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Role of Non-HDL Cholesterol and LDL_C/HDL_c Ratio to Assess cardio vascular risk in Type- II Diabetic Patients

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Abstract

Diabetes mellitus is the commonest metabolic abnormality in the world. It has been estimated that more than 33 million people in India are affected by diabetes. Patients with type- II diabetes have 2 - 4 times higher risk of experiencing cardiovascular disease than adults without diabetes. Diabetic dyslipidemia consists of elevated LDL_C, Triglycerides and decreased levels of HDL_C. Recent data suggests that measurement of Non–HDL Cholesterol level (Total Cholesterol minus HDL_C) could be more representative of all atherogenicapolipoprotein (Apo) B containing lipoproteins. Although Apo-B can be assessed directly, measurement of Non-HDL_C can be considered as a surrogate marker for Apo B in routine clinical practice. Also LDL_C to HDL_C ratio > 4:1 is predisposing to heart disease whereas a ratio < 4:1 is protective. To evaluate the Lipid profile including Non-HDL_C levels and LDL_C/ HDL-C ratio in type II Diabetic Patients as markers of diabetic dyslipidemia. Age and sex matched 100 cases and 100 controls were included in the study. All the samples were analysed forFasting plasma glucose, Triglycerides, Total cholesterol,$LDL_c$and HDL_c. whereas Non-HDL cholesterol and LDL_c/ HDL_c ratio were calculated. There was a significant increase in Non-HDL cholesterol ($p<0.001$) and LDLC/ HDL-C ratio ($P<0.05$) in diabetic patients compared to age and sex matched controls. Type II diabetis mellitus leads to dyslipidemia causing increased levels of Non-HDL Cholesterol along with LDL_C/HDL_C ratio which indicates increased impending cardiovascular risk.

Keywords: Diabetes mellitus, Atherogenic lipoproteins, Diabetic Dyslipidemia, LDL-C/HDL-Cratio, Non-HDL Cholesterol.

Introduction

Diabetes mellitus (DM) is a clinical syndrome characterized by hyperglycemia in the fasting state[1,2] affecting a higher percentage of people in many developing countries than it does in Western countries. There are currently 135 million people with diabetes in the world, and India leads the world with 40.9 million people in diabetes in 2007. Moreover, it is projected that, by the year 2025, 80.9 million will have diabetes in India[3].

Type-II DM currently accounts for at least 90-95% of those with diabetes worldwide and its prevalence is rapidly increasing[4] Indeed there was a five-fold excess prevalence of Type II DM in Asians aged 40–60 years[5].

The most common pattern of dyslipidemia in Type-II diabetic patients is elevated triglyceride (TG) levels and decreased HDL cholesterol (HDL-c) levels and typically has a preponderance of smaller, denser LDL particles, which possibly increases atherogenicity even if the absolute concentration of LDL cholesterol (LDL-c) is not significantly increased[6]. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. The determination of the serum lipid levels in people with diabetes is now considered as a standard of the diabetes care[7].

The orange arrows signify the three common changes that occur in diabetic dyslipidemia: i. An increase in VLDL triglycerides, ii. An increase in small, dense LDL, and iii. A decrease in HDL cholesterol. In many cases, diabetic dyslipidemia is associated with hepatic dysfunction, including increased VLDL secretion and an increase in hepatic lipid content (orange circles) Figure adapted from Lusis et al. [8].

Although LDL is widely accepted as the major atherogenic lipoprotein, the TG-rich lipoproteins (TGRLP), including the very low-density lipoproteins (VLDL) and intermediate-density lipoprotein (IDL), still plays a pivotal role in atherogenesis[9]. Non-HDL cholesterol measurement (calculated as provides a single index of all the atherogenic, apolipoprotein (apo) B containing lipoproteins – LDL, VLDL, intermediate-density lipoprotein (IDL), and lipoprotein (a), and it can be used as a predictor of CHD in individuals with and without high TG levels, Type II DM and metabolic syndrome[10].

