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NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL VERSUS APOLIPOPROTEIN B AS PREDICTORS FOR CORONARY HEART DISEASE

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ABSTRACT

Recently non high density lipoprotein cholesterol (non-HDL-C) and apolipoprotein B (apoB) have been found to be better and more reliable markers of atherosclerotic risk than LDL-C (low density lipoprotein cholesterol).Very few studies, as of now, are available in India which compared their predictability as a cardiovascular risk marker. The aim of our study was to evaluate and compare apoB and non-HDL-C and to find out the superiority of one over the other, if any. Lipid profile and apoB were determined in 110 subjects of coronary heart disease (CHD). Non-HDL-C was calculated. ApoB and non-HDL-C were compared in terms of receiver operating characteristic (ROC) curves. Within the constraints of this study there was statistically no difference between apoB and non-HDL-C in predicting CHD; therefore, this study supports the use of non-HDL-C as an initial screen for coronary risk as it incurs no additional cost, takes less time to report and is simple to calculate.

KEY WORDS: **Apolipoprotein B, Coronary heart disease, LDL-C, Non-HDL-C**

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INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) concentration has been established as an independent risk factor for the development of atherosclerosis; consequently, multiple practice guidelines have recognized LDL-C as the primary target of therapy [1, 2]. For decades, considerable efforts have been directed towards educating physicians and the general public about the importance of lowering LDL-C levels. Despite the extensive available data relating LDL-C to atherosclerosis, there is enough evidence to suggest otherwise [3].Many limitations exist for focusing alone on LDL-C: (1) At elevated triglyceride concentration LDL-C is not the optimal strategy for risk estimation as it was erroneously estimated to the tune of 17% and 25% in patients having triglyceride concentrations from 151-200mg/dl and 201-300mg/dl respectively [4].(2) A good number of patients with atherosclerotic vascular disease have LDL-C in the optimal range [5].(3)Other triglyceride-rich lipoproteins, including very low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL) are also atherogenic which are not taken into consideration while estimating LDL-C [6,7]. Recently the focus has been shifted to alternative estimations of atherogenic cholesterol which takes into account all atherogenic particles, among which non-HDL-C and apoB has been shown to be better cardiovascular risk indicators than LDL-C [8]. Non–HDL-C is calculated by subtracting the HDL-C from the total cholesterol, and it represents the cholesterol concentration of all atherogenic lipoproteins [9, 10]. Although non–HDL-C is a good surrogate measure of apo B, it does not measure the same thing. Non–HDL-C measures the “cholesterol” content of all atherogenic lipoproteins (LDL, IDL, and VLDL), whereas apoB represents the total number of circulating atherogenic particles. Canadian guidelines endorse the use of non-HDL-C or apoB as an alternative to LDL measurement when triglyceride levels are elevated [2, 11].The US and European guidelines also recommend non-HDL cholesterol or apo B as acceptable markers for cardiovascular risk prediction[1, 12]. Besides, the superiority of apoB [13, 14-16,] and non-HDL-C[17, 18, 19] has been established over LDL-C. The controversy regarding the superiority of non-HDL-C and apoB as predictors of coronary risk has not been settled yet. Few studies have made a comparative assessment of these two [13, 17, 20]. To address this controversy we have compared these biomarkers in predicting coronary events.

METHODS

Subjects: From November 2011 to August 2013, a total of 110 patients (aged 20-60,mean age 41.01$\pm$ 9.20) of coronary heart disease of either sex with a history of acute chest pain, non ST-segment elevation, unstable and stable angina, examined and treated at advanced cardiac centre, PGIMER, Chandigarh were enrolled in the present study. 50 age and sex matched healthy controls (mean age 33$\pm$10.32 years) were randomly selected. 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 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, dietary habits) 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 estimation was 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.

Statistical analysis

Results were presented as mean $\pm$ 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 non-HDL-C and apoB was analyzed by comparing 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. The area under the ROC curve was considered a global performance indicator for a prognostic factor [21]. All p-values <0.05 were considered 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 I).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 I
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) and apolipoprotein B levels were measured for all subjects, non-HDL-C levels were calculated and the results are summarized in (Table II).The levels were not affected by age, gender, diet or life style).

