evaluated the level of sdLDL particles among diabetic patients associated with (50.73 ± 15.56, p< 0.001) and without (50.12 ± 17.01, p<0.001) CAD and found that there is significant rise of sdLDL in DM patients compared with controls (24.72 ± 5.49).

When we correlated sdLDL with the other lipid parameters, it showed highly significant positive correlation with TC, TG, LDL-C, LDL-D and VLDL (p<0.001) in both diabetics with and without CAD. We found negative correlation between sdLDL and HDL-C (p 0.008) in diabetic individuals without CAD while in patients with CAD the association was found to be very significant (p<0.001) as given in Table 4. All these findings are corresponding to various studies showing association of sdLDL and CAD.

Similar study by **V. Mohan et al** [27] , **Anju Sharma et al** [4], **Ya-Ching Huang** [28] ,**Shinji Koba et al (2004)**[29] , **Ron C Hoogeveen et al**[30] found that sdLDL-C levels were strongly correlated with atherogenic lipid profile and were higher in diabetic than non-diabetic patients.

In the contrary to the findings of our study, **Ip et al** [31] found that LDL particle size and small dense LDL particle fraction were not consistently associated with CVD incidence. **Gazi et al** [32] also found no definite causal relationship between sdLDL and CVD, probably because of the close association between sdLDL and TG and other risk factors like differences in age, ethnicity, sex and geographical distribution among the study populations.

However on comparing the sdLDL-C among diabetic patients associated with and without CAD (Pearson chi square test, p=0.00 with Odds ratio 11.81 with 95% *CI* 3.3-41.0) which gives 11 times increased risk of raised sdLDL value in DM with CAD patients shown in Table 5. Thereby indicating sdLDL-C as strong predictor for CAD in diabetes patients.

SUMMARY & CONCLUSION:

Diabetes and its life threatening complications like cardiovascular events have spectacularly increased in the recent decades which can be prevented by regular monitoring of the various blood indices like glycemic control and lipid profile monitoring, of course with healthy lifestyle changes. In addition to that, as in our study, sdLDL is proved to be the better index for the early detection and monitoring of cardiac health in diabetic individuals. However further studies are needed to confirm the same.

