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Research Article

RELATIONSHIP BETWEEN THE GLYCEMIC CONTROL, VITAMIN D STATUS AND CARDIOVASCULAR RISK FACTORS INCLUDING sLDL IN TYPE 2 DIABETES MELLITUS

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A B S T R A C T

Background: T2DM, affects 5.9% of the world's population. Diabetics suffering from complications of chronic hyperglycaemia are also susceptible to deadly cardiovascular diseases (CVD). sLDL, a subtype smaller and heavier than LDL, easily penetrates the walls of arteries. Being atherogenic, it is an emerging risk factor for CVD in T2DM. **Objectives:** To analyse and correlate glycemic control with vitamin D status, lipid profile including sLDL among T2DM patients and also to clarify the association of sLDL with glycemic control in T2DM. **Methods:** Total 60 subjects; 30 with T2DM and 30 normal were selected based on the levels of HbA1c. Lipid profile, sLDL, FBS, HbA1c, Profile and Vitamin-D were analysed. **Results:** HbA1c and FBS ($p< 0.0001$) and lipid profile parameters, TC($p < 0.05$), TG($p <0.001$), LDL, VLDL, Non-HDL, LDL/HDL, TC/HDL, TG/HDL ratios, sLDL ($p < 0.0001$) and the sLDL/HDL ratio increased significantly and in contrast HDL and Vitamin D levels were significantly decreased ($p < 0.05$) in Type 2 Diabetic group compared to the control group. HbA1C showed a positive correlation with VLDL($r=0.45$, $p< 0.001$), sLDL($r=0.28$; $p< 0.05$), sLDL/HDL($r=0.53$; $p< 0.0001$), TG($r=0.46$; $p<0.001$), TG/HDL($r=0.50$; $p< 0.00001$) but a negative significant correlation with HDL ($r= -0.27$; $p<0.05$), and Vitamin D (although insignificant) in Type 2 Diabetic patients. sLDL in T2DM showed a positive relationship with sLDL/HDL($r=0.655$; $p<0.00001$), TG($r=0.46$; $p<0.0001$), VLDL($r=0.466$; $p<0.0001$), TC ($r=0.44$; $p<0.001$), Non-HDL-cholesterol($r=0.421$; $p<0.001$). In contrast, sLDL showed a significant negative correlation with HDL($r= -0.499$; $p<0.00001$), LDL/HDL($r = -0.25$; $p<0.05$)), TC/HDL($r= -0.162$) indicating that these decrease while sLDL may increase or vice-versa. sLDL has insignificantly inverse relation with Vitamin D in Type 2 Diabetic patients. **Conclusion:** sLDL was directly correlated with HbA1c and Triglycerides while inversely related to Vitamin D levels in T2DM. T2DM patients with increased HbA1c due to uncontrolled diabetes showed invariable increase in lipid profile and sLDL although decreased HDL and Vitamin D levels.

INTRODUCTION

Diabetes Mellitus is a serious and a majorly growing public health problem worldwide. According to WHO, the number of adults with diabetes in the world would increase alarmingly from 135 million in 1995 to 300 million in 2025 [1]. Type 2 Diabetes Mellitus, which contributes to more than 90% of all diabetic cases, affects 5.9% of the world's adult population with almost 80% of the total in developing countries [2]. India leads the world with the largest number of diabetic subjects earning the dubious distinction of being termed as the "diabetes capital of the world" [3].

Diabetic patients apart from suffering from numerous complications of chronic hyperglycaemia are also susceptible to deadly cardiovascular disease (CVD). CVDs are one of the major reasons that reduce the life expectancy in these groups of patients. The enhanced levels of glycated hemoglobin (HbA1c) are considered an independent risk factor for stroke and coronary heart disease (CHD) in diabetic and non-diabetic patients. If glycemic control is improved it can reduce the risk of cardiovascular events in diabetics to a major extent. [4]

Interestingly, based on recent studies, a new type of LDL, namely, small dense LDL- cholesterol (sLDL-C) is considered as an emerging risk factor for cardiovascular disease in diabetes mellitus type 2. [5, 6] It is a type of LDL which is smaller and heavier than typical LDL and can increase the risk of Atherosclerosis because it is small enough to penetrate the walls of arteries. Being more susceptible to oxidize, it stays

