ORIGINAL RESEARCH PAPER Volume - 9 | Issue - 11 | November - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SMALL DENSITY LDL (SD LDL) AND ITS CORRELATION WITH HBA1C IN NEWLY DIAGNOSED TYPE 2 DIABETES MELLITUS PATIENTS

Biochemistry

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ABSTRACT

Introduction: Low density lipoprotein (LDL) is the major source of atherosclerotic lipid storage and it plays a key role in the development and progression of atherosclerosis and cardiovascular disease. LDL consists of several subclasses of particles with different sizes and densities, including large buoyant (lb), intermediate and small dense (sd) LDLs. Type 2 DM patients have been found to have a preponderance of sd LDL which increases the atherogenicity despite the normal LDL. **Materials & Methods:** 80 newly diagnosed T2DM patients and equal number of age and gender matched healthy individuals were included in the study. sd LDL along with routine lipid profile, fasting and post prandial blood sugar (FBG & PPBG) and HbA1c were estimated in all subjects. **Results:** The values of FBG, PPBG, TG, TC, LDL, VLDL, sd LDL and HbA1c were significantly ($p<0.001$) higher in newly diagnosed diabetic cases as compared to controls. In newly diagnosed T2DM cases, HbA1c had strong positive correlation with TG, TC, LDL, VLDL, sd LDL ($r=0.895$, $r=0.921$, $r=0.881$, $r=0.895$, $r=0.713$, respectively) and a strong negative correlation with HDL ($r=-0.578$). **Conclusion:** Our study shows statistically strong correlation of small dense LDL with HbA1C and is a metabolically better parameter than conventional lipid parameters in assessing the risk of CVD in diabetic patients. Metabolic as well as genetic association of non conventional lipid parameters would help in the early detection of future risk of CVD in type 2 DM patients.

KEYWORDS

sd LDL, HbA1C, Type 2 Diabetes Mellitus, Cardiovascular disease

Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia due to insulin deficiency or insulin resistance resulting in altered metabolism of carbohydrates, lipids and proteins. DM is a complex, chronic illness that requires multifactorial risk reduction, medical care and medical treatment.[1] By the year 2045 India is expected to become the diabetic capital of the world, with almost 134.3 million diabetics according to the estimates of International Diabetic Federation.[2] Indians are more prone for the development of diabetes due to fast socio economic development, rapid urbanization, genetic susceptibility, environment and also because of their lifestyle and dietary habits. Indians are genetically susceptible to CAD from early childhood.[3]

Hypertension and dyslipidemia also play a significant role in increased risk of CAD in type 2 DM patients in addition to hyperglycemia. Various studies have shown the association of high plasma level of total cholesterol, and triglycerides and the occurrence of cardiovascular events. Low density lipoprotein cholesterol (LDL-C) makes up about 70-75% of the total cholesterol level.[4] High levels of LDL cholesterol are principal risk factors in the pathogenesis of atherosclerosis.[5] But it has been found that certain people having normal lipid profile develop cardiovascular diseases.[6] So, the lipid profile assessed routinely, was found to be inadequate in finding the status of CVD in both diabetic and non diabetic patients. Hypertriglyceridemia and oxidation of lipoproteins play an important role in various steps of atherogenesis and this eventually leads to cardiovascular diseases.

Small dense LDL particles are formed from the processing of larger VLDL particles via series of steps including lipolysis.[7] Insulin resistance and type 2 DM leads to increase efflux of free fatty acids from adipose tissue. Further triglyceride enrichment of the lipolytic products through the action of cholesteryl ester transfer protein, together with hydrolysis of TG and phospholipids in LDL and HDL by hepatic lipase, leads to increased production of small dense LDL particles.[8] LDL particles are heterogeous in respect to size, density and lipid composition. LDL particles have been divided into 2 distinct phenotypes. The first one is pattern A which has large more buoyant LDL (lb LDL) particles and the other is pattern B which has prominence of small dense LDL.[9,10] sd-LDL is more atherogenic as it highly penetrates the arterial wall and greater arterial retention because of increased binding to proteoglycans, has a short plasma half life and little affinity for the LDL receptor and is more prone to oxidation as compared to the large buoyant fraction.[11,12] sd LDL particles are smaller and contain less cholesterol, increased levels of plasma sd LDL represents increased atherogenic particles, not reflected by LDL-C. Type 2 DM patients have been found to have a preponderance of sd LDL which increases the atherogenicity despite the normal LDL.[13]

Glycated hemoglobin is regarded as a independent risk factor in the risk of CVD in diabetic patients. It can be used as a predictor of dyslipidemia and thus early diagnosis of dyslipidemia can be used as a preventive measure for the development of CVD in patients with T2DM.[14] This study is an attempt to find a correlation between HbA1C and ox LDL in newly diagnosed type 2 DM patients. In this study we assessed the conventional lipid profile (TC, TG, LDL, HDL and VLDL) and sdLDL. We correlated the values of conventional lipid parameter and sd LDL with HbA1c.