Unlike TG, Non-HDL cholesterol requires measurements of only Total cholesterol and HDLc which can be measured reasonably accurately in the non-fasting state and it is readily derived from the routine lipoprotein profile. For these reasons, although apolipoprotein B can be assessed directly, measurement of Non-HDL Cholesterol can be considered as a

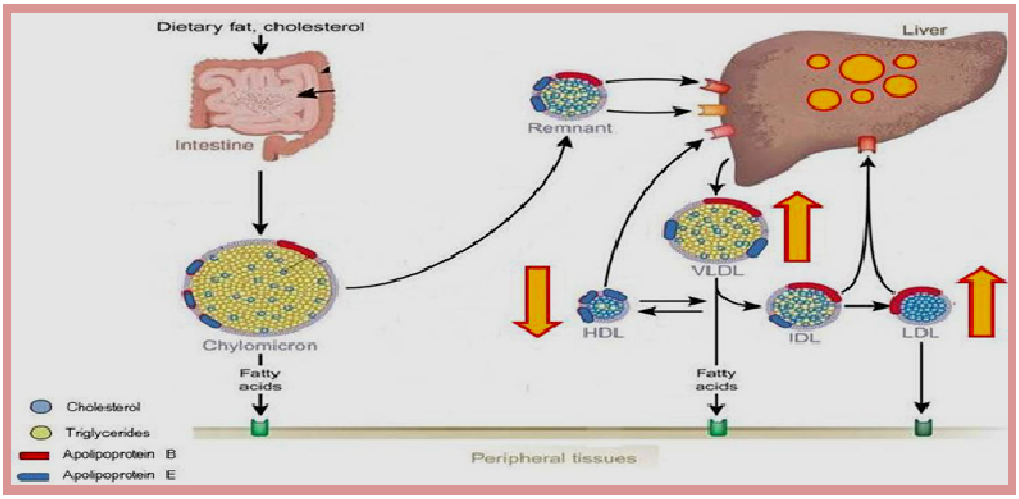

Figure-1
Diabetic Dyslipidemia[8]

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[11].

A more tenable option that has been proven to be an accurate predictor of cardiovascular risk is the 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[12].

In the present study an attempt was made to demonstrate the pattern of Non-HDL Cholesterol and ratio in Type II DM patients. Further, to assess the cardiovascular risk among the most prevalent diabetic groups.

Material and Methods

Materials: The Institutional Ethical Committee approved the study and informed consent was obtained from each participant in the study. Our study group comprised of hundred patients attended the outpatient department of General Medicine at Kamineni Institute of Medical Sciences,Narketpally is included in the study.

Inclusion criteria: Hundred patients(fifty males and fifty females) diagnosed with Type- II diabetes mellitus for atleast five years, and aged between 40 – 65 years. The patients were diagnosed as per the ADA criteria of FBG > 126 mg/dl andGlycosylated hemoglobin >6%.

Exclusion criteria: Type- II Diabetic patients on hypolipidemic drugs. Patients with thyroid disorders and obstructive liver disorders which will alter the lipid profile.

Controls: Hundred (fifty males and fifty females) apparently healthy, age and sex matched subjects who come for routine health checkup to Kamineni Institute of Medical Sciences Hospital.

Methods: Plasma Glucose and lipid profile: 2ml of venous blood was collected from ante-cubital vein under aseptic precautions and was transferred in to prepared fluoride tube. The sample was allowed to clot and serum was separated by centrifugation at 3000rpm for 10 minutes and stored at 2-8^{0}C until processed. The estimation of glucose in the sample was carried out within 6-8 hours.

Glycosylated Hemoglobin: 2 ml venous sample was drawn into a sterile disposable syringe which was transferred in to a tube containing EDTA and mixed well.

Samples were stored in refrigerator at 2 - 8°C until further processed. The following biochemical parameters were estimated in all patients and controls: Fasting plasma glucose by GOD-POD (glucose oxidase-peroxidase) method. Glycosylated hemoglobin (HbA_{1c}) by ion exchange method. Total cholesterol and HDL cholesterol by CHOD/POD (cholesterol oxidase/peroxidase) method. Direct LDL cholesterol by direct enzymatic method. Triglycerides by GPO-POD (Glycerol 3 Phosphate oxidase –peroxidase) method. Calculation of Non-HDL cholesterol, Calculation of ratio.

The data obtained was analysed and the differences in the mean of various parameters were compared using student's t-test. Statistical analysis was performed using software SPSS windows.

Results and Discussion

Table-1showed that in the current study the Mean and Standard deviation (SD) of age among diabetic cases was 52 ± 6.3 years and that of controls was 51.8 ± 7.91 years. As shown in table-1, the mean age of male cases was high when compared to female cases.

