Evaluation of non-HDL-c and total cholesterol: HDL-c Ratio as Cumulative Marker of Cardiovascular Risk in Diabetes Mellitus

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ABSTRACT

Background

Cardiovascular disease (CVD), is the primary cause of morbidity and mortality in patients with diabetes and have approximately - two to four times higher CVD rate than adult without diabetes. Low density lipoprotein cholesterol (LDL-C) is primarily used as the marker of cardiovascular risk in diabetes despite its several limitations. Although several newer markers of CVD are emerging, no marker has been established in Nepal.

Objectives

The study was designed to evaluate the non-high-density-lipoprotein- cholesterol(Non-HDL-C) and Total Cholesterol to High density lipoprotein cholesterol (TC:HDL-C ratio) as CVD risk marker in diabetes mellitus.

Methods

The study was conducted in the Department of Bbiochemistry, Kathmandu University School of Medical Sciences. The study comprised of 76 diabetic subjects and 60 non-diabetic subjects. The anthropometric and biochemical parameters were measured. The Non-HDL-C and TC:HDL-C ratio were also calculated employing their respective formula.

Results

Body mass index (BMI), waist circumference (WC), blood pressure and lipid parameters were significantly different between diabetic subjects and non-diabetic subjects. There was increased non-HDL-C and TC:HDL-C ratio in subjects with diabetes mellitus. Furthermore, statistically significant correlations of non-HDL-C and TC:HDL-C ratio were obtained with BMI, WC, total cholesterol, HDL-C and LDL-C in diabetic subjects.

Conclusions

The present study observation revealed that the Non-HDL-C and TC: HDL-C strongly correlate with established independent risk factors such as obesity(WC), elevated blood pressure, HDL-C and LDL-C in diabetes. Thus, the evaluation of Non-HDL-C and TC: HDL-C ratio can be used as the simple, cost-effective and cumulative marker of cardiovascular risk in diabetes mellitus.

Key Words

cardiovascular risk, diabetes mellitus, Hypertension, lipid profile, Obesity, Non-HDL-cholesterol

INTRODUCTION

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 29% of all deaths worldwide.[1] CVD is the primary cause of morbidity and mortality in patients with diabetes and accounts for approximately 65% of overall death with diabetic complications. Adults with diabetes have approximately two to four times higher CVD rate than adults without diabetes.[2] Considering this data, it is crucial to identify and address CVD risks. Although considerable advancement has been made from the diagnostics, the current approach to evaluating CVD risk in asymptomatic diabetic individuals remains suboptimal. The ambiguities in the recommendation and utility of recent metabolic markers such as apolipoproteins, C- reactive proteins (CRP) and markers of insulin resistance in cardiovascular risk assessment are still present.[3] This understanding is based on the interpretation of the conventional lipid profile panel, while several consensus documents backing up this argument also exist.[4-7] This current dilemma may be simplified by using the non-HDL-cholesterol (Non-HDL-C) and Total Cholesterol: High Density Cholesterol (TC:HDL-C) ratio because these parameters have shown to have better predictors of atherogenic dyslipidemia and CVD.[8-10] As a result, the objective of the study is to evaluate the Non-HDL-C and TC:HDL-C ratio in diabetes mellitus and its relation to the anthropometric and biochemical parameters in the context of Nepal.

METHODS

A cross sectional study was conducted in the Department of Biochemistry, in collaboration with the Department of Internal Medicine, Kathmandu University School of Medical Sciences (KUSMS/ Dhulikhel Hospital-Kathmandu University Hospital (DH-KUH). 76 diabetic subjects consulted at DH-KUH during the study period of April 2010 to November 2010 were recruited for the study. The inclusion criteria were the previously diagnosed and confirmed cases of diabetes mellitus according to definition of diabetes mellitus. Criteria published by American Diabetes Association/WHO were used for defining diabetes mellitus.[11] 60 non-diabetic, non-hypertensive individuals who presented in the biochemistry laboratory for general health check-ups were also enrolled. The non-probability sampling method was used for the selection of the participants. Informed consent was taken from all the participants and ethical approval was also obtained from the Institutional Review Committee.