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longer in the blood stream. Hence it can be considered as an emerging risk factor for cardiovascular disease in type 2 Diabetes Mellitus. While Vitamin D plays a pivotal role in calcium/phosphorus homeostasis and bone physiology, several lines of evidence also suggest that Vitamin D deficiency has been linked to the onset of diabetes. It has been found that Vitamin D levels are inversely related to the occurrence of Type- 2 diabetes mellitus. [7]

AIMS AND OBJECTIVES

1) To analyse glycemic control, vitamin D status, lipid profile picture including sLDL among type 2 diabetes mellitus patients.
2) To correlate glycemic control with the vitamin D status and lipid profile in type-2 diabetic patients.
3) To clarify small, dense LDL (sLDL) association with glycemic control in type 2 diabetes, it being a specific emerging risk factor for cardiovascular disease.

MATERIALS AND METHODS

The study was conducted on 60 subjects among which 30 Type 2 Diabetic patients were selected based on levels of HbA1c and 30 normal healthy subjects were considered as control group.

Selection criteria:

Inclusion criteria

Adult male and female patients suffering with Type 2 Diabetes will be included in the study.

Exclusion criteria

Subjects with Type 1 diabetes or recently diagnosed diabetes, cardiac problems, other chronic disorders and on Vitamin D supplementation will be excluded from the study.

After obtaining the clearance from the Institutional Ethical Committee [IEC/ASR/016/2018] to carry out the research study in the institution, written informed consents along with predefined questionnaires were obtained from the Diabetic patients and the normal subjects.

The blood samples were collected in sterile labeled plain vacutainer (12 h fasting) for serum, centrifuged at 3000rpm for 10 mins. and also separately 2 ml of blood sample was collected in an EDTA-coated tube for HbA1C analysis. All lipid profile parameters, sLDL and HbA1c were analyzed by using Beckman Coulter AU 480 analyzers.

The parameters are analysed by the methods of Triglycerides (Tg) - Enzymatic, colorimetric method, GPO- PAP method, total cholesterol (TC) - CHO-POD method, high-density lipoprotein cholesterol (HDL-C) - CHE &CHO-POD method , low density lipoprotein choesterol (LDL-C) - calculated by using Friedwald's formula and fasting blood glucose (FBG) – Hexokinase G-6-PD, HbA1c – Turbidometric Immunoinhibition by using Beckman Coulter AU 480 automated biochemistry analyser and also sLDL by automated chemical analyzers where the sLDL-EX "SEIKEN" test is a direct method. Serum 25(OH) D3 or vitamin-D is measured CLIA using Beckman Coulter Access 2.

Statistical analysis

The statistical methods used in the study were done in the software Microsoft Excel 2007 version. The quantitative data was expressed as Mean ± Standard Deviation and the significance of the difference in the mean values between the test and control groups was calculated by "Unpaired Student t-Test" at the significance of p value 0.05. The correlation between different analytes with HbA1c, sLDL and Vitamin D values in both the groups was done by "Pearson Correlation Test "at the significance of p value 0.05

RESULTS

The present study anticipates the association of mean±SD values of FBS, HbA1C, Vitamin D, fasting lipid profile (TC, TG, VLDL, LDL, sLDL, HDL, non HDL, non HDL/HDL, LDL/HDL, sLDL/HDL,TG/HDL and TC/HDL) levels in patients with T2DM. Total 60 subjects, sex and age related, were include in the study of which, 30 had T2DM and another 30 normal healthy as control group.

Table 1 Comparison of Glycemic Indices, Lipid profile, SLDL and Vitamin D among the Controls & Type 2 Diabetic Patients

Parameters	Control	Diabetic	t Value	p- value
FBS	98.3+20.7	183.9+73.47	6.24	<0.00001
HbA1c	5.34 + 0.35	8.8 + 1.8	10.06	< 0.0001
MBG	112.7+12.8	234 + 66	9.906	< 0.0001
Total cholesterol	166.1 + 63.4	200.7+ 31.5	1.71	< 0.05
HDL	43.7+9.27	39.6 +7.27	2.02	< 0.05
LDL	101.5+ 24.7	124.6 + 32.3	3.1	<0.001
VLDL	21.9 + 6.9	33.1+16.6	3.38	<0.001
LDL/HDL	2.38 + 0.61	3.18 + 0.94	4.077	< 0.0001
sLDL	24.35 + 3.78	50.53 + 11.8	11.52	< 0.0001
sLDL/HDL	0.56 + 0.14	1.28 + 0.14	12.46	< 0.0001
TG	110 + 34.72	166.2 + 82.9	3.376	<0.001
TC/HDL	3.89 + 0.74	5.17 + 0.91	5.93	< 0.0001
Non –HDL	123.37 + 68.4	157.73 + 35	4.31	< 0.0001
Non-HDL/HDL	2.8816 + 0.72	4.076 + 1.07	4.95	<0.00001
TG/HDL	2.54 + 1.11	4.38 + 2.54	3.32	< 0.001
Vitamin D	26.56 + 2.53	11.65 + 2.23	24.1	< 0.00001