Table-1
Mean age distribution among diabetics and controls

	Age (years)	
	Diabetics (n=100)	**Controls (n=100)**
Males (Mean±SD)	54.1±5.44 (n=50)	51.22±7.92 (n=50)
Females (Mean±SD)	50.6±6.68 (n=50)	52.28±7.94 (n=50)
Total (Mean±SD)	52±6.3 (n=100)	51.8±7.91 (n=100)

Table- 2
Sex distribution based on 5 yrs and>5yrs duration of diabetes

Duration of disease	**Males (%)**	**Females (%)**	**Total (%)**
5 years	15(30%)	14(28%)	29(29%)
>5years	35(70%)	36(72%)	71(71%)
Total	50	50	100

Table-2 showed the sex distribution based on duration of diabetes among the cases. Out of 100 diabetic patients, 15 (30%) male cases and 14 (28%) female cases were with 5 years of disease duration whereas 35 (70%) male cases and 36(72%) female cases were with >5 years of disease duration. Among the total diabetics maximum number of cases belonged to >5 years duration of disease group.

Table-3 showed that the levels of Fasting blood glucose (FBG) and HbA_{1c}% of glycosylated hemoglobin in the study population. FBG levels were significantly raised in diabetic cases (182.15 ± 28.95) as compared to controls (97.88 ± 9.21) with p-value <0.001. Even HbA_{1c} levels showed a similar increase in diabetics as compared to controls.

Table-3
Comparison of FBG and HbA_{1c} (Mean ± SD) levels in diabetics and controls

Parameters	**Diabetics (n=100)**	**Controls (n=100)**	**p-value**
FBG (Normal value: 70-110mg/dl)	182.15±28.95**	97.88±9.21	< 0.0001
HbA1c (Normal value: 3.5-5.5%)	7.27±0.81**	4.92±0.25	< 0.0001

**p-value < 0.001 was considered as highly significant. @ HbA1c levels were projected from GHb% values using the conversion chart provided with the kit.

Table -4 showed that the levels of Fasting blood glucose and HbA_{1c}% of glycosylated hemoglobin among the male and female cases and controls in the study population. FBG levels were significantly raised in both male (182.58±28.37) and female (194.83±24.00) diabetic cases as compared to male (95.70±7.30) and female (100±10.40) controls with p-value <0.001. Even HbA_{1c} levels showed a similar increase in diabetics as compared to controls in both the sex groups.

Table-5 shows the levels of lipid profile in the study population. The levels of Total Cholesterol, LDL-Cholesterol, and Triglycerides were significantly raised and HDL-Cholesterol levels were significantly decreased in diabetic cases as compared to controls with p-value <0.001.

Table-6 showed the levels of lipid profile among the male and female cases and controls in the study population. The levels of Total Cholesterol, LDL-Cholesterol, and Triglycerides were significantly raised and HDL-Cholesterol levels were significantly decreased in both male and female diabetic cases as compared to sex matched controls with p-value <0.001.

Table-4
Comparison of FBG and HbA_{1c}levels within the sex matched diabetics and controls

Parameters	**Males (n=100)**		**Females (n=100)**		
	Diabetics (n=50)	**Controls (n=50)**	**Diabetics (n=50)**	**Controls (n=50)**	**p-value**
FBG (Normal value:70-110mg/dl)	182.58±28.37**	95.70±7.30	194.83±24.00**	100±10.40	< 0.0001
HbA1c (Normal value: 3.5-5.5%)	7.30±0.82**	4.86±0.20	7.61±0.67**	4.98±0.29	< 0.0001

**p-value < 0.001 was considered as highly significant

Table-5
Comparison of Lipid Profile levels in diabetics and controls

Parameters	**Diabetic (n=100)**	**Control (n=100)**	**p-value**
TotalCholesterol (Normal value: 130 - 200mg/dl)	226.65 ± 20.48**	167.85 ± 15.49	< 0.0001
LDL-c (Normal value: <130mg/dl)	151.42 ± 19.99**	94.8 ± 16.44	< 0.0001
HDL-c (Normal value: 35-60mg/dl)	31.26 ± 6.06**	46.3 ± 7.18	< 0.0001
Triglycerides (Normal value: <150mg/dl)	219.79 ± 32.22**	133.85 ± 17.11	< 0.0001

**p-value < 0.001 was considered as highly significant.

Table-6
Comparison of lipid profile () levels in sex matched diabetics and controls