Anthropometric measurements such as Height (metres/m), weight (kilograms/kg), waist circumference (WC) (centimetres/cm) and blood pressure (milligrams of mercury/mmHg) were measured from participants by standard protocol.[12,13] Body mass index (BMI) was calculated as weight (in kilograms)/height (in metres) squared. Waist circumference was used as a measure of central obesity and the BMI was used as a measure of general obesity.[14] Hypertension was considered when the systolic blood pressure was greater than 140 mmHg and the diastolic blood pressure was greater than 90 mmHg.

After 12 hours of fasting, 5ml of blood was collected in a glass? vial for the separation of serum. The glucose (glucose oxidase peroxidase method), Triglycerides (glycerol phosphate oxidase peroxidase method), Total Cholesterol (Cholesterol oxidase peroxidase method) were measured in serum by the standard kits provided by RFCL Diagnostics, India. For the estimation of serum High Density Lipoprotein Cholesterol (HDL-C), the Very Low Density Lipoprotein (VLDL) and the LDL fraction was measured by the precipitating reagent (Dextran sulfate and Magnesium ion) and the remaining HDL-C fraction was measured by the cholesterol oxidase method as provided by RFCL diagnostic kit. The LDL-C, Non-HDL-C and TC:HDL-C were calculated on employing their respective formula. The LDL-C was calculated by the *Friedewald formula*[16] *i.e.* $\{LDL\text{-}C = TC - (HDL\text{-}C + TG/5)\}$. The Non-HDL- C can be calculated as total cholesterol minus HDL-C. And the TC:HDL-C ratio can be calculated by dividing the total cholesterol by HDL-C.

Statistical Analysis

The data was analysed using Software Package for Social Science (SPSS-11.5 version). The data was presented as a mean number with standard deviation. The student 't' test, Mann Whitney U test and Analysis of Variance (ANOVA) were applied for comparison of various parameters. The correlation coefficient was also determined. The findings were considered significant when the value of 'P' was less than 0.05.

RESULTS

The present cross sectional study comprised of 76 confirmed cases of diabetes mellitus and 60 non-diabetic, non-hypertensive subjects. The comparison various anthropometric and biochemical parameters with non diabetic subjects and diabetes mellitus using students 't' test as represented in Table 1 and Table 2. The study found significant differences in the age, BMI, blood pressure

(both systolic and diastolic). Likewise, the TC, HDL-C, LDL-C, Non-HDL- C and TC:HDL-C ratios were statistically significant between non-diabetic subjects and diabetes mellitus.

In the present study, the TC: HDL-C ratio was classified as belonging to a low risk group and the high risk group of cardiovascular diseases is as shown in Table 3. For the purposes of the study, the low risk group was classified as any result when the ratio was less than four, whereas the high risk group was considered when a result was any ratio greater than four.[15] Table 3 represents the comparison of anthropometric measurement and biochemical parameters in two risk groups of diabetes mellitus using Mann Whitney Test. BMI, WC, fasting blood glucose, TC, HDL-C, TG and LDL-C were significantly different at $P<0.05$. The age and the blood pressure (both systolic and diastolic) results were not considered statistically significant in two groups when categorised according to the TC:HDL-C ratio.

According to the Non HDL-C level, the diabetic subjects were categorised into three groups according to the National Cholesterol Education Program-Adult Treatment Panel-III (NCEP-ATP-III) criteria[17] as Group I (<130 mg/dL), Group II (130-160 mg/dL) and Group III (>160 mg/dL) and compared using ANOVA test. The anthropometric and biochemical parameters were compared and the statistical differences were obtained in WC, TC, TG and LDL-C between these three groups in diabetes mellitus, as represented in Table 4.