Table 1 depicts the comparison between Glycemic Indices, Lipid profile, sLDL and Vitamin D among the Controls & Type 2 Diabetic Patients. The levels of HbA1c (Fig.1) and MBG (Fig.2) increased significantly in Type 2 Diabetic patients compared to normal subjects ($p< 0.0001$). Similar to HbA1c, the lipid profile parameters (fig.3) like, total cholesterol ($p< 0.05$), LDL ($p<0.001$), VLDL ($p<0.001$), Non –HDL ($p<0.0001$) and triglyceride ($p<0.001$) levels increased significantly compared to normal subjects. The LDL/HDL, sLDL/HDL, Non- HDL/HDL, TC/HDL and TG/ HDL ratio (Fig.4) were found to be increased significantly in Type2 Diabetic patients compared to normal subjects ($p<0.0001$).

In contrast to these parameters, the serum levels of HDL decreased (Table 1) significantly in Type 2 Diabetic patients compared to normal subjects ($p<0.05$). Like HDL, the serum levels of Vitamin D levels(Table 1) were found decreased significantly in Type 2 Diabetic patients compared to normal subjects ($p<0.00001$).

The sLDL levels (Table 1) in serum increased significantly in Type 2 Diabetic patients compared to normal subjects ($p< 0.0001$).

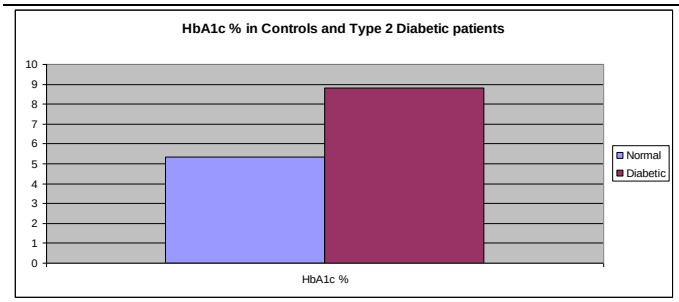

Figure 1 HbA1c % in Controls and Type 2 Diabetic Patients

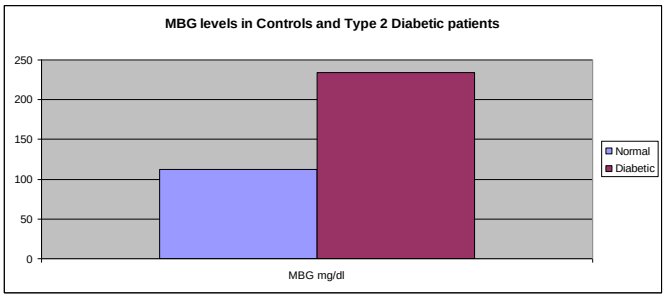

Figure 2 MBG levels in Controls and Type 2 Diabetic Patients

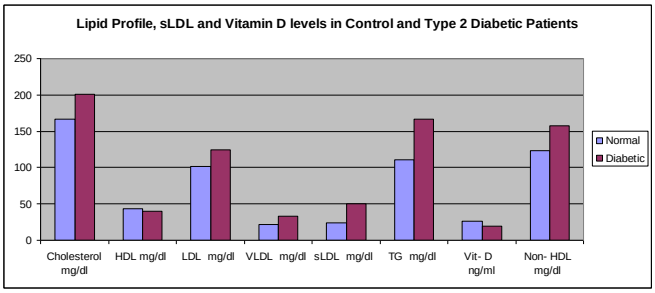

Figure 3 Lipid profile, sLDL and Vitamin D levels in Controls and Type 2 Diabetic Patients