Material & Methods

The study was conducted in the Departments of Biochemistry and Medicine of SGT Medical College, Hospital & Research Institute, Budhera, Gurugram after taking ethical clearance from the ethical commitee. It was a hospital based study which included 80 newly diagnosed Type 2 DM patients attending medicine OPD and 80 age and gender matched healthy individuals from general population were taken as controls. The diagnosis of new patients of T2DM was based on American Diabetic Association criteria[15] i.e Fasting blood sugar level $\geq$ 126 mg/dl, Random blood glucose $\geq$ 200 mg/dl or HbA1c $\geq$ 6.5 % with classical symptoms of diabetes (polyuria, polydipsia, polyphagia, weight loss) or hyperglycemic crisis and two hour plasma glucose $\geq$ 200 mg/dl following a 75g oral glucose load.

Patient with history of smoking and chronic alcoholism and on medications such as hypolipidemic drugs, hormone replacement therapy, steroids, drugs that induce hyperglycemia, liver or kidney diseases and those suffering from acute or chronic inflammatory disorders, dyslipidemias and HIV were excluded from the study.

Written and informed consent was taken from all the subjects included in the study after explaining the details of the study and its purpose.

5ml of venous blood was drawn after 12-14 hours of overnight fasting, under aseptic conditions in suitable vaccutainers from the selected cases and controls. The plasma/serum was separated by centrifugation at 3000 rpm for 15 minutes. Plasma/ Serum were used for analysis of FBG, PPBG, TG, TC, LDL, HDL, HbA1c.

FBG, PPBG and lipid parameters were measured on Erba Mannheim EM-200 Fully-automatic analyser with ERBA XL System Pack. HbA1c was measured by Ion Exchange Resin Method and sd LDL was calculated by using the formula given by Hattori et al.[16]

$$\text{sd LDL} == \frac{0.94\text{Chol} - 0.94\text{HDL-chol} - 0.19\text{TG}}{\text{ApoB} - 0.09\text{Chol} + 0.09\text{HDLchol} - 0.08\text{TG}}$$

This formula gives the radius of the sd LDL. The concentration is inversely proportional to the radius. So, if the radius decreases the concentration increases.

Data was collected and mean and SD for all the parameters was calculated. Statistical analysis was performed by SPSS 21 program for Windows. Continuous variables were summarized in the form of Mean ± SD and categorical variables are presented as frequencies and percentages. Graphically data was presented by bar diagrams. Student independent t tests were employed for comparing continuous variables. Chi square tests or Fisher's test, whichever appropriate, was applied for comparing categorical variables. A p value <0.001 was considered highly significant.

Results

The mean age of newly diagnosed T2DM cases was 52 years and the mean age of controls was 52.3 years. The difference of age between cases and controls was not significant (p= 0.678). (Table: 1) 65% of cases were males and 35% were females while 57% of patients control group were males and 23% of healthy controls were females. There was no significant correlation between cases and healthy people on the basis of sex of individuals. The values of serum FBG, PPBG, TG, TC, LDL, VLDL were significantly higher (p<0.001) while the levels of serum HDL was significantly lower (p<0.001) in newly diagnosed Type 2DM cases as compared to controls. The radius of sd LDL was lower in cases as compared to controls. As the radius is inversely proportional to the concentration, sd LDL concentration was significantly higher in cases as compared to controls. (p<0.001) (Table: 2) In newly diagnosed T2DM cases, HbA1c had strong positive correlation with FBG, PPBG, TG, TC, LDL, VLDL, (r=0.937, r=0.930, r=0.895, r=0.921, r=0.881, r=0.895, respectively). HbA1c had a strong negative correlation with HDL (r=-0.578). HbA1c had a strong positive correlation with sd LDL concentration (r=0.713) as the values of sd LDL represent the radius of the sd LDL, which relates inversely to its concentration. (Table: 3)

Table 1: Showing mean age (years) among cases and controls

Age (years)	N	Mean	SD	Range
Cases	80	52.0	3.75	46-62
Controls	80	52.3	2.56	47-59

Table 2: Showing various biochemical parameters among cases and controls

Parameter	Cases		Controls		p-value
	Mean	SD	Mean	SD	
FBG (mg/dl)	279.05	25.97	87.95	6.13	<0.001*
PPBG (mg/dl)	310.00	31.45	120.73	6.86	<0.001*
HbA1C (%)	6.95	0.46	5.09	0.37	<0.001*
TG(mg/dl)	320.6	38.99	118.5	16.05	<0.001*
TC(mg/dl)	289.4	27.12	174.9	16.63	<0.001*
LDL(mg/dl)	192.3	28.38	111.5	16.71	<0.001*
HDL (mg/dl)	32.6	4.68	39.7	4.96	<0.001*
VLDL(mg/dl)	64.1	7.80	23.7	3.21	<0.001*
sd LDL(no units)	0.76	0.15	1.29	0.27	<0.001*

