Pattern of dyslipidemia and evaluation of non-HDL cholesterol as a marker of risk factor for cardiovascular disease in type 2 diabetes mellitus

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ABSTRACT

People with type 2 Diabetes have an elevated risk for developing cardiovascular disease (CVD) for which dyslipidemia is the major contributor. Diabetic patients have characteristic pattern of dyslipidemia with decreased level of high density lipoprotein cholesterol (HDL-C) and elevated triglycerides (TG) level. However, in diabetes mellitus, low density lipoprotein cholesterol (LDL-C) which is used as one of the markers for the risk of CVD, is underestimated so in such cases the levels of non-High density lipoprotein cholesterol (non-HDL-C) can be a stronger predictor of CVD as it strongly correlates with atherogenic lipoproteins. Therefore, an attempt has been made to evaluate the level of non-HDL-C as a newer marker for the risk of cardiovascular disease and to find out the pattern of dyslipidemia in diabetes mellitus. The present study comprised of 82 type 2 Diabetic cases and 81 non-diabetic controls. Among the diabetics, the majority of the subjects (61.0%) were HDL-C dyslipidemic. However, among the controls, the maximum numbers of individuals (40.7%) were TG dyslipidemic. Diabetics have significantly elevated ratio of total cholesterol to high density lipoprotein cholesterol (TC/HDL-C) and the significant increased levels of non-high density lipoprotein cholesterol (non-HDL-C) compared to controls which can be used as markers of dyslipidemia and can also be used to predict the risk of cardiovascular disease in type 2 Diabetes Mellitus.

Keywords: Type 2 diabetes mellitus, diabetic dyslipidemia, non-HDL-C and Cardiovascular disease.

INTRODUCTION

Type 2 diabetes and its complications lead to an elevated cardiovascular risk globally. Adults with diabetes have a two to four times higher risk of experiencing cardiovascular events than adults with no diabetes.[1,2] 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 increased prevalence of small, dense low density lipoprotein cholesterol (LDL-C) particles.[3] National Cholesterol Education Program Adult treatment panel III (NCEP ATP III) has recognized hypertriglyceridemia as 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.[4] 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).[5] 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.[6] 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.[7] In patients with type 2 diabetes, the level of LDL-C may not be significantly elevated thus cardiovascular risk is not accurately identified. This adds to the fact that importance of calculating of non-HDL-C is especially important and it can serve as an additional tool to assess cardiovascular risks.[7,8] Moreover, it stands superior to LDL-C in predicting CVDs and should be used as the primary lipid target in persons with diabetes.[9] The lipid research clinic (LRC) program reported Non-HDL-C as a predictor of CHD events in women and all cause mortality in either sex while that of LDL-C failed to do so.[10]

Diabetes continues to be regarded as a equivalent of CAD, so adult patients are automatically in the high risk category, hence the aim is to achieve an LDL-C <2.5 mmol/L (97.5 mg/dL) and the ratio of TC to HDL-C <4.[11] Thus, the present study is an attempt to evaluate the levels of non-HDL-C, the ratio of TC to HDL-C as the markers of CVDs risk and to shed light on the pattern of dyslipidemia in type 2 diabetes mellitus.

Table-1: Comparison of different parameters between cases and controls

Parameters	Cases (N= 82) Mean±SD	Controls (N=81) Mean±SD	P-value
Age (years)	53.3±10.6	50.3±9.9	0.067
BMI (kg/m2)	25.7±3.6	23.5±2.6	<0.001
TC (mg/dL)	196.1±45.7	169.9±37.6	<0.001
TG (mg/dL)	206.5±104.7	158.8±87.3	0.002
HDL-C (mg/dL)	40.0±4.9	45.8±7.2	<0.001
LDL-C (mg/dL)	114.7±46.8	92.3±36.7	0.001
Non HDL-C (mg/dL)	156.0±46.2	124.1±39.0	<0.001
TC: HDL-C	5.0±1.3	3.8±1.0	<0.001

P <0.05: significant and P <0.001: highly significant.