REFERENCES

1) Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. J Intern Med 2001; 249:225-235
2) Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; 16:434-44
3) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of The National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97
4) Anju Sharma, Neha, Shailja et al. Study od dyslipidemia in patients of type 2 diabetes mellitus. BCAIJ,9(1),2015[036-040]
5) Anber V, Griffin BA, McConnell M, Packard CJ, Shepherd J. influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. Atherosclerosis. 1996;124:261-271
6) Dejager S, Bruckert E, Chapman MJ. Dense low density lipoprotein subspecies with diminished oxidative resistance predominance in combined hyperlipidemia. J Lipid Res. 1993;34:295-308
7) Hurt-Camejo E, Camejo G, Rosengren B, Lopez F, et al. Differential uptake of proteoglycan-selected subfractions of low density lipoprotein by human macrophages. J Lipid Res. 1990;31:1387-1398
8) Nordestgaard BG, Zilversmith DB. Comparison of arterial intimal clearance of LDL from diabetic and non-diabetic cholesterol-fed rabbits. Differences in intimal clearance explained by size differences. Arteriosclerosis. 1989;9:176-183
9) Sattar N, Petrie JR, Jaap AJ. The atherogenic lipoprotein phenotype and vascular endothelial dysfunction. Atherosclerosis. 1998;138:229-235
10) Myers GL, ed. Laboratory Medicine Practice Guidelines: Emerging Biomarkers for Primary Prevention of Cardiovascular Disease and troke. Washington, DC: The National Academy of Clinical Biochemistry; 2009
11) Akosah KO, Schaper A, Cogbill C, et al. preventing myocardial infarction in the young adult in the first place: how do the NAATIONAL Cholesterol Education Panel III guidelines perform? J Am Coll Cardiol. 2003;41:1475-1479
12) Lamarche B, Lemieux I, Despres JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. Diabetes Metab. 1999;25:199-211.
13) Koba S, Hirano T, Kondo T, et al. significance of small dense loe-density lipoproteins and other risk factors in patients with various types of coronary heart diseases. Am Heart J. 2002;144:1026-1035.
14) St-Pierre AC, Cantin B, Dagenais GR,et al. Low-density lipoprotein subfractions and the long term risk of ischaemic heart disease in men:13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol. 2005;25:553-559.
15) Pornpen Srisawasdi, Sirrirat Chaloeysup, Yaovalak Teerajetgul, et al. Estimation of plasma small dense LDL cholesterol from classic lipid measures. Am J Clin Pathol. 2011;136:20-29.
16) Wild S, Roglic G, Green A, et al. Global prevalence of Diabetes: estimates for the year 2000 and projection for 2030. Diabetes Care 2004;27:1047-1053 [PubMed: 15111519]
17) Haffner SM, Letho S, Ronnemaa T, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229-34.
18) Almdal T, Scharling H, Jensen JS, et al. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20yrs of follow-up. Arch Intern Med 2004;164:1422-1426.
19) C A Abbott, M I Mackness, S Kumar, A O Olukoga, C Gordon, S Arrol, D Bhatnagar, A J M Boulton and P N Durrington, Relationship between serum butyrylcholinesterase activity, hypertriglyceridemia and insulin sensitivity in diabetes mellitus, Clinical Science (1993) 85, 77-81
20) Sapna Smith and Alok M Lall, A Study on Lipid profile Levels of Diabetics and Non-Diabetics Among Naini Region in Allahbad, India, Turk Biyokimya Dergisi, Turkish Journal of Biochemistry, 33(4), 138-141 (2008).
21) Indumati.V, Vidya.S.Ratil,Krishnaswamy.D, Satishkumar.D, Vijay.V, Mahesh.S, Rajeshwari.V. International Journal of Pharma and Bio Sciences Vol 2/Issue 2/Apr-Jun 2011
22) Rakesh M Parikh, Sashank R Joshi, Padmavathy S, et al. Prevalence and pattern of diabetic dyslipidemia in Indian type 2 diabetic patients. Diabetes and Metabolic syndrome; Clinical Research and Review (2010);4(1):10-12
23) Vicky Jocelyne Ama Moor, Sylvie Ndongo Amougou, Sebastein Ombotto, et al. Dyslipidemia in patients with a cardiovasculae risk and disease at the University Teaching Hospital of Yaounde, Cameroon. International Journal of Vascular Medicine, vol.2017, article ID 6061306, https://doi.org/10.1155/2017/6061306
24) Dr. Musa Khan, Dr. P. Sakuntala, Dr. R. siddeswari, et al. pattern of dyslipidemia in Diabetes Mellitus. International Journal of Scientific and Research Publications, vol. 5, issue 5, may2015; ISSN 2250-3153.
25) Li Qi, Xianbin Ding, Wenge Tang, et al. Prevalence and risk factors associated with dyslipidemia in Chongqing, China. Int j Environ Res. Public Health 2015,12,13455-13465; doi:10.3390/ijerph121013455.
26) Ito Y, Fujimura M, Ohta M, et al. development of a homogeneous assay for measurement of small dense LDL cholesterol. Clin Chem. 2011;57:57-65.
27) V Mohan, R Deepa, K Velmurugan, et al. Association of small dense LDL with coronary artery disease and diabetes in urban Asian Indians- The Chennai Urban Rural Epidemiology Study (CURES-8). JAPI. Vol.53, February 2005. www.japi.org
28) Ya-Ching Huang, Pi-Yueh Chang, Jawl-Shan Hwang, et al. Association of small dense LDL- cholesterol in type 2 diabetics with coronary artery disease. Biomed J 2014;37:375-379.
29) Shinji Koba, Yuuya Yokota, Tsutomu Hirano, et al. Small LDL-cholesterol is superior

to LDL-cholesterol for determining severe coronary atherosclerosis. J Atheroscler Thromb, 2008;15:250-260.
30) Ron C. Hoogeveen, John W. Gaubatz, Wensheng Sun, et al. Small dense LDL-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Arterioscler Thromb Vasc Biol, 2014;34:1069-1077.
31) Ip S, Lichtenstein AH, Chung M, et al. systematic review: association of LDL subfractions with cardiovascular outcomes. Ann Intern med 2009;150:474-484
32) Gazi IF, Tsimihodimos V, Tselepis AD, et al. Clinical importance and therapeutic modulation of small dense LDL particles. Experr Opin Biol Ther 2007;7:53-72.