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Original Research Article

Evaluation of Non-HDL Cholesterol and Total Cholesterol/HDL-C Ratio, LDL-C/HDL-C Ratio as Cumulative Marker of Cardiovascular Disease risk in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Cardiovascular disease (CVD) is the leading cause of death worldwide with an estimated 17.3 million deaths from CVDs in 2008, representing 30% of all global deaths. The main cause of CVD development is atherosclerosis and among the various factors for developing increased cardiovascular risk in diabetes; lipid abnormalities, the dyslipidemia is the major contributor. The common type of dyslipidemia in diabetes is characterized by elevated triglyceride (TG), low levels of high density lipoprotein cholesterol (HDL-C) and typically has a preponderance of smaller, denser low density lipoprotein cholesterol (LDL-C) particles, which possibly increases atherogenicity even if the absolute concentration of LDL cholesterol (LDL-C) is not significantly increased.

Materials & Methods: Total of 100 diagnosed type 2 diabetes mellitus ambulatory patients of both sexes were taken as cases. Their age ranged between 30 to 70 years. 100 healthy subjects, with their age ranging between 30 to 70 years were selected as control group. Serum sample was used for the estimation of study parameters such as FBS, PPBS, Total Cholesterol, Triglycerides, HDL Cholesterol, LDL Cholesterol.

Results: In this study, total cholesterol, triglycerides, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C were found to be statistically significantly increased in type 2 diabetes mellitus patients when compared to controls. HDL-C was significantly decreased in type 2 diabetes mellitus patients when compared to controls (p-value 0.001).

Conclusion: In this study, type 2 diabetes mellitus is associated with dyslipidemia with increased levels of TC, TG, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C ratio, but a lower levels of HDL-C compared to that of controls. Increased levels of Non-HDL-C, TC/HDL-C and LDL-C/HDL-C ratio which indicates increased impending cardiovascular disease risk in type 2 diabetes mellitus.

Keywords: Cardiovascular disease, Diabetic Dyslipidemia, LDL-C/HDL-C ratio, Non-HDL-Cholesterol, TC/HDL-C ratio, Type 2 Diabetes Mellitus.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide with an estimated 17.3 million deaths from CVDs in 2008, representing 30% of all global deaths. Cardiovascular diseases have been gaining importance in India because of increased incidence of the disease [1]. It is the first among top 5 causes of death in Indian population. Recent estimation states that CVD accounts for more than 25% of the total deaths in India. It has been found that over 80% of deaths and 85% of disability from CVD occur in low and middle income countries. Among these, CVD affects Indians with greater frequency and at a younger age than counterparts in developed countries, as well as many other developing countries [2]. Cardiovascular disease (CVD) is the primary cause of morbidity and mortality in patients with diabetes and have approximately 2 to 4 times higher CVD rate than adult without diabetes [3].

According to the World Health Organization (WHO), cardiovascular disease (CVD) is defined as "a group of disorders of the heart and blood vessels and

includes coronary heart disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism" and the estimated mortality from CVD accounts for 30% of all deaths worldwide. CVD is the primary cause of morbidity and mortality in patients with diabetes and accounts for approximately 65% of overall deaths with diabetic complications. [3]. It is important to identify risk factors that may increase CVD risk in diabetic patients [4].

The main cause of CVD development is atherosclerosis and among the various factors for developing increased cardiovascular risk in diabetes; lipid abnormalities, the dyslipidemia is the major contributor. The common type of dyslipidemia in diabetes is characterized by elevated triglyceride (TG), low levels of high density lipoprotein cholesterol (HDL-C) and typically has a preponderance of smaller, denser low density lipoprotein cholesterol (LDL-C) particles, which possibly increases atherogenicity even if the absolute concentration of LDL cholesterol (LDL-C) is not significantly increased. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance [5]. National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has recognized hypertriglyceridemia is a risk factor for coronary artery disease (CAD). It has stated that LDL-C level is not a valid basis for therapeutic purposes at the TG level over 200 mg/dL, rather Non-High density lipoprotein cholesterol (Non-HDL-C) is identified as the therapeutic target at that level of TG. Measurement of Non-HDL-C could be more representative of all atherogenic apolipoprotein B (apoB) containing lipoproteins- LDL-C, very low density lipoprotein cholesterol (VLDL-C), intermediary density lipoprotein cholesterol (IDL-C) and lipoprotein (a). It has been suggested that Non-HDL-C may be a strong predictor of coronary heart disease (CHD) mortality and non-fatal coronary events than LDL-C in people with diabetes. Elevated Non-HDL-C signifies increased cardiovascular disease (CVD) risk even if LDL-C levels are at or below the NCEP goal or appear to be normal. And Non-HDL-C can also be used as a predictor of CHD in individuals with and without high triglyceride levels, type 2 diabetes mellitus and metabolic syndrome [6].