MATERIALS AND METHODS

This cross-sectional study was conducted in National Public Health Laboratory (NPHL), Kathmandu Nepal from December 2010 to March 2011. A total of 163 subjects were recruited for the study. Among them, 82 were known cases of type 2 diabetes mellitus and 81 were healthy controls (non-diabetics and normotensives). The informed consent was obtained from all the participants. Height and weight were measured and body mass index (BMI) was calculated according to standard nomograms. Blood pressure measurement was done in the seated position with feet resting on the floor and the arm support at the heart level. The fasting blood sample was collected and then serum was separated by centrifuging for the estimation of TG, TC and HDL-C. TG and TC were measured by enzymatic method. HDL-C was measured by precipitation followed by enzymatic analysis. The LDL-C was calculated using Friedewald formula.[12] However, in case of TG more than 400 mg/dL, the LDL-C was estimated by direct kit method. The Non-HDL-C was calculated by subtracting HDL-C from TC. And the TC: HDL-C ratio was calculated by dividing TC by HDL-C. Data were analyzed using SPSS for Windows (Version 16). The group association was determined by Chi-square test and correlation was analyzed by Spearman rank correlation. P-value of <0.05 was considered to be significant.

RESULTS

A total of 163 subjects were recruited for the study purpose of which 82 were diabetic cases and the rest were healthy controls. The mean characteristics of the cases versus (vs.) controls are shown in Table-1. The age of cases and controls were statistically similar however the BMI and the lipid parameters were significantly higher in cases compared to controls.

The serum lipid profile of cases and controls is shown in Table-2. Among the cases, the majority of individuals i.e. 61.0% had low HDL-C levels (HDL-C < 40 mg/dL), 59.8% had high LDL-C levels (LDL-C ≥100 mg/dL), 57.3% had hypertriglyceridemia (TG ≥150 mg/dL) and 44.0% had hypercholesterolemia (TC ≥200 mg/dL). Among the controls, the maximum number of individuals (40.8%) had increased TG levels whereas the least number of individuals (29.6%) had raised TC levels.

Table-3 illustrates the gender wise distribution of dyslipidemia in cases and controls. Among the cases, the highest numbers of individuals, 64.6% males and 64.7% females were HDL-C and LDL-C dyslipidemic respectively while, the majority of males (39.2%) were LDL-C dyslipidemic and the majority of females (46.7%) were TG dyslipidemic amongst the controls.

As depicted in Table-4, the markers of dyslipidemia like non-HDL-C and TC/HDL-C were positively correlated with TC, TG, LDL-C and BMI. Furthermore, their correlation with TC and LDL-C was statistically significant. However, non-HDL-C and TC/HDL-C were negatively correlated with HDL-C and the correlation of TC/HDL-C with HDL-C was statistically significant.

Table-5 depicts the distribution of subjects according to BMI categories with 50% of the diabetics and only 28% of the controls were overweight and obese.

Table-2: Serum lipid profiles of Type 2 Diabetic cases and controls

Parameters	Cases N=82 (%)	Controls N=81 (%)	P-value
TC			
<200	46 (56.1)	57 (70.3)	0.04
200-239	19 (23.2)	18 (22.2)	
≥240	17(20.7)	6 (7.4)	
Triglyceride			
<150	35 (42.7)	48 (59.2)	0.19
150-199	23 (28.0)	18 (22.2)	
200-499	22 (26.8)	14 (17.3)	
≥500	2 (2.4)	1 (1.2)	
LDL-C			
<100	33 (40.2)	51 (63.0)	0.012
100-129	20 (24.4)	17 (21.0)	
130-159	14 (17.0)	10 (12.3)	
160-189	9 (11.0)	2 (2.5)	
≥190	6 (7.3)	1 (1.2)	
HDL-C			
≥40	32 (39.0)	51 (63.0)	0.002
<40	50 (61.0)	30 (37.0)	

P <0.05: significant and P <0.001: highly significant.

