

RESEARCH ARTICLE

BIO CHEMISTRY

NON-HDL CHOLESTEROL AND LDL-C/HDL-C RATIO IN TYPE II DIABETIC PATIENTS

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ABSTRACT

Diabetic dyslipidemia consists of elevated LDL cholesterol, Triglycerides and decreased levels of HDL Cholesterol. More recent data suggests that measurement of Non -HDL Cholesterol level (calculated as Total Cholesterol minus HDL Cholesterol) could be more representative of all atherogenic, apolipoprotein (apo) B containing lipoproteins. Although apolipoprotein B can be assessed directly, measurement of Non-HDL Cholesterol can be considered as a surrogate marker for apolipoprotein B in routine clinical practice. Here an attempt is to evaluate the Lipid profile including Non-HDL Cholesterol levels and LDL-C/HDL-C ratio in type II Diabetic Patients as markers of diabetic dyslipidemia.

Our study group comprised of age and sex matched 50 normal, 50 type II diabetic subjects. There was a significant increase in Non-HDL cholesterol ($p < 0.001$) and LDL-C/HDL-C ratio ($P < 0.05$) in diabetic patients compared to age and sex matched controls. Hence Non-HDL Cholesterol along with LDL-C/HDL-C ratio can be used as markers of dyslipidemia in Type II Diabetic patients.

KEY WORDS

Atherogenic lipoproteins, Diabetic Dyslipidemia, LDL-C/HDL-C ratio, Non-HDL Cholesterol.

INTRODUCTION

In India, diabetes is not an epidemic anymore but has turned into a pandemic. According to the International Journal of Diabetes in developing Countries which labeled India as the diabetic capital of the world. The International Diabetes Federation estimates that the number of diabetic patients in India more than doubled from 19 million in 1995 to 40.9 million in 2007. It is projected to increase to 69.9 million by 2025. Type II diabetes and its complications constitute a major worldwide public health problem. Patients with type II diabetes have 2 - 4 times higher risk of experiencing cardiovascular disease (CVD) than adults without diabetes^{1,2} and their relative risk of dying from CVD is about twice as high³, much of which may be preventable with appropriate treatment of dyslipidemia.

The elevated CVD risk affecting patients with Type II diabetes may be attributed to a combined dyslipidemia characterised by elevated triglycerides, elevated triglyceride rich remnant lipoproteins (TGRLP), elevated apolipoprotein (apo) B and low levels of HDL cholesterol, with a predominance of small, dense LDL particles amid relatively normal LDL Cholesterol levels⁴. More recent data suggests that measurement of Non-HDL Cholesterol level (Calculated as Total Cholesterol minus HDL Cholesterol) could be more representative of all atherogenic, apolipoprotein (apo) B containing lipoproteins – LDL, VLDL, IDL and Lipoprotein(a). Although apolipoprotein B can be assessed directly, measurement of Non-HDL Cholesterol is more practical, reliable, inexpensive and can be considered as a surrogate marker for apolipoprotein B in routine clinical practice^{5,6}. Hence in the present study an attempt is made to study Non-HDL Cholesterol levels as a marker of dyslipidemia in diabetic patients.

Materials: The Institutional Ethical Committee approved the study and informed consent was obtained from each participant in the study. Our study group comprised of age matched 50 normal, 50 type II diabetic subjects in males and 50 normal and 50 type II diabetic subjects in females.

Inclusion Criteria: Only old cases of Type II Diabetic subjects with fasting glucose above 126mg/dl and glycosylated hemoglobin (HbA_{1c}) levels > 6% were considered as per the recommendations of American diabetic association (ADA).

Exclusion criteria: Type II Diabetic patients on hypolipidemic drugs were excluded from the study. Patients with thyroid disorders and obstructive liver disorders also were excluded from our study group.

All participants underwent complete physical examination including measurement of height and weight. BMI was calculated as weight in kilograms divided by height in squared meters.

Methods: 12 hours fasting venous blood was collected from antecubital vein under aseptic precautions. 2 ml blood was allowed to clot and serum was separated by centrifugation and stored at 4°C. The estimation was carried out within 6-8 hours. Another 2 ml blood was collected in a bulb containing EDTA for estimation of Glycosylated hemoglobin (HbA_{1c}).