Parameters	Males (n=100)		Females (n=100)		p-value
	Diabetics (n=50)	Controls (n=50)	Diabetics (n=50)	Controls (n=50)	
TotalCholesterol (Normal value:130-200mg/dl)	224.4±21.02**	164.5±14.91	228.88±19.89**	171.2±15.48	<0.0001
LDL-c (Normal value: <130mg/dl)	149.4±21.18**	91.96±15.61	150.68±19.40**	97.64±16.91	<0.0001
HDL-c (Normal value: 35-60mg/dl)	26.79±3.97**	44.96±6.75	35.74±4.19**	48.00±7.40	<0.0001
Triglycerides (Normal value: <150mg/dl)	227.33±34.70**	138.00±15.00	212.25±27.88**	130.00±18.30	<0.0001

(**p-value <0.001 was considered as highly significant)

Table-7
Comparison of Non-HDL and LDL/HDL () levels in diabetics and controls

Parameter	Diabetics (n=100)	Controls (n=100)	p-value
Non-HDL[#] (Normalvalue: <130mg/dl)	195.38±22.65**	121.57±16.11	< 0.0001
LDL/HDL ratio (Normal ratio: <3)	5.07±1.51**	2.55±0.66	< 0.0001

** p-value < 0.001 was considered as highly significant. [#] Non-HDL-C levels were projected from lipid profile using the formula

Table-8
Comparison of Non-HDL and LDL/HDL () levels in sex matched Diabetics and Controls

Parameter	Males (n=100)		Females (n=100)		p-value
	Diabetics (n=50)	Controls (n=50)	Diabetics (n=50)	Controls (n=50)	
Non-HDL (Normalvalue: <130mg/dl)	197.63±23.21**	119.54±15.46	193.13±22.07**	123.60±16.64	< 0.0001
LDL/HDL (Normalratio:< 3)	5.86±1.53**	2.69±0.63	4.27±0.98**	2.40±0.66	< 0.0001

**p-value <0.001 was considered as highly significant

Table-7 showed the levels of Non-HDL cholesterol and LDL/HDL ratio among the cases and controls in the study population. The levels of Non-HDL cholesterol and LDL/HDL ratio were significantly raised in diabetic cases as compared to controls with p-value <0.001.

Table-8 showed the levels of Non-HDL cholesterol and LDL/HDL ratio among the male and female cases and controls in the study population. The levels of Non-HDL cholesterol and LDL/HDL ratio were significantly raised in both male and female diabetic cases as compared to sex matched controls with p-value <0.001.

Discussion: The present study was conducted on 100 Type II DM cases clinically diagnosed according to ADA guidelines and were aged between 40-65 years of which 50 cases were males and 50 cases were females. The findings were compared with 100 apparently healthy age and sex matched controls.

Diabetic 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.

In the present study the levels of Fasting blood glucose were significantly raised in diabetics as compared to Non-diabetics. These results are similar to the findings of Aryal M et al. (2010)[13] and Dr PurviPurohit et al. (2012)[14]. They observed, that type-2 diabetic patients to have significantly raised FBS, insulin, HOMA–IR and C – peptide levels as compared with the healthy controls. Hyperinsulinemia is a condition seen due to high glucose levels in blood–itself an independent cause of atherosclerosis as it promotes atherogenicdyslipidaemia hinting an enhanced CVD risk in such individuals[14].

In the present study the levels of HbA_{1c} were significantly high in diabetics as compared to non-diabetics. The findings were similar to the study conducted by Indumati et al. (2011)[6]. These studies suggest that diabetics have poor glycaemic control. HbA1c concentration indicates average blood glucose concentrations over the preceding 3 months and is also the marker for assessing the importance of glycaemic control in the development of diabetic complications[15].

In an observational study on new aspects of HbA1c as a risk factor for cardiovascular diseases in type 2 diabetes by K. Eeg-Olofsson et al. (2010) stated that patients with type 2 diabetes in clinical practice showed progressively increasing risks for CHD, stroke, CVD and total mortality with higher HbA1c values due to poor metabolic control during hyperglycemia[16].