Table-3: Gender wise distribution of dyslipidemia in cases and controls

Dyslipidemia	Cases (N=82)		Controls (N=81)	
	Male (N=48) (%)	Female (N=34) (%)	Male (N=51) (%)	Female (N=30) (%)
TC	20 (41.7)	16 (47.0)	19 (37.2)	5 (16.7)
TG	28 (58.3)	19 (55.9)	18 (35.3)	14 (46.7)
LDL-C	27 (56.2)	22 (64.7)	20 (39.2)	10 (33.3)
HDL-C	31 (64.6)	19 (55.9)	18 (35.3)	12 (40.0)

DISCUSSION

Present study showed a statistically significant increase in BMI in diabetics compared to controls. Similar results were reported in other studies by Aryal *et al.* and Sert *et al.*[13,14] Likewise, a study by Bays *et al*. depicted an association between increased BMI and increased prevalence of diabetes that was highest among morbidly obese individuals.[15] Obesity is believed to generate few diabetogenic substances which further deteriorate the insulin resistance process.[16] This insulin resistance could be the link in clustering of the CVD risk factors.[17] Obesity is associated with insulin resistance and is a major risk factor for CVD. Weight loss can improve cardiovascular health by decreasing insulin concentration and increasing insulin sensitivity. Therefore, exercising and weight loss can prevent or delay the onset of type 2 diabetes and help reduce the chances CVDs.

Among the various factors responsible for the risk of CVDs in diabetes, lipid abnormalities are the major contributors. The lipid profile in the present study was found to be altered with statistically significant increase in TC and non-HDL-C but with significant decrease in HDL-C in diabetics compared to controls (P<0.001). Similarly, TG and LDL-C were significantly increased in diabetics compared to controls (P<0.05). The present study showed 44% of the diabetics to be hypercholesterolemic (TC ≥200 mg/dL) which is in consistent with the study by Sert *et al*. [14] Our study showed 57% of diabetics had hypertriglyceridemia (TG ≥150mg/dL) and this finding is also supported by few other recent studies by Sert *et al.* and Regmi *et al.* [14,18] Our study revealed 60% of Diabetic patients had serum LDL-C ≥100 mg/dL, a similar study from Turkey reported 69.1% of diabetics had serum LDL-C ≥100mg/ dL.[14] Similarly, this study showed 35.0% of diabetics had serum LDL-C levels ≥130mg/dL and this data seem to be in line with reports from previous study in Turkey and India with the value of 34.9% and 45.2% respectively. [14,19] Results of our study depicted 61.0% of diabetics were HDL-C dyslipidemic (HDL-C <40mg/ dL) whereas the study by Sert et al. showed a less number of diabetics (55.4%) to be HDL-C dyslipidemic.[14]

Table-4: Correlation of non-HDL-C and the ratio of TC to HDL-C with other variables in diabetics

Variables	Non-HDL-C r(P)	TC/HDL-C r(P)
TC	0.992 (<0.001)	0.881 (<0.001)
TG	0.194 (0.081)	0.202 (0.068)
HDL-C	-0.202 (0.069)	-0.505 (<0.001)
LDL-C	0.882 (<0.001)	0.810 (<0.001)
BMI	0.246 (0.026)	0.343 (0.002)

In case of 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 Coenzyme 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.[20,21]

In the present study, even, among the controls, 37%

Table-5: Distribution of cases and controls according to BMI

Category	Cases (N=82) (%)	Controls (N=81) (%)
Normal Weight	41 (50.0)	58 (71.6)
Overweight	32 (39.0)	21 (25.9)
Obese	9 (11.0)	2 (2.5)

were TC and HDL-C dyslipidemic, 32% were LDL-C dyslipidemic and 29% were TG dyslipidemic and this trend of dyslipidemia in controls seem to be in line with the previous report of Sert *et al.*[14] Moreover; there is no distinction of distribution of dyslipidemia according to gender. Diets with higher fat and calorie intake accompanied by lack of physical activity among this age group of urban people could be the major contributing factor for dyslipidemia.