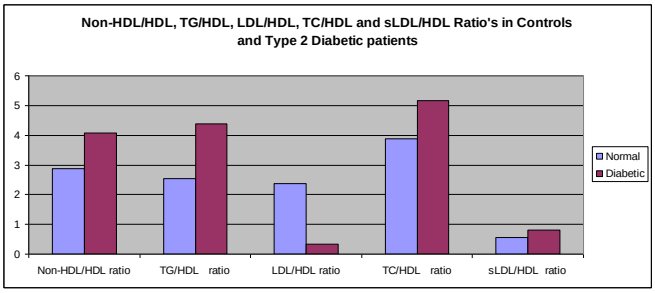

Figure 4 Non-HDL/HDL , TG/ HDL, LDL/HDL, TC/HDL and sLDL/HDL ratios in Controls and Type 2 Diabetic Patients

Table 2 Correlation between HbA1c with Lipid Profile, sLDL and Vitamin D in Controls and Type 2 Diabetic subjects.

PARAMETERS	CONTROL		TYPE 2 DIABETIC	
HbA1C	Pearson Correlation r	P value	Pearson Correlation r	P value
Cholesterol	-0.04	0.76	-0.05	0.70
HDL	-0.1	0.44	-0.27	0.03
LDL	0.01	0.9	-0.10	0.44
VLDL	-0.07	0.59	0.45	0.0003
LDL/HDL	-0.006	0.96	0.066	0.6
sLDL	-0.17	0.19	0.288	0.02
sLDL/HDL	-0.08	0.54	0.53	0.00001
TG	-0.07	0.59	0.46	0.0002
TC/HDL	0.02	0.96	0.18	0.76
Non –HDL	-0.005	0.76	0.12	0.36
Non –HDL/HDL	0.039	0.65	0.26	0.036
TG/HDL	-0.058	0.19	0.50	0.00001
Vitamin D	0.17	0.87	-0.04	0.76

The Pearson Correlation Analysis between HbA1c with Lipid Profile including sLDL and Vitamin D in Controls and Type 2 Diabetic subjects is depicted in Table 2. The Pearson correlation of HbA1c with lipid profile, sLDL and Vitamin D does not show any statistical significance in the normal control group. In contrast to these, the HbA1C showed a positive correlation with VLDL(r=0.45, p< 0.001), sLDL(r=0.28; p< 0.05), sLDL/HDL(r=0.53; p< 0.0001), TG(r=0.46; p<0.001), TG/HDL(r=0.50; p< 0.00001) but a negative significant correlation with HDL (r= -0.27; p<0.05), and Vitamin D (although insignificant) in Type 2 Diabetic patients.

Table 3 Correlation between sLDL with Lipid Profile and Vitamin D in Controls & Type 2 Diabetic patients.

Parameters	Control		Type 2 Diabetic	
sLDL	Pearson Correlation r	p Value	Pearson Correlation r	p value
Cholesterol	0.51	0.000001	0.44	0.0004
HDL	0.188	0.16	- 0.49	0.00001
LDL	0.45	0.0003	0.21	0.10
VLDL	0.39	0.002	0.46	0.0001
LDL/HDL	0.29	0.02	-0.25	0.05
sLDL/HDL	0.44	0.0004	0.65	0.00001
TG	0.39	0.002	0.469	0.0001
TC/HDL	0.27	0.03	-0.16	0.22
Non –HDL	0.53	0.00001	0.42	0.0008
Non – HDL/HDL	0.24	0.064	-0.02	0.87
TG/HDL	0.23	0.07	0.24	0.06
Vitamin D	0.101	0.44	-0.03	0.82

Table 3 depicts Correlation between sLDL with Lipid Profile and Vitamin D in Controls & Type 2 Diabetic patients. Statistical correlation of sLDL was seen positive with all the variables namely, Non-HDL (r=0.53;p<0.00001), total cholesterol(r=0.51, p<0.00001), LDL (r=0.45; p<0.0001), sLDL/HDL(r=0.44; p<0.0001), Triglycerides (r=0.39; p<0.001), VLDL (r=0.39; p<0.001), LDL/HDL (r=0.29; p< 0.05), TC/HDL (r= 0.27; p<0.05) in the normal control group. The Pearson correlation analysis revealed the relationship of the parameters with sLDLin T2DM to be sLDL/HDL (r=0.655; p<0.00001), followed by triglycerides (r=0.46; p<0.0001), VLDL(r=0.466; p<0.0001), total cholesterol (r=0.44;p<0.001), Non-HDL-cholesterol(r=0.421; p<0.001). In contrast, sLDL showed a significant and negative correlation with HDL(r= - 0.499; p<0.00001), LDL/HDL (r = -0.25; p<0.05)), TC/HDL(r= -0.162) indicating that these decrease while sLDL may increase or vice-versa. sLDL has insignificantly inverse relation with Vitamin D in Type 2 Diabetic patients.

