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RESEARCH ARTICLE

Evaluation of Non-HDL-Cholesterol/HDL-cholesterol ratio as a predictor of coronary artery disease

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Abstract

Background and Objectives: Higher prevalence of coronary artery disease (CAD) has been reported in Indian population, which cannot be accounted for by the traditional risk factors. Identification of new risk factors which would help in treatment and prevention of CAD is of utmost importance in India. Many cardiovascular risk predictors which also include some lipid ratios are already in use. Recently apoB/apoA-1 ratio has been found to be good predictor for assessing cardiovascular risk. Our aim of this study was to find the relevance of non-HDL-C/HDL-C ratio, in comparison to apoB/apoA-1 ratio which has already been established as a predictor of coronary artery disease. **Methods:** 110 patients of CAD (coronary artery disease) of mean age (41.01$\pm$ 9.20) and 50 healthy controls (mean age-33.14$\pm$10.32) were enrolled for the study. Extended lipid profile with serum apolipoprotein A-1 and B was undertaken for each subject. Non-HDL-C was calculated (Total cholesterol-HDL cholesterol). Ratios of apoB/apoA-1 and non-HDL/HDL were calculated and statistically evaluated. **Statistical Analysis:** Unpaired t test was used to compare the means. A p value<0.05 was considered significant. Area under the receiver operating characteristic curve was calculated to assess the utility and to compare the predictive value of the ratios of apoB/apoA-1 and non-HDL-C/HDL-C. **Results:** Both the ratios were significantly higher in cases compared to the controls (P<0.001). The greater AUC of the ROC curve was obtained with the non-HDL/HDL ratio. **Conclusion:** Based on our findings we recommend the use of non-HDL/HDL ratio in Indian population for assessing risk of coronary artery disease.

Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality in developed countries and is emerging as an epidemic in developing countries [14]. It is predicted that there will be an increase of 111% in cardiovascular deaths in India by the year 2020 when compared to the year 1990 [9].This is much higher than that predicted to any other region both in Asia as well as outside Asia. This high prevalence warrants probing into the presence of various risk factors and their association with CAD. Although traditional risk factors accounts for the majority of the cases of coronary artery disease but sizable number of patients remain, in whom risk is not quantified yet. This led to studies on newer risk factors like Lp(a),Non-HDL-C, apolipoproteins B and A-1 etc.

Although low density lipoprotein cholesterol (LDL-C) is a well established atherogenic factor for coronary artery disease, it does not completely represent the risk associated with atherogenic lipoprotein in the presence of high triglycerides (TG) levels. In routine clinical practice, non-HDL-C is regarded as a surrogate marker for apoB [3, 11].

Both apoB and non-HDL-C have been reported to be better predictor of CVD than LDL-C [1, 12]. Although measurement of apolipoprotein does not require fasting conditions, these apolipoproteins are not currently part of routine laboratory lipid assessment [7]. Thus, non-HDL-C is an easy and cost effective means to estimate apoB levels, besides its original usefulness for estimating LDL-C atherogenicity in hypertriglyceridemic patients [4].
Various lipid and lipoprotein ratios are being utilized for assessing the risk of CAD such as the ratios of total or LDL cholesterol to HDL-cholesterol and that of apoB to apoA-1. We attempted to present non-HDL-C/HDL-C ratio which to the best of our knowledge has not been evaluated so far in Indian population as a risk predictor in coronary artery disease although its utility in predicting cardiovascular risk in type 2 diabetes [2] and in patients with metabolic syndrome [6] has been reported.

Methods

Subjects: From November 2010 to August 2013, a total of 110 patients (aged 20-60, mean age 41.01± 9.20) of coronary artery disease of either sex with a history of acute chest pain, non ST-segment elevation, unstable and stable angina, examined and treated at were enrolled in the present study. 50 healthy individuals free of any symptoms of CAD served as controls. Patients with diabetes mellitus, nephrotic syndrome, acute or chronic renal failure, thyroid disorders, acute infection or any other systemic illness and on lipid lowering drugs for the past 3 months were excluded from the present study. Tobacco/Regular Smokers and alcohol abusers were also excluded. The same exclusion criteria were also applied for the selection of controls. The institutional Ethical Committee approved the study and informed consent was obtained from all the participants. The patient's demographic profile, socioeconomic status, behavioural risk factors (sedentary life style, non vegetarian diet) and disease risk factor histories were recorded. Fasting venous blood samples were collected and analyzed by using enzymatic procedures with Johnson & Johnson's Vitros 250 auto analyzer for serum Total Cholesterol, Triglycerides, HDL and LDL-Cholesterol by direct assay. Serum apolipoprotein B and apolipoprotein A-1 estimations were carried out in NEPHSTAR (PROTEIN ANALYSIS SYSTEM) by using kit from Goldsite diagnostic inc. Non-HDL-C was calculated by subtracting HDL-C from total cholesterol. The ratios of non-HDL-C/HDL-C and apoB/apoA-1 were calculated.
Statistical analysis: Results were presented as mean ± standard deviation. The Unpaired't' test was used to compare the levels of both the ratios between the test and control group. A comparison of the ratios of non-HDL-C/HDL-C and apoB/apoA-1 was analyzed by comparing with the cut off values for dyslipidemia (total cholesterol, LDL-C, HDL-C, triglycerides, and non-HDL-C) according to National Cholesterol education Program (NCEP) Adult Treatment Panel (ATP) III criteria and in terms of receiver operating characteristic (ROC) curve. A ROC curve is a plot with the 1-specificity on the x-axis and sensitivity on the y-axis obtained for different cut off points. Areas under the curve (AUC) and their 95% confidence intervals (CI) were evaluated as a measure of diagnostic accuracy. Greater AUC of the ROC curve indicated better markers of the study. All p-values <0.05 were considered as significant. All analyses were performed using the SPSS version 16.0