The Parameters studied were: Glucose by GOD-POD method, Total Cholesterol by CHOD-PAP method, Triglyceride By GPO method, HDL Cholesterol by Phosphotungstate method, LDL Cholesterol was calculated by Friedwald et al formula (LDL Cholesterol (mg/dl) = Total Cholesterol – (HDL Cholesterol + Triglycerides/5), Non-HDL



Cholesterol(mg/dl)= Total Cholesterol – HDL Cholesterol⁷, Glycosylated hemoglobin(HbA1c) by Ion Exchange resin method and LDL-C/HDL-C ratio.

The data obtained was analysed and the differences in the mean of various parameters were compared using students-t- test. Statistical analysis was performed using software SPSS windows.

RESULTS

Table 1 shows the baseline characteristics of the 100 type II Diabetic patients.

Table 1
Baseline Characteristics of Type II Diabetic Patients

No. of Diabetic patients(n)	100
Male/Female	50/50
Age(years)	52.9 ± 12.0
Duration of Diabetes(yrs)	10.8 ± 4.4
BMI(kg/m ²)	29.8 ± 4.5

Table 2 shows the comparison of fasting blood sugar, HbA1c, Total Cholesterol, LDL, HDL Cholesterol, triglycerides, Non-HDL cholesterol and LDL-C/HDL-C ratio.

Table 2
FBS, HbA1c and Lipid profile levels in age and sex matched Type II Diabetic Cases and Controls.

Parameters	Male(n=100)		Female(n=100)	
	Control(n=50)	Diabetic(n=50)	Control(n=50)	Diabetic(n=50)
FBS (mg/dl)	95.46 ± 12.92	189.26 ± 60.18***	97.42 ± 11.69	191.46 ± 78.56***
HbA1c(%)	5.65 ± 0.30	7.54 ± 1.25***	5.40 ± 0.42	8.35 ± 1.86***
Total Cholesterol(mg/dl)	165.77 ± 30.39	198.02 ± 48.63***	184.96 ± 35.85	205.72 ± 42.33***
LDL-C (mg/dl)	108.15 ± 20.46	120.83 ± 20.35**	120.19 ± 23.21	146.56 ± 38.24***
HDL-C (mg/dl)	37.58 ± 0.89	34.58 ± 0.56***	42.5 ± 0.99	38.04 ± 0.71***
Triglycerides(mg/dl)	100.24 ± 45.23	218.66 ± 44.18***	121.25 ± 58.24	202.7 ± 106.87***
Non-HDL Cholesterol(mg/dl)	128.19 ± 29.5	164.56 ± 49.19***	144.44 ± 36.84	169.1 ± 43.04***
LDL/HDL ratio	3.22 ± 1.91	3.89 ± 1.87*	3.04 ± 1.16	3.74 ± 1.45*

All values are expressed as Mean ± Standard Deviation (S.D).

Significantly different from control: * p<0.05; ** p<0.01; *** p<0.001.

There was a significant increase in Total Cholesterol ($p < 0.001$), LDL-Cholesterol ($p < 0.01$), Triglycerides ($p < 0.001$), HDL-Cholesterol ($p < 0.001$), Non-HDL cholesterol ($p < 0.001$) and LDL-C/HDL-C ratio ($p < 0.05$) in diabetic patients compared to age and sex matched controls.

DISCUSSION

Coronary heart disease and Diabetes mellitus are both chronic metabolic diseases whose pathophysiology remains extremely complex and multifactorial. Yet of the many risk factors to blame, much attention has been focused on the elevated lipid profile and its atherogenic potential as a very powerful risk factor.