Table 4 Correlation between Vitamin D with Lipid Profile in Controls & Type 2 Diabetic patients.

Parameters	Control		Diabetic	
Vitamin D	Pearson Correlation r	p value	Pearson Correlation r	p value
Cholesterol	-0.18	0.16	0.06	0.64
HDL	-0.16	0.19	0.016	0.90
LDL	-0.003	0.98	0.24	0.06
VLDL	0.22	0.08	-0.26	0.04
LDL/HDL	0.25	0.05	0.403	0.0015
sLDL	0.101	0.44	-0.03	0.82
sLDL/HDL	0.206	0.11	-0.09	0.49
TG	0.22	0.09	-0.27	0.03
TC/HDL	0.09	0.49	0.04	0.76
Non –HDL	0.066	0.64	0.100	0.44
Non –HDL/HDL	0.237	0.07	0.09	0.46
TG/HDL	0.23	0.07	-0.23	0.07

Table 4 depicts the correlation between Vitamin D with Lipid Profile in Controls & Type 2 Diabetic patients. Status of Vitamin D was also compared with other biochemical variables using Pearson Correlation Analysis. In T2DM, a positive significant correlation was found with LDL/HDL($r=0.40$; $p<0.001$) although a negative correlation of Vitamin D was found with VLDL ($r= -0.26$; $p<0.05$). The results obtained reveal the negative correlation between the serum levels of vitamin D with HbA1C and sLDL.

DISCUSSION

In the present study, the levels of different entities were analyzed and compared between Type 2 diabetic patients and control group. We focused on independent correlations between glycemic index, vitamin D status and the cardiovascular risk factors including sLDL in patients with Type2 Diabetes Mellitus. The results obtained from the tests performed for glycemic index reveal that levels of HbA1c and MBG in Type2 Diabetic group were significantly increased compared to control group. Several other studies also found similar findings in Type2 Diabetics.[8,9,10] In addition to HbA1c, FBS was significantly increased in the Type 2 Diabetic group compared to control which was similar to the study presented by Ghazanfari Z *et al*; that stated about the increased reliability of Fasting Blood Sugar to separate diabetic from non-diabetic subjects compared to HbA1c.[11]

Similar to HbA1c and FBS, the parameters of lipid profile like TC, TG, LDL, VLDL, Non-HDL, also increased significantly in Type 2 Diabetic group compared to the control group which was corresponding to the findings of various studies of Ozder A *et al*. [12] and Ronald *et al*. [13] The LDL/HDL, TC/HDL, TG/HDL ratios of both the groups showed significant increase among the Type2 Diabetics. This was homogenous to the study done by Nita Garg *et.al*; who also concluded TC/HDL, LDL/HDL ratios being more specific and accurate for assessing Coronary Artery Disease than considering TC, TG, HDL, LDL levels alone. [14]

The increased lipid profile in Type 2 DM patients was due to increased lipolysis in adipocytes due to deficiency of insulin resulting in increased fatty acids which are transported to the liver.

In the present study, sLDL and the sLDL/HDL ratio were significantly increased in the T2DM patients when compared to the controls and our findings correlate with that of Wahid Ali *et.al*.,2017[15]. The increased sLDL in Type2 DM, may be due to its increased generation from intravascular processing of specific larger triglyceride-rich very-low density lipoprotein (VLDL) precursors. Hepatic production of VLDL is stimulated by insulin resistance, and hence the prevalence of sLDL is found to be associated with insulin resistance. Therefore sLDL has been highlighted as a useful new marker for the risk of CHD or type 2 diabetes mellitus [16]. The significant relationship between sLDL/LDL and plasma triglyceride indicates that triglyceride rich VLDL, a precursor of sLDL, contributes to the appearance of sLDL particles in the plasma [17]. A study based on the Low-density lipoprotein subclasses and the risk of Myocardial Infarction presented by Austin MA *et.al*. attested that the elevation in sLDL has a 3-fold increased risk of Myocardial Infarction[18,19].