Results

Among the 110 subjects participating in the study males constituted 67% of the total population compared with females constituting 33% (**Table 1**). Among the demographic variables considered the test group showed significantly larger number of sedentary life style subjects and majority of which were non vegetarian as compared to control group.

Table 1. Demographic data of CHD patients and control groups

		Test (n=110) n (%)	Control (n=50) n (%)
Mean Age		41.01±9.20	33.26±10.66
Male		81(74)	26(52)
BMI		26.15±4.23	23.84±4.44
Young	≤40	56(51)	41(82)
Higher	>40	54(49)	9(18)
Urban		86(78)	42(84)
Rural		24(22)	8(6)
	Act	12(11)	6(12)
Life Style	Mod	26(24)	33(66)
	Sed	72(65)	11(22)
Diet	V	45(41)	23(46)
	NV	65 (59)	27(54)
HTN		57(52)	Nil
F/H DM		49(45)	28(56)
F/H CHD		52(47)	21(42)

CHD, coronary heart disease; BMI, body mass index; Act, active; Mod, moderate; Sed, sedentary; V, vegetarian; NV, non-vegetarian; HTN, hypertension; F/H, family history; DM, diabetes mellitus

Assay of blood lipids: Blood lipids (Total Cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein B, apolipoprotein A-1, non-HDL-C, ratio apoB/apoA-1 and non-HDL-C/HDL-C) levels were measured for all subjects and the results are summarized in (**Table 2**).The levels were not affected by age, gender, diet or life style).

Table 2. Lipid profile and Ratios in Study and Control Group

Parameters	Group	N	Mean	Std. Deviation	95% Confidence Interval	P
TC	C	50	152.54	29.62	144.12 - 160.96	<0.001**
	T	110	199.49	54.14	189.26 - 209.72	
LDL-C	C	50	91	25.30	83.81 - 98.19	<0.001**
	T	110	132.40	48.88	123.16 - 141.63	
HDL-C	C	50	45.30	6.37	43.50 - 47.11	<0.001**
	T	110	40.28	8.43	38.69 - 41.88	
TG	C	50	97.78	30.05	89.24 - 106.32	<0.001**
	T	110	189.89	87.25	173.40 - 206.38	
Non-HDL-C	C	50	107.23	27.65	99.38 - 115.09	<0.001**
	T	110	157.59	52.18	147.73 - 167.45	
Apo-B	C	50	79	21	73 – 85	<0.001**
	T	110	110	31	104 – 116	
Apo-A1	C	50	141	34	132 – 151	<0.05*
	T	110	129	29	123 – 134	
ApoB/A1	C	50	0.58	0.18	0.53 - 0.63	<0.001**
	T	110	0.90	0.37	0.82 - 0.97	
Non-HDL-C/HDL-C	C	50	2.40	0.65	2.21 - 2.58	<0.001**
	T	110	4.07	1.64	3.76 - 4.38	

All values in (mg/dl).p-value<0.001= ***, p-value<0.05=*

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; Non-HDL-C, Non-high density lipoprotein cholesterol; Apo-B, apolipoprotein B; Apo-A-1, apolipoprotein A-1; ApoB/A-1,ratio apolipoprotein B/apolipoprotein A-1; Non-HDL-C/HDL-C, ratio non-high density lipoprotein cholesterol/high density lipoprotein cholesterol

Comparison of the ratios apoB/apoA-1 and non-HDL-C/HDL-C was done with the varying (normal as well as elevated) levels of total cholesterol, LDL-Cholesterol, HDL-cholesterol, triglycerides and non-HDL-Cholesterol as shown in (**Table 3**) and it clearly indicates that the normal as well as elevated levels of above mentioned parameters were associated significantly with the ratio of non-HDL-C/HDL-C but not with the ratio of apoB/apoA-1.