Emerging novel risk factors were nevertheless recognised by National Cholesterol Education Program (NCEP). One of them is the Non-HDL Cholesterol, whose value can be calculated by subtracting HDL-Cholesterol from the Total Cholesterol. Because the amount of LDL-Cholesterol inside the lipoprotein particle varies in individuals, the serum LDL-Cholesterol measurement does not reflect the number of particles and therefore the true level of cardiovascular risk. Two individuals with the exact same LDL-Cholesterol concentrations may have different risk factors. Accordingly, a more precise way to determine risk would be to measure the number of atherogenic lipoprotein particles in the serum that is apolipoprotein-B. In fact, once triglyceride levels exceed 100 mg/dl, the atherogenic small, dense LDL particles predominate⁸. Non-HDL Cholesterol is a better predictor of the risk of CVD than a simple measure of LDL Cholesterol. Our study showed a significant increase in Non-HDL cholesterol and LDL/HDL ratio in Diabetic patients compared to controls. In fact of the 100 diabetic patients we studied, nearly 44 patients had normal LDL-Cholesterol levels. Our findings are consistent with the findings of other studies^{9,10,11}. In the Strong Heart Study, multivariable analyses indicated that non-HDL cholesterol is a strong predictor of CVD in men and women with diabetes and is particularly indicative of coronary events. Hazard ratios for the highest tertile of non-HDL cholesterol in men and women with diabetes (2.23 and 1.80, respectively) were higher than those for either

LDL cholesterol or triglycerides alone in both men and women¹². Non-HDL Cholesterol and total apolipoprotein B are more potent predictors of CVD than LDL Cholesterol alone in Diabetic patients^{13,2}. Many reports confirm a strong correlation between Non-HDL Cholesterol and apolipoprotein B⁹.

The assay of apolipoprotein B is not routinely available to the clinicians. This may be due to a general unfamiliarity with its interpretation outside of the research setting and also because of its cost relative to its potential advantages for clinical decision making. Because of its simple calculation, the Non-HDL Cholesterol level is easily available to the clinician with every lipid profile ordered, thus eliminating any additional costs. Also, its derivation does not require a lipid profile to be done in the fasting state, and it avoids the potential inaccuracy caused by the inherent intraindividual variability of the triglyceride measurements.

A routine calculated LDL Cholesterol level using Friedewald formula cannot circumvent most of these limitations. The Friedewald equation requires fasting triglyceride level < 400 mg/dl in order to accurately calculate LDL Cholesterol. Thus in many cases of fasting hypertriglyceridemia common in diabetes, the clinician has no reliable estimate of LDL cholesterol and therefore no objective index of lipid associated CVD risk, unless Direct LDL cholesterol is assayed¹⁴.

In contrast, the Non-HDL Cholesterol level of a hypertriglyceridemia patient would still be available to the clinician and could potentially be more accurate than either the directly measured or the calculated LDL Cholesterol levels¹⁵. Non-HDL Cholesterol thus represents a readily obtainable, inexpensive and convenient measure of CVD risk that may be superior to LDL Cholesterol in many respects. In order for Non-HDL Cholesterol to replace LDL Cholesterol as the primary lipid target, strong evidence of its superiority will be

needed. At present such evidence is not yet available. One of the studies has concluded that both Non-HDL cholesterol and apo-B are more potent predictors of CVD incidence among diabetic men than LDL Cholesterol¹⁴.

The Adult Treatment Panel (ATP) III of the National Cholesterol Education Program

(NCEP) has recommended that Non-HDL cholesterol be used as a secondary target of therapy in people with Triglyceride levels >200 mg/dl, especially those with diabetes or metabolic syndrome¹⁶.

Comparison of LDL Cholesterol and Non-HDL Cholesterol Goals for Three Risk Categories¹⁶

Risk Category	LDL Goal(mg/dl)	Non-HDL Goal(mg/dl)
CHD and CHD Risk Equivalent(10- year Risk For CHD > 20%)	<100	<130
Multiple(2+) Risk factors and 10-year risk < 20%	<130	<160
0-1 Risk factor	<160	<190