In contrast to these parameters, the HDL levels were found to be decreased significantly in the Type2 DM patients compared to the controls. The decrease in plasma HDL cholesterol levels is due to increased hydrolysis by hepatic lipase which result in rapid catabolism and clearance from plasma. Reduced HDL levels may be due to the transfer of cholesterol from HDL to triglyceride-rich lipoproteins, with the reciprocal transfer of triglyceride to HDL commonly found in Type2 DM patients which is associated with insulin resistance [20].

The levels of vitamin D were observed to be drastically decreased in the Type 2 DM patients when compared to controls. Our findings are in similarity with the study conducted by Bayani MA *et al*; whose study demonstrated that the mean vitamin D values were significantly lower in the patients with Diabetes Mellitus than the healthy controls, and that 89.2% of Diabetic patients had insufficient vitamin D values[21] while it was found to be 98.4% according to SerdarOlt's findings [22]. The cause for the decrease of Vitamin D in T2DM might be due to the decrease in the rate of hydroxylation vitamin D3 in the kidneys which favors a decrease in the synthesis of this vitamin as mentioned in the study by Mathieu *et.al*; 2005 [23].

The presence of elevated HbA1C, sLDL and LDL levels in Type 2 Diabetic patients with low vitamin D levels in our study suggest the role of vitamin D deficiency in the pathogenesis of type 2 diabetes mellitus and its complications which was earlier proved by taking into consideration the elevated levels of only HbA1c and LDLc [24]. In the present study, weak negative correlation was found between glycated hemoglobin and Vitamin D which was similar to other studies[25].

In the present analytical study, an inverse association was observed between fasting blood glucose, HbA1c and Vitamin D3 levels which is in par with the findings of Dhia J. Al-Timimi *et.al*. 2013[26] and Kostoglou *et al.*2012[27], implying that low serum 25(OH) D3 may be associated with impaired glucose metabolism.

The glycated hemoglobin was found to have direct relations with sLDL, VLDL and Triglycerides in Type 2 DM patients which was in similarity with the findings of Wahid Ali *et.al*.,2017 [15]. In contrast, the glycated hemoglobin was found to have indirect relations with Vitamin D and HDL. Elevation in HbA1c shows an apparent decrease in both HDL and Vitamin D levels. The diabetic components are shown to be significantly related to high lipid profile including sLDL and their ratios.

Positive correlations were observed between sLDL and triglycerides and thus increase in triglycerides also increases the levels of sLDL. sLDL was also found to have a significant association with glycemic index in T2DM, it being an emerging risk factor for CHD. Comparatively, the increase in sLDL associates with the dual decrease in HDL and Vitamin D levels.

Independent inverse correlations were observed between Vitamin D and HbA1c which was insignificant and between Vitamin D and sLDL which was significant.

CONCLUSIONS

The present study showed significant increase in glycemic index, and sLDL levels associated with very low serum Vitamin D levels. Abnormal lipid profile was observed which

includes Total cholesterol, VLDL, LDL, Triglycerides, Non-HDL, LDL/HDL, TC/HDL, TG/HDL ratios, which were found increased and in contrast the HDL levels were decreased in Type2 DM patients. Among the various metabolic parameters, high levels of HbA1C, TG, VLDL and LDL had a consistent association while sLDL had weakly significant relation with the reduced Vitamin D status in T2DM patients.

sLDL was directly correlated with HbA1c and Triglycerides. Type2 DM patients with increased HbA1c due to uncontrolled diabetes show invariable increase in the lipid profile and sLDL levels although HDL levls decrease. The atherogenicity of sLDL may be due to reduced LDL receptor affinity, easier penetration into the walls of arteries, increased binding to proteoglycans in the arterial wall and increased susceptibility to oxidation. The increase in sLDL levels associated with dyslipidemic features in Type 2 DM patients may contribute to the formation of atherosclerosis plaque or arterial damage.

Further Scope of Study

Studies are required on Vitamin D supplementation which may improve the glycemic control and also the consequences of increased sLDL in Type 2 Diabetes Mellitus.

Acknowledgment

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Abbreviations

DM(Diabetes Mellitus), CVD(Cardiovascular disease), CHD(Coronary heart disease), CAD(Coronary Artery disease), HbA1C (Glycated Hemoglobin), MBG(Mean Blood Glucose), FBS(Fasting Blood Sugar), TC(Total Cholesterol), TG(Triglycerides), VLDL(Very Low Density Lipoprotein), LDL(Low Density Lipoprotein), sLDL (small, dense Low Density Lipoprotein), HDL (High Density Lipoprotein), T2DM(Type 2 Diabetes Mellitus)

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