Unlike triglycerides, Non-HDL cholesterol requires measurements of only total cholesterol and HDL-C which can be measured reasonably accurately in the non-fasting state and it is readily derived from the routine lipid profile. For these reasons, although apolipoprotein B can be accessed directly, measurement of Non-HDL cholesterol can be considered as a surrogate marker for apolipoprotein B in routine clinical practice as it is a highly useful lipid measure for predicting the risk of CHD and evaluating response in treatment of hyperlipidemia, LDL-C/HDL-C ratio, which can be obtained from a standard lipid profile and is more accurate than LDL-C or HDL-C alone. The LDL-C to HDL-C ratio > 4.1 is predisposing to heart disease whereas a ratio < 4.1 is protective [7, 8]. The present study is an attempt to study the levels of Non-HDL-C and TC/HDL-C and LDL-C/HDL-C ratio in type 2 diabetes mellitus patients.

2. Materials and methods

This study was conducted at Maheshwara Medical College & Hospital, Patancheru, Medak District, Telangana. Total of 100 diagnosed type 2 diabetes mellitus ambulatory patients of both sexes were taken as cases. Their age ranged between 30 to 70 years. 100 healthy subjects, with their age ranging between 30 to 70 years were selected as control group. Patients with type 1 diabetes mellitus, Type 2 Diabetes Mellitus patients with hypolipidemic drugs, patients with thyroid disorders and obstructive liver disorders were excluded from the study. The clinical history and other necessary details were obtained from the patients records.

5 ml of venous blood was collected from anti-cubital vein under aseptic precautions. Samples were left for 20 minutes at room temperature, and serum was separated by centrifugation at 3000 rpm for 10 minutes. The following biochemical parameters were estimated in all cases and controls: FBS & PPBS by GOD-POD method, Total Cholesterol by CHOD-POD (cholesterol oxidase/peroxidase) method, Triglycerides by GPO-POD (Glycerol 3 phosphate oxidase-peroxidase) method, High-density lipoprotein cholesterol (HDL-C) and Low-density lipoprotein cholesterol (LDL-C) by enzymatic colorimetric assay.

Non-HDL-C is calculated by using the formula: Non-HDL-C (mg/dl) = Total Cholesterol – High-Density Lipoprotein Cholesterol. Calculation of ratio. All the parameters were analyzed by using automated chemistry analyzer Erba chem – 5X. Data were expressed as mean ±SD. P value <0.001 is considered as statistically significant. Statistical analysis was performed using SPSS 20.0.

3. Results

Total of 100 type 2 diabetes mellitus ambulatory patients were selected for the study. Among them 58 were males (58%) and 42 were females (42%) with average age of (51.7±8.9) years, ranging between 30 to 70 years. 100 healthy subjects were taken as controls. Among them 50 were males (50%) and 50 were females (50%) with average of (48.01±10.2) years, their ages ranging between 30 to 70 years.

In this study, total cholesterol, triglycerides, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C were found to be statistically significantly increased in type 2 diabetes mellitus patients when compared to controls. HDL-C was found significantly decreased in type 2 diabetes mellitus patients when compared to controls. The lipid profile measurements in T2DM patients were total cholesterol (201.5±34.5), triglycerides (197.3±91.7), LDL-C (128.1±31.1), Non-HDL-C (166.6±32.1), TC/HDL-C (5.9±1.2) and LDL-C/HDL-C (3.7±1.0) and HDL-C (34.8±7.4) as illustrated in table 1.