Table 3. Comparison of ratios of ApoB/A-1 and Non-HDL-C/HDL-C with varying (normal as well as elevated) levels of total cholesterol, LDL-C, HDL, triglycerides and Non-HDL-C

Parameters		Varying levels	N	Mean	Std. Deviation	P
	TC	< 200	54	0.83	0.33	NS
		>200	56	0.95	0.40	
	LDL-C	< 100	28	0.87	0.38	NS
		>100	82	0.90	0.37	
Apo B/A-1	HDL-C	< 40	62	0.97	0.39	<0.05*
		>40	48	0.80	0.32	
	TG	< 150	48	0.85	0.33	NS
		>150	62	0.93	0.40	
	Non-HDL-C	<130	32	0.86	0.37	NS
		>130	78	0.91	0.38	
	TC	< 200	54	3.21	0.91	<0.001***
		>200	56	4.90	1.77	
	LDL-C	<100	28	2.75	0.81	<0.001***
		>100	82	4.52	1.61	
Non-HDL-C/HDL-C	HDL-C	< 40	62	4.41	1.99	<0.001***
		< 40	48	3.63	0.88	
	TG	<150	48	3.26	0.98	<0.001***
		>150	62	4.69	1.78	
	Non-HDL-C	< 130	32	2.82	0.81	<0.001***
		>130	78	4.58	1.63	

(All values in mg/dl).p-value<0.001=***, p-value<0.05=*

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; Non-HDL-C, Non-high density lipoprotein cholesterol; Apo-B, apolipoprotein B; ApoA-1, apolipoprotein A-1; ApoB/A-1,ratio apolipoprotein B/apolipoprotein A-1; Non-HDL-C/HDL-C, ratio non-high density lipoprotein cholesterol/high density lipoprotein cholesterol

ROC Curve Analysis: It was shown that the ratios of apoB/apoA-1 and non-HDL-C/HDL-C were effective diagnostic markers for coronary artery disease and the area under the ROC curve of non-HDL-C/HDL-C was higher than those of apoB/apoA-1 (**Fig. 1**) and (**Table 4**).

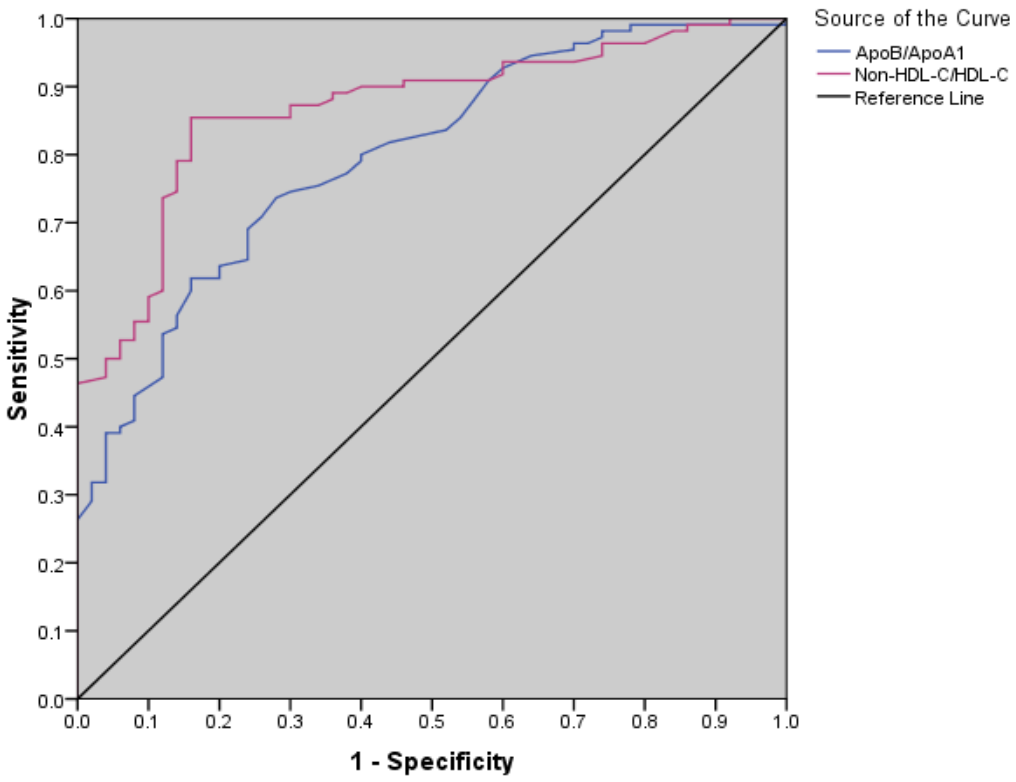

Figure 1: Receiver Operating Characteristic curve for apoB/apoA-1 and non-HDL-C/HDL-C in CHD patients. Area under the curve is greater for non-HDL-C/HDL-C than for apoB/apoA-1.