Table-9
Comparative studies on lipid profile levels in diabetics and controls with other authors

Authors	Subjects	Lipid profile (mg/dl)			
		TC	LDL	HDL	TG
SapnaSmithet al. (2008)[17] n= 60	Diabetics (n=30)	229.36±13.46**	164.15±5.98**	31.12±1.10**	178.32±10.12**
	Controls (n=30)	165.54±16.03	112.69±4.69	46.12±0.73	158.21±7.48
S.Zabeen et al. (2012)[18] n=150	Diabetics (n=103)	219.78±41.74**	129.37±39.22**	38.23±7.56^{NS}	265.53±67.58**
	Controls (n=47)	178.38±38.24	108.89±31.71	36.00±8.64	165.74±69.85
PresentStudy (2012) n=200	Diabetics (n=100)	222±20.13**	147.20±20.40**	33.95±6.38**	218.13±31.21**
	Controls (n=100)	167.85±15.49	94.8±16.44	46.3±7.18	133.85±17.11

**P – value < 0.001 was considered highly significant, NS= not significant

In the present study, serum lipid profile levels showed a significant rise in Total cholesterol, LDL and Triglyceride levels and significant decrease in HDL levels among the diabetics as compared to controls. The observed increase and decrease in serum lipid profile associated with diabetes mellitus are in accordance with findings of Sapna Smith et al.(2008)[17]. Where as in the study conducted by S Zabeen et al. (2012)[18]HDL levels were raised but not significantly as shown in table-9.

In the study done by Sapna Smith et al. 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 triglycerides and due to this there was reduction in HDL cholesterol levels[18].

Table-10
Comparative studies on Non-HDL cholesterol levels in Diabetics and Controls with other authors

Authors	Non-HDL (mg/dl)		p-value
	Diabetics	Controls	
Aryal.M et al. (2010)[13]n=136	148.13±70** (n=76)	100.58±19.47 (n=60)	0.000
S.Zabeen et al. (2012)[18] n=150	181±40.11** (n=103)	142.47±36.27 (n=47)	0.000
Present study (2012) n=200	188.04±22.53** (n=100)	121.57±16.11 (n=100)	<0.0001

** p-value <0.001 was considered highly significant

In this study our attempt was to explore the role of Non-HDL cholesterol in diabetic population. Our study revealed that Non HDL-c was significantly higher in diabetic subjects than non diabetic subjects. As shown in table-10 our finding was similar to the findings of Aryal et al. (2010)[13] and S Zabeen et al. (2012)[18]. They suggested Non-HDL levels were associated with risk of atherosclerosis in type 2 DM and it can be a simple and cumulative marker of cardiovascular disease. Because Non-HDL cholesterol includes an assessment of all lipoproteins that are considered to be atherogenic, i.e. VLDL, IDL, LDL, and lipoprotein (a), Non HDL-c could be considered as a marker for atherogenicity.

Table-11
Comparative studies on LDL/HDL ratios in diabetics and controls with other authors

Authors	LDL/HDL ratio		p-value
	Diabetics	Controls	
Indumati et al. (2011)[6] n=200	3.81±1.67* (n=100)	3.15±1.53 (n=100)	<0.05
PresentStudy (2012) n=200	4.54±1.27** (n=100)	2.55±0.66 (n=100)	<0.0001

*p-value <0.05 was considered significant, **p-value <0.001 was considered highly significant

Our study showed a significant rise in LDL/HDL ratio in diabetics compared to controls. These results are similar to findings of Indumati et al. (2011)[6]. The PROSPER trial, a retrospective analysis of 6,000 patients done in 2005 found that the LDL/HDL ratio was the most powerful measure of cardiovascular disease risk in elderly people[5]. In fact, in a study by Wilson W et al. in 1990 showed that a 1 percent greater LDL value was associated with slightly more than a 2 percent increase in coronary artery disease over 6 years, and a 1 percent lower HDL value was associated with a 3 to 4 percent increase in coronary artery disease, even at total cholesterol levels less than 200 mg/dl. Additionally, low HDL levels are associated with increased heart attacks and death from coronary artery disease[19-24].

Conclusion

From the findings of the present study it can be concluded that diabetes mellitus was associated with significant increase in Non-HDL levels and LDL/HDL ratio as compared to controls.

The Non-HDL and LDL/HDL parameters were significantly increased with increase in duration of disease.

As Non-HDL cholesterol was more representative of all atherogenic lipoproteins, and LDL/HDL ratio was a significant predictor of atherosclerotic burden, more emphasis should be placed on considering Non-HDL cholesterol and LDL/HDL ratio as markers of diabetic dyslipidaemia and Cardiovascular risk markers than LDL alone. These parameters are cost effective and affordable comparative to some new markers in assessing cardiovascular risk in diabetic patients.

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