Table 1: Comparison of age, lipid profile parameters between type 2 diabetes mellitus patients and healthy controls

Parameters	Control (n=100)Mean ± SD	Study (n= 100)Mean ± SD	p-value
Age	48.01±10.2	51.7±8.9	0.007
FBS(mg/dl)	93.3±9.5	180.5±58.6	0.001*
PPBS(mg/dl)	114.06±25.5	274.2±84.3	0.001*
Serum cholesterol (mg/dl)	134.4±24.7	201.5±34.5	0.001*
Serum Triglycerides (mg/dl)	124.9±50.5	197.3±91.7	0.001*
HDL Cholesterol (mg/dl)	36.1±10.6	34.8±7.4	0.315
LDL Cholesterol (mg/dl)	74.1±22.7	128.1±31.1	0.001*
Non-HDL-Cholesterol (mg/dl)	98.8±22.6	166.6±32.1	0.001*
TC/HDL-C	3.9±1.2	5.9±1.2	0.001*
LDL-C/HDL-C	2.1±0.95	3.7±1.0	0.001*

*Statistically significant

As shown in table 2, statistically significant correlation was observed between FBS and total cholesterol, triglycerides, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C in cases and negative correlation was observed between FBS and HDL-C in cases.

Table 2: Correlation between FBS and lipid profile parameters

Parameters	Correlation coefficient	p value
PPBS	0.878**	0.001
Serum Cholesterol	0.517**	0.001
Serum Triglycerides	0.391**	0.001
HDL Cholesterol	-0.169*	0.017
LDL Cholesterol	0.489**	0.001
Non-HDL-Cholesterol	0.563**	0.001
TC/HDL-C	0.534**	0.001
LDL-C/HDL-C	0.515**	0.001

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

As shown in table 3, statistically significant correlation was observed between PPBS and total cholesterol, triglycerides, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C in cases and negative correlation was observed between PPBS and HDL-C in cases.

Table 3: Correlation between PPBS and lipid profile parameters

Parameters	Correlation coefficient	p value
FBS	0.878**	0.001
Serum Cholesterol	0.537**	0.001
Serum Triglycerides	0.404**	0.001
HDL Cholesterol	-0.120	0.091
LDL Cholesterol	0.496**	0.001
Non-HDL-Cholesterol	0.571**	0.001
TC/HDL-C	0.500**	0.001
LDL-C/HDL-C	0.487**	0.001

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Type 2 diabetes mellitus patients have greatly increased CVD risk compared with non-diabetic individuals; therefore it is important to identify factors that may increase CVD risk in these patients. [6] The lipid profile in the present study was found to be altered with statistically significant increase in total cholesterol, triglycerides, LDL-C, Non-HDL-C, TC/HDL-C & LDL-C/HDL-C ratio in type 2 diabetes mellitus patients compared to controls. HDL-C was statistically significantly decreased in type 2 diabetes mellitus patients compared to controls. The observed increase and decrease

in serum lipid profile in type 2 diabetes mellitus are in accordance with findings of Sapna Smoth et al. (2008) [8].

In Diabetes Mellitus, cells of skeletal muscle, heart and adipocytes cannot uptake and utilize glucose, instead obtain energy from the oxidation of fatty acids, thus producing increased level of acetyl Co-enzyme - A that can be funnelled for Cholesterol biosynthesis thus, resulting in hypercholesterolemia. Similarly, in case of insulin resistance to fat cells, the activity of hormone sensitive lipase increases resulting in enhanced lipolysis in adipose tissue to release (FFAs) into circulation which are taken up by different organs including liver wherein FFAs leads to the synthesis of TG which along with cholesterol and apoproteins are incorporated into very low density lipoprotein (VLDL). Insulin deficiency is associated with decreased clearance of VLDL. Since, VLDL is rich in TG and cholesterol resulting into hypercholesterolemia and hypertriglyceridemia. The increased number of VLDL and increased plasma TG levels decrease the level of HDL-C and increase the concentration of small dense LDL-C particles which can be explained as VLDL transported TG is exchanged for HDL- transported cholesteryl ester through the action of the cholesteryl ester transfer protein (CETP), which results in increased amounts of both atherogenic cholesterol-rich VLDL remnant particles and TG-rich, cholesterol-deplete high density lipoprotein (HDL) particles. The TG-enriched HDL is subsequently hydrolysed by hepatic lipase or lipoprotein lipase; Apo A-I dissociates from the reduced size HDL, which is filtered by the renal glomeruli and degraded in renal tubular cells [9, 10].