As recorded in Table 5, the TC:HDL-C ratio was positively correlated with WC, TC, TG and LDL-C and these were statistically significant. In contrast, TC:HDL-C ratio was negatively correlated with HDL-C and it was statistically significant. In the case of Non HDL-C, there was a positive correlation between TC, HDL-C, TG and LDL-C with high statistical significance.

DISCUSSION

In this study, the statistically significant increase in BMI was observed in the subject with diabetes mellitus, while the non-significant increase in the WC in diabetic subjects was recorded in order to be compared to non-diabetic subjects. The results proved that the tendency of having abdominal obesity is higher in both diabetic and non-diabetic subjects in Nepal. Nowadays, obesity is recognised as a worldwide problem, with more than 300 million people suffering from it, and being one of the identifiers for diabetes mellitus. Obesity is known to generate few diabetogenic substances which further deteriorate the insulin resistance process.[18] This insulin resistance could be the link to the reason behind the cardiovascular disease risk factors.[19] Few studies suggested that the association of diabetes with central obesity is stronger than the association with general fat.[20,21] Contrary results were observed in another study where increased BMI was associated with increased prevalence of diabetes and was highest among morbidly obese individuals.[22] The occurrence of obesity leads to decreased glucose tolerance, alteration in glucose insulin homeostasis, reduced metabolic clearance of insulin and decreased insulin stimulated glucose disposal progressing to insulin resistance and diabetes.[23-25]

The present study observed significant increase in the blood pressure of diabetic subjects which shows a greater probability of incidences of diabetes and hypertension. A positive association between insulin resistance and hypertension has been reported by several studies.[26-28] Recently it was established that hypertension is a multi-factorial disorder, and that there are mechanistic connections between insulin resistance and hypertension. When hypertension coexists with overt diabetes, the risk for CVD, including nephropathy, is twice as likely .[28, 29]

Table 1. Comparison of Anthropometric and Biochemical Parameters in Non diabetic controls and Diabetes Mellitus

Anthropometric Parameters	Non-diabetic subjects	Diabetes Mellitus	P value
Age (years)	36.90 ± 5.373	49.20 ± 12.891	0.000
BMI (kg/m2)	24.98 ± 2.508	25.54 ± 3.624	0.002
Waist Circumference (cm)	88.80 ± 9.824	89.64 ±8.127	0.221
Systolic Blood Pressure (mmHg)	121.57 ± 9.294	127.00±12.511	0.026
Diastolic Blood Pressure (mmHg)	80.40±6.872	87.47±10.153	0.000

Table 2. Comparison of Anthropometric and Biochemical Parameters in Non diabetic controls and Diabetes Mellitus

Biochemical Parameters	Non-diabetic subjects	Diabetes Mellitus	P value
Fasting Blood Glucose (mg/dl)	83.95± 14.826	135.92±65.848	0.000
Total Cholesterol (mg/dl)	141.25±18.671	195.79±74.681	0.000
HDL-Cholesterol (mg/dl)	42.66±11.522	40.67±4.725	0.000
Triacylglycerol (mg/dl)	186.70±119.607	164.96 ± 97.578	0.447
LDL-Cholesterol (mg/dl)	70.83 ± 15.397	115.14± 66.942	0.000
Non HDL Cholesterol (mg/dl)	100.58±19.475	148.13 ± 70.331	0.00
TC: HDL-C ratio	3.51 ± 0.61	4.18 ±1.24	0.00

Table 3. Comparison of anthropometric parameters and biochemical parameters in risk group according to TC: HDL-C in diabetes mellitus