Both the NCEP and the American Diabetes Association recommend reducing LDL cholesterol to a goal of <100mg/dl and Non-HDL Cholesterol to a goal of 130mg/dl in patients with diabetes². Elevated Non-HDL Cholesterol signifies increased CVD risk, even if LDL Cholesterol levels are at or below the NCEP goal or appear “normal”⁵. In the strong Heart Study, patients with diabetes in the highest tertile of Non-HDL Cholesterol had a higher hazard ratio for Myocardial infarction(3.17) than they did with any other lipid parameter(1.96 for LDL Cholesterol and 2.04 for triglycerides) compared with those in the lowest tertile¹¹. There is also evidence to suggest that, in patients with diabetes, Non-HDL Cholesterol is a stronger predictor of mortality from coronary disease than LDL Cholesterol. In a post hoc analysis of patients with diabetes from four prospective cohort studies—the Framingham Cohort Study, the Framingham Offspring Study, the Lipid Research Clinics Prevalence Follow-Up Study, and the usual-care group of the Multiple Risk Factor Intervention Trial—the relative risk of death for diabetic (compared with nondiabetic) patients was 7.2 for those with elevated Non-HDL cholesterol (≥ 130 mg/dl) and low LDL-Cholesterol (< 100 mg/dl) and 5.7 for those with low Non-HDL cholesterol (< 130 mg/dl) and elevated LDL-Cholesterol (≥ 100 mg/dl)². Non-HDL cholesterol seems to be a better choice, as it includes Triglyceride rich Lipoproteins (TGRLP), which plays an

important role in atherogenesis in type II diabetic patients¹⁷. Appropriate attention to measuring, targeting and treating Non-HDL cholesterol in patients with diabetes can help limit instances in which high-risk lipid profiles remain unrecognized and unaddressed.

Our study showed a significant difference (P<0.05) in LDL-C/HDL-C ratio in diabetics compared to controls both in men and women. The LDL/HDL ratio is actually a purer ratio than total cholesterol/HDL, because LDL is a measure of "bad" cholesterol and HDL is a measure of "good" cholesterol, whereas the total cholesterol is the sum of HDL, LDL, and the VLDL. Several large epidemiological and clinical studies have found the LDL-C/HDL-C ratio to be an excellent predictor of CHD risk. The PROSPER trial, a retrospective analysis of 6,000 patients; found that the ratio of LDL-C/HDL-C was the most powerful measure of cardiovascular disease risk in elderly people¹⁸. The researchers also concluded that changes in LDL-C/HDL-C ratio as a result of statin treatment appeared to account for the beneficial effects of therapy and suggested that statin therapy could usefully be targeted to those with an LDL-C/HDL-C ratio of 3.3. The PROCAM Study, which included almost 11,000 men aged 36 to 65 years who were studied for 4 to 14 years, found a continuous and graded relationship between the LDLC/HDL-C ratio and CVD mortality¹⁹. Coronary deaths spiked when the LDL-/HDL-C ratio reached between 3.7 and 4.3. In the Physicians' Health Study,



which involved almost 15,000 men ages 40 to 84 years, a 1-unit increase in the LDL-C/HDL-C ratio was associated with a 53% increase in risk of MI²⁰. In the Boston Area Health Study, which analyzed a group of men and women less than 76 years of age with no prior history of CVD but who had experienced a first MI, a 1-unit increase in the LDL-C/HDL-C ratio was associated with a 75% increase in risk of MI²¹. In addition, comparison of individual LDL-C/HDL-C ratios from subjects in the Framingham Study clearly indicates that the ratios are significantly more robust predictors of CVD than the individual levels of LDL-C or HDL-C. The existing focus on LDL-C as the primary culprit in atherogenesis may divert attention from the more efficient lipid profile of LDL-C/HDL-C ratio. The LDL-C/HDL-C ratio reflects the two-way traffic of cholesterol

entering and leaving the arterial intima in a way that the individual levels of LDL-C and HDL-C do not²².

CONCLUSION

Careful analysis of the lipid profile can make a significant difference in reducing cardiovascular risk. As Non-HDL Cholesterol is more representative of all atherogenic lipoproteins, more emphasis should be placed on considering Non-HDL cholesterol and LDL-C/HDL-C ratio as markers of Diabetic dyslipidemia than LDL Cholesterol alone. Further prospective follow up studies are needed to study the Non-HDL Cholesterol levels in comparison to apolipoprotein B as a predictor of CVD in Type II Diabetic patients.

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