Our study revealed that Non-HDL-C was significantly higher in diabetic subjects than non diabetic subjects. Non-HDL-C levels were associated with risk of atherosclerosis in type 2 diabetes mellitus. It has been suggested that Non-HDL-C may be a strong predictor of coronary heart disease (CHD) mortality and non-fatal coronary events than LDL-C in people with diabetes [11] Since, diabetic dyslipidemia is most commonly manifested as elevated TG and a decreased level of HDL-C with a predominance of small dense LDL-C particles and relatively normal LDL-C levels,[12] elevated Non-HDL-C signifies an increased CVD risk even if LDL-C levels are at or below the NCEP goal or appear normal [13]. Both the NCEP and the ADA recommended reducing LDL-C and Non-HDL-C to a goal of <100 mg/dL and <130mg/dL respectively in patients with Diabetes [14]. Although LDL-C remains the primary target of therapy in dyslipidemic patients, the NCEP considers Non-HDL-C a secondary target in people with elevated TG i.e TG 200 mg/dL, many of whom are diabetics [13].

Non HDL-C is calculated by subtracting HDL-C from TC and therefore includes not only LDL-C but also cholesterol contained in all other apoB containing potentially atherogenic lipoproteins in blood, including cholesterol in lipoprotein (a), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL-C) and cholesterol-enriched remnant lipoproteins. Experimental evidence supports a more important role for apolipoprotein B (apoB) and apoB-containing lipoproteins than for LDL-C content in mediating atherogenesis. Lipoproteins containing apoB must first enter the arterial wall and undergo oxidative modification before they can contribute to atherogenesis. This modification affects the structure of the apoB molecule or the phospholipid membrane of these lipoproteins, yielding ligands for the scavenger receptors of macrophages in the arterial wall [15]. Subsequently, cholesterol accumulation and crystallization in macrophage cytoplasm leads to the formation of foam cells and progression to atherosclerotic plaque [16]. Interestingly, measured apoB and Non HDL-C have been found to be highly correlated in a number of studies [17, 18]. Recent studies involving subjects of different age groups have also shown the importance of Non-HDL-C as a reliable, less costly parameter that is strongly correlated with cardiovascular risk because Non-HDL-C includes all atherogenic lipid subfractions. [18].

Hence, in patients with Diabetes, Non-HDL-C may be a stronger predictor of CVD than either LDL-C or TG. The present study showed significantly increased levels of TC/HDL-C ratio and LDL-C/HDL-C ratio in type 2 diabetes mellitus patients compared to controls. Non-HDL-C and the ratio of TC to HDL-C and LDL-C/HDL-C may be superior to LDL-C in diabetic patients.

5. Conclusion

From the findings of the present study it can be concluded that type 2 diabetes mellitus is associated with dyslipidemia with increased levels of TC, TG, LDL-C, Non-HDL-C, TC/HDL-C and LDL-C/HDL-C ratio, but a lower levels of HDL-C compared to that of controls. Type 2 diabetes mellitus patients are at high risk for cardiovascular morbidity and mortality, adequate risk assessment and management is imperative. The simple Non-HDLC measurement, which can be conducted in non-fasting state and can be determined regardless of triglyceride concentration, may be of particular clinical utility. The adult treatment panel (ATP-III) of the national cholesterol education program recommended a therapeutic goal for Non-HDL-C of 30 mg/dl higher than the goal for LDL-C; therefore, in patients with diabetes, the goal would be a Non-HDL-C target of <130 mg/dl. For the management of diabetic dyslipidemia, lifestyle changes including increased physical activity and dietary modifications are needed.

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