Parameters	TC:HDL-C ratio		P value
	Lower Risk group	Higher Risk group	
Age (years)	50.59± 11.245	47.73±14.437	0.226
BMI (kg/m2)	24.62±3.337	26.51±3.702	0.041
Waist Circumference (cm)	85.82±7.236	93.68±7.056	0.000
Systolic Blood Pressure (mmHg)	126.92±13.95	127.08±10.98	0.932
Diastolic Blood Pressure (mmHg)	85.85±11.30	89.08±8.64	0.256
Fasting Blood Glucose (mg/dl)	149.72±71.605	121.38±56.547	0.038
Total Cholesterol (mg/dl)	162.23±35.306	231.16±88.303	0.000
HDL-Cholesterol (mg/dl)	51.24±11.846	43.89±9.999	0.021
Triacylglycerol (mg/dl)	138.59±85.89	192.76±102.478	0.009
LDL-Cholesterol (mg/dl)	83.28±24.703	148.72±80.206	0.000

Table 4. Comparison of various anthropometric and biochemical parameters in diabetes mellitus according to Non HDL Cholesterol level

Parameters	Non HDL Cholesterol			P value
	Group I <130 mg/dL	Group II 130-160mg/dL	Group III >130mg/dL	
Age (years)	50.76±13.319	44.38±8.742	51.27±14.476	0.106
BMI (kg/m2)	25.00±3.882	24.92±3.020	26.73±3.680	0.156
Waist Circumference (cm)	87.17±7.092	88.42±8.124	94.18±8.045	0.006
Systolic Blood Pressure (mmHg)	127.93±13.944	124.50±12.417	128.36±10.966	0.512
Diastolic Blood Pressure (mmHg)	86.62±12.480	86.67±8.165	89.82±8.792	0.475
Fasting Blood Glucose (mg/dl)	140.31±63.690	130.21±72.864	136.64±64.778	0.861
Total Cholesterol (mg/dl)	145.41±22.441	193.21±18.706	269.64±95.632	0.000
HDL-Cholesterol (mg/dl)	45.21±10.982	50.83±13.927	47.82±8.803	0. 214
Triacylglycerol (mg/dl)	102.17±35.905	178.21±119.052	236.45±74.368	0.000
LDL-Cholesterol (mg/dl)	79.77±18.100	106.73±28.846	174.53±94.098	0.000

Table 5. Correlation of Anthropometric and biochemical parameters with TC/HDL-C ratio and Non HDL-C in diabetes mellitus

Parameters	TC/HDL-C ratio r (P value)	Non-HDL-C r (P value)
Age (years)	-0.039 (0.739)	0.049 (0.673)
BMI (kg/m2)	0.093 (0.425)	-0.032 (0.782)
Waist Circumference (cm)	0.226 (0.490)	0.033 (0.775)
Systolic Blood Pressure (mmHg)	0.027 (0.814)	0.056 (0.631)
Diastolic Blood Pressure (mmHg)	0.129 (0.267)	0.178 (0.123)
Fasting Blood Glucose (mg/dl)	-0.151 (0.193)	-0.077 (0.534)
Total Cholesterol (mg/dl)	0.726 (0.000)	0.989(0.000)
HDL-Cholesterol (mg/dl)	-0.251(0.029)	0.307(0.007)
Triacylglycerol (mg/dl)	0.428(0.000)	0.308(0.007)
LDL-Cholesterol (mg/dl)	0.728(0.000)	0.961(0.000)

Among the lipid profile parameter, there was increased TC and LDL-C and the decreased HDL-C in diabetic subjects was observed compared to non-diabetic groups. The study has shown the typical picture of dyslipidemia in diabetes mellitus and the findings also concur with studies.[30,31] Atherogenic dyslipidemia is often associated with diabetes mellitus and it is characteried by elevated cholesterol, decreased HDL-C and moderately elevated LDL-C level.[32,33] Hypertension, diabetes, and dyslipidemia are all factors individually associated with increased risk for mortality from cardiovascular disease and all-cause mortality.[34,35]

The finding of the present study revealed the clustering of various risk factors such as obesity, blood pressure and lipid profile parameters in diabetes mellitus as compared to non-diabetic subjects. Other studies have also found strong associations between obesity, hypertension and abnormal lipids and they confer an even greater risk for CVD development.[34,35] Furthermore, there is overwhelming evidence that an elevated LDL-C concentration in plasma is atherogenic whereas high HDL-C levels are cardio-protective.[36-38] The present study observed significant decrease in HDL-C and increased LDL-C which signifies the greater risk of CVDs.