The present study showed significantly increased level of serum non-HDL-C (P<0.001) and TC to HDL-C (P<0.001) ratio in diabetics compared to the values in control group. Increased level of LDL in diabetes results from its raised level of its precursor-VLDL leads to the increased level of non-HDL-C. Similar result has been reported by Aryal *et al.* that shows statistically increased levels of non-HDL-C and the TC to HDL-C ratio in diabetics compared to control group.[13] Likewise, study by Indumati *et al.* also reported significant increased levels of non-HDL-C in diabetics compared to age and sex matched controls.[22]

Non-HDL-C and TC/HDL-C showed strong positive correlation with TC and LDL-C and is statistically significant and is negatively correlated with HDL-C. 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.[6] 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 amid relatively normal LDL-C levels,[7] elevated non-HDL-C signifies increased CVD risk even if LDL-C levels are at or below the NCEP goal or appear normal[5]. 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.[23,24] 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.[3,5] Non-HDL-C on itself is a single index of all the atherogenic apolipoprotein B (apoB)- containing lipoproteins- LDL, VLDL, IDL and lipoprotein(a). Hence, in patients with Diabetes, non-HDL-C may be a stronger predictor of CVD than either LDL-C or TG. Non-HDL-C and the ratio of TC to HDL-C may be superior to LDL-C in diabetic patients for several reasons. First, diabetes is often associated with atherogenic dyslipidemia. Single LDL-C measurement neglects the significant contribution of atherogenic VLDL and IDL cholesterol to CVD. Second, the LDL-C level is usually calculated from the Friedewald formula based on the measurement of TC, HDL-C, and TG.[5,25] LDL-C calculated by Friedewald formula,[12] may create a false impression by underestimating the LDL-C level when TG level is high. As the level of TG increases, the LDL-C level mathematically decreases and vice versa. Hence, NCEP guidelines state that the LDL-C level is not a valid basis for therapeutic decisions when the TG level is over 200 mg/dL, instead non-HDL-C is the therapeutic target.[4] So non-HDL-C, the elevated level of which signifies increased CVD risk, can be used as a part of lipid profile.

Among the diabetics of our study, 58% of males and 56% of females are TG dyslipidemic and this will obviously results in unreliable LDL-C calculation. So, relying on LDL-C targets alone can be misleading in such patients hence, a new parameter on lipid profile, the non-HDL-C can be used as a marker of dyslipidemia since it reflects the sum of serum cholesterol carried by all of the potentially atherogenic lipoproteins— LDL, VLDL, IDL, and other remnant lipoproteins. NCEP ATP III guidelines recommend lowering non-HDL–C as a secondary goal when TG is > 200 mg/dL. Measurement of Non-HDL-C and the ratio of TC: HDL-C nullifies the interference of elevated TG and even more their evaluation does not require any fasting samples.[5,25] Hence, calculation of non-HDL-C and TC/HDL-C is considered simple and cost effective.

Lastly, it can be concluded that type 2 diabetes mellitus is associated with dyslipidemia with increased levels of TC, TG, LDL-C and Non-HDL-C but a lower levels of HDL-C compared to that of controls. Because of having hypertriglyceridemia (TG>200mg/dL), a common finding in type 2 diabetes mellitus, the calculated LDL-C cannot give a real value. Since, measurement of LDL-C has some limitations; inclusion of non-HDL-C may be considered a better marker for predicting the risk of CVD in diabetes mellitus. Thus, failure to consider the importance of non-HDL-C in type 2 diabetes may result in poor treatment of patients.

For the management of dyslipidemia in diabetes mellitus, lifestyle changes including increased physical activity and dietary modifications are needed. It has also been known that the replacement of carbohydrates with proteins lead to modest increase in HDL-C and a reduction in TG levels. Increased physical activity or exercise helps maintain weight loss and also improve insulin sensitivity and increases HDL-C levels that may be beneficial in diabetes mellitus.

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