Table IV. The area under the receiver-operating characteristic curves for ApoB/A-1 and Non-HDL-C/HDL-C

Variable	Area under the ROC Curve ± SE	95% Confidence Interval	P value
ApoB/A-1	0.798 ± 0.036	0.727 - 0.868	<0.001
Non-HDL-C/HDL-C	0.869 ± 0.029	0.813 - 0.926	<0.001

The optimal cut off value of the non-HDL-C/HDL-C ratio for the detection of coronary heart disease was 2.84 with a sensitivity of 84.5% and specificity of 84% (**Table 5**).

Table 5. The cut off value corresponding to the highest % sensitivity and % specificity as were calculated from ROC curves

Variable	Cut off value	Sensitivity	Specificity
ApoB/A-1	0.645	73.6%	74%
Non-HDL-C/HDL-C	2.845	70.9%	72%

Discussion

Our study demonstrated that the ratio of non-HDL-C/HDL-C is a better marker than apoB/apoA-1 ratio for identifying the risk for coronary artery disease. We analyzed the correlation of these two with other lipid parameters commonly used and significant correlation was observed for non-HDL-C/HDL-C ratio. We also observed that the AUCs of the non-HDL-C/HDL-C ratio for the prediction of coronary artery disease was significantly higher than those of the apoB/apoA-1 ratio. The clinical importance of these findings suggested strong association of non-HDL-C/HDL-C ratio with the coronary artery disease.

Early and accurate estimation of risk holds pivotal role in cardiovascular prevention. Despite the multifactorial aetiopathogenesis of atherosclerosis, around 50% of risk burden for cardiovascular disease can be attributed to lipid profile abnormalities [15]. So far reliance has been on LDL, but now epidemiologists and clinicians are in almost unanimous agreement that CAD risk estimation based solely on low-density lipoprotein (LDL) cholesterol is not adequate [13]. This hoisted search for emergence of new cardiovascular risk factors to improve early disease prediction[15].Towards this aim, in an attempt to optimize the predictive capacity of the lipid profile, several lipoprotein ratios or "atherogenic indices" have been defined which could be more transparent depicters' of clinically relevant metabolic interactions between lipid fractions and lipoproteins.

Besides the conventional lipid ratios (TC/HDL, LDL-C/HDL-C) the ratio of apolipoproteinB (apoB) to apolipoprotein A-1 (apoA-1) an indicator of the balance between atherogenic and atheroprotective cholesterol transport [8] has been found to be independently associated with CVD [15]. This ratio has been found to predict cardiovascular disease more accurately and more strongly than either apoB or apoA1 alone or any of the other cholesterol. At triglyceride levels >200 mg/dl, non-HDL-C better defines atherogenic risk, since associated changes in VLDL-TG/VLDL-C ratio may lead to under calculation of LDL-C with the friedewald formula [10]. Non-HDL-C actually being estimation of the level of all apoB-carrying lipoproteins; can be a simple and inexpensive replacement for apoB measurement, especially in patients with hypertriglyceridemia or diabetes [4]. Clinical relevance and equivalence of apoB and non-HDL measurements has been reiterated by ATP III-NCEP [10] and also in patients on statin therapy [5]. In addition to its merits, non-HDL-C is readily available from a routine lipid profile in the fasting state irrespective of the triglyceride levels, thus, obviating the need to perform additional and more expensive apo-B measurement.

Although a few studies have evaluated this lipoprotein ratio for assessing cardiovascular disease risk. Our findings are in consistent with the findings of Kim SW et al [6] who found this ratio, a good predictor for CV risk in patients of metabolic syndrome and with Eliasson B et al [2] who suggested that CHD risk can be better predicted using the ratio of non-HDL-C to HDL-C than LDL-C, HDL-C, and non-HDL-C. We have attempted to evaluate this ratio in the patients of coronary artery disease in India. There is no Indian study as of now, to our knowledge, which has evaluated non-HDL-C/HDL-C ratio as a predictor of coronary artery disease.

Limitations

Considering the diagnostic importance both for the clinicians and the patients, larger sample sized trial is needed for providing more precise information and accuracy of the non-HDL-C/HDL-C ratio as a predictive marker of coronary artery disease.

Conclusion

Our findings suggest that the non-HDL-C/HDL-C ratio is a better marker than apoB/apoA-1 ratio for identification of coronary heart disease. We also determined that the optimal cut off value of non-HDL-C/HDL-C ratio for the detection of coronary heart disease is 2.84

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