Table 3: Correlation of HbA1c with FBG, PPBG and lipid parameters

Parameter	Pearson Correlation	P-value
FBG(mg/dl)	0.937	<0.001*
PPBG(mg/dl)	0.930	<0.001*
TG(mg/dl)	0.895	<0.001*
TC mg/dl	0.921	<0.001*
LDL mg/dl	0.881	<0.001*
HDL (mg/dl)	-0.578	<0.001*
LDL/HDL	0.847	<0.001*
VLDL(mg/dl)	0.895	<0.001*
sd LDL(no units)	-0.713	<0.001*

Discussion

Diabetes Mellitus is the most common endocrinal disorder worldwide with high incidence and prevalence. Diabetics have two to three times higher rate of cardiovascular diseases than those without diabetes.[17] In our study the levels of FBG, PPBG and HbA1c were significantly higher in newly diagnosed T2DM patients as compared to controls. In newly diagnosed T2DM cases the mean values of HbA1c was 6.95±0.46 % whereas the mean value of HbA1c in controls was 5.09% ±0.37%. Fasting blood sugar is the cheapest biomarker to separate diabetic from non diabetic individuals. Our study showed a marked increase in FBG and HbA1c in the diabetic patients as compared to the controls, which is similar to study of Ghazanfari Z et al[18] who presented that FBG and HbA1c are used as diagnostic biomarker to separate diabetic from non-diabetic subjects. Hyperglycemia leads to formation of sorbitol, glycosylation of hemoglobin and various lipoproteins and later formation of advanced glycosylated end products (AGEs). The AGEs form cross links with extracellular matrix proteins like collagen and cause endothelial dysfunction, reduces nitric oxide (NO) synthesis and accelerate atherosclerosis.

In our study sd LDL was found to have significantly higher values in newly diagnosed T2DM cases as the radius of sd LDL is 0.76 ± 0.15 in T2DM cases as compared to controls (1.29± 0.27). HbA1c had a strong positive correlation with sd LDL concentration. (r=0.713) as the values of sd LDL represent the radius of the sd LDL, which relates inversely to its concentration. Our results are similar to Hayashi Toshiyuki et al,[19] Inaku Kenneth O et al[20] Soji Tetsuo et al[21] and Hoogeveen Ron C et al[22] who concluded that plasma small dense LDL cholesterol (sd LDL–C) was strongly correlated and higher in patients with diabetes mellitus than healthy people. Hoogeveen Ron C et al[22] also concluded that elevated plasma levels of sd LDL-C were associated with increased risk of CHD. (HR, 1.51;95 CI,1.21-1.88) and increased sd LDL-C also predicted this risk of incident CHD in patients with normal LDL-C. sd LDL is more susceptible to glycation than large buoyant fraction. This greater suspectibity to glycation occurs because a larger portion of lysine residues of Apo B are exposed at the surface of sd LDL particles. LDL glycation and oxidation both lead to atherogenesis.[23] Our results are similar to Toft-Peteron Anne P et al[24] who found that CAD patients had significantly higher sd LDL as compared to patients without CAD (mean 50.1% and 40.0%, respectively; p < 0.001). They found that the proportion of small dense LDL particles is a strong univariate predictor of clinically significant coronary luminal stenosis.

The formation of sd LDL increases in the presence of insulin resistance and hyper triglyceridemia.[25] sd LDL is more susceptible to glycation than large buoyant fraction because a larger portion of lysine residues of Apo B are exposed at the surface of sd LDL particles. Hyperglycemia leads to glycation of sd LDL and glycated sd LDL is more prone to oxidation. Both glycation and oxidation of sd LDL promote atherogensis. sd LDL is associated with increased risk of cardiovascular diseases. Even if the LDL-C is normal the concentration of sd LDL particles may be increased as sdLDL particles contain less cholesterol than large buoyant LDL (lbLDL) particles. Hence, there are more sd LDL particles than lb LDL particles at a given LDL-C concentration. Increased sd LDL concentration, is a strong predictor of CVD in T2DM patients. sd LDL-C is a better risk marker of atherosclerosis than the standard lipid parameters.[21] sd LDL can be used a risk predictor for atherosclerosis in T2DM patients HbA1C is a strong predictor of concentration of sd LDL in T2DM patients.

Conclusion

Our study shows statistically strong correlation of small dense LDL

with HbA1C and is a metabolically better parameter than conventional lipid parameters in assessing the risk of CVD in diabetic patients. Metabolic as well as genetic association of non conventional lipid parameters would help in the early detection of future risk of CVD in type 2 DM patients. sd LDL can used to assess the risk of CVD in T2DM but larger sample size and further research needs to be carried out before using this parameter routinely in diabetes patients.

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