Non-HDL-C and TC-HDL-C ratios were significantly higher in diabetes mellitus as compared to non-diabetic subjects. When the variables were compared with the various groups according to TC:HDL-C ratio, significant difference were obtained in the both anthropometric and biochemical parameters viz. WC, blood pressure, TC, TG, HDL-C and LDL-C. Furthermore they study showed that there is a strong correlation of the TC:HDL-C ratio with WC, TC, TG, HDL-C and LDL-C ratios. Similarly, when the diabetic subjects were categorised according to Non-HDL-C levels, there were significant differences in WC, TC, LDL and TG. The present study showed that TC:HDL-C and Non-HDL-C, the newest marker of dyslipidemia, strongly correlated with abdominal obesity, high blood pressure and LDL-C. The abdominal obesity, high blood pressure and LDL-C are independent risk factors for CVD in diabetes mellitus.[39] There is escalating evidence 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.[40] Jiang R et al. (2004) reported that Non-HDL-C was a stronger predictor of CVD in diabetes mellitus but TC: HDL-C ratio was most strongly associated with CVD risk.[8] Higher levels of Non-HDL-C doubled the risk of CHD morbidity or mortality. Literature showed that the association of LDL-C with CVD was weak and LDL was not a significant predictor of CHD mortality.[41] Recently, Non-HDL-C has been considered as secondary target therapy in diabetes and other risk of cardiovascular diseases by NCEP-ATP III panel. The target goal for diabetes for Non-HDL-C is recommended as less than 130 mg/dL.[42] This is due to the fact that LDL-C is not usually raised in diabetes mellitus, thus making Non-HDL-C more reliable. [43, 44]

The Non-HDL-C and TC:HDL-C ratios may be superior to LDL-C in diabetic patients for several reasons.[43,45] Firstly, diabetes is often associated with atherogenic dyslipidemia. Single LDL-C measurement neglects the significant contribution of atherogenic VLDL and IDL cholesterol to CVD. Secondly, the LDL-C level is usually calculated from the Friedewald formula based on the measurement of total cholesterol, HDL cholesterol, and triglycerides. However, for accurate measurement based on Friedewald formula, a fasting triglyceride level must be <400 mg/dL. In DM, there are often elevated triglyceride levels in diabetic patients which results in unreliable LDL-C calculation.[15] Measurement of Non-HDL-C and TC: HDL-C is simple and cost effective. It also nullifies the interference of elevated triglycerides. Finally, Non-HDL-C and TC: HDL-C estimation does not require fasting samples. [43,45]

The study has few limitations, but the sample size was limited. Should a further study be considered,, the sample size should be increased. The utility of Non-HDL-C and TC: HDL- C as a marker of cardiovascular risk should also be assessed in non-diabetic subjects. Other risk factors such as smoking, lifestyle, duration of diabetes, and incidences of myocardial infarction should also be considered.

CONCLUSION

From the findings presented in this study, it can be concluded that diabetes mellitus is associated with obesity (both visceral and general), elevated blood pressure and atherogenic dyslipidemia all of which are independent risk factors of cardiovascular disease. Non-HDL-C and TC:HDL-C were shown to be associated with these risk factors and simultaneous measurements of Non-HDL-C and TC: HDL-C ratio can be a simple and cumulative marker of cardiovascular disease in diabetes in Nepal. These tests are cost-effective, and affordable compared to some newer markers, and have advantages over the existing markers of cardiovascular disease in Nepal.

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