Table II
Lipid profile and Ratios in Study and Control Group

Parameters	Group	N	Mean	Std. Deviation	95% Confidence Interval	P value
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	91.00	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	
ApoB	C	50	79.08	21.37	73.00 – 85.15	<0.001***
	T	110	110.16	30.82	104.34 – 116.98	

All values in mg/dl).p-value<0.001=, p-value<0.01=**, 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

ROC Curve Analysis

Non-HDL-C and apoB were found to be effective diagnostic markers for coronary heart disease and the area under the ROC curve of non-HDL-C was almost equal to that of apoB (Fig. I) and (Table III).

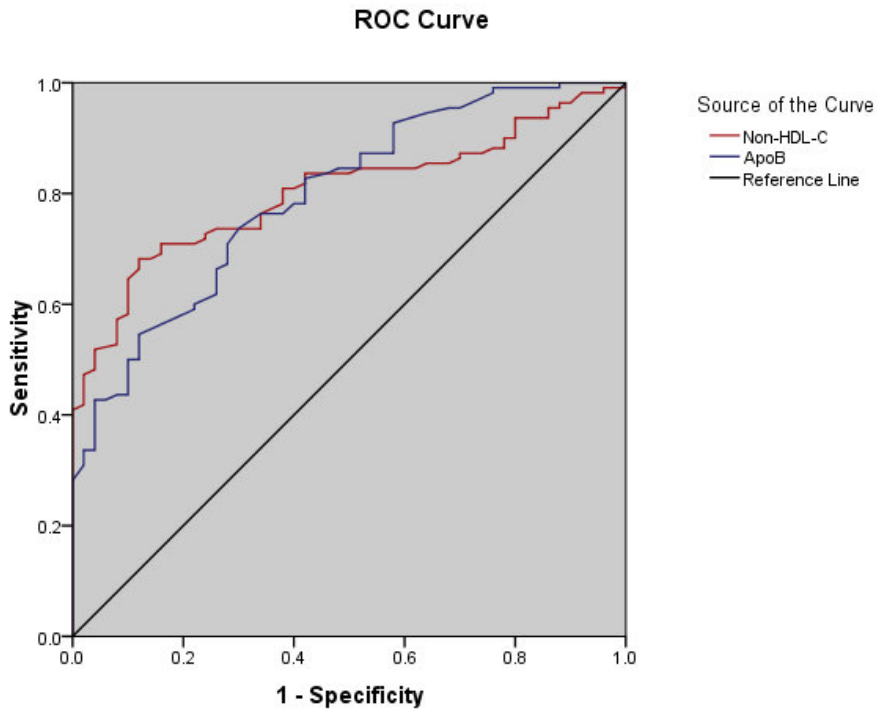

Figure 1
Receiver Operating Characteristic curve for non-HDL-C and apoB in CHD patients.

Table III
The Area under the receiver-operating characteristic curves for apoB and non-HDL-C

Variable	Area under the ROC Curve ± SE	95% Confidence Interval	P value
ApoB	0.796 ± 0.036	0.727 - 0.868	<0.001***
Non-HDL-C	0.802 ± 0.034	0.736 - 0.869	<0.001***

The optimal cut off values of the non-HDL-C and apoB for the detection of coronary heart disease were 124.5 and 0.89 with a sensitivity of 73.6% and specificity of 74% and 70.9% and 72% respectively (Table IV).

Table IV
The Cut off points corresponding to the highest % sensitivity and %specificity were calculated from the ROC curves

Variable	Cut off value	Sensitivity (%)	Specificity (%)
Apo B	0.89	70.9%	72%
Non-HDL-C	124.5	73.6%	74%

DISCUSSION

Although LDL-C is a well-recognized target, emerging findings suggest that it has fallen in favour as a predictor of cardiovascular disease for a number of reasons [9, 22]. ApoB and non-HDL-C have increasingly been considered as better predictors for assessing cardiovascular risk than LDL-C being a single measurement that includes all atherogenic lipoproteins. Apolipoprotein B (apoB) is a key structural component of all the atherogenic lipoprotein particles (LDL, VLDL and IDL). Each of these particles carries one apoB molecule; thus, the total apoB level represents the total number of circulating atherogenic lipoprotein particles and would assist the clinician with a more accurate index of cardiovascular risk [23].Various studies reported the strongest association of apoB with cardiovascular risk compared with conventional measures[13,24-26]. Alternatively, some other experts have advocated the use of non–high-density lipoprotein cholesterol (non–HDL-C) instead of LDL-C in cardiovascular risk prediction, particularly in those with elevated triglyceride values[1].The National Cholesterol Education Program (NCEP)/Adult Treatment Panel (ATP) III guidelines have recommended the use of non-HDL-C in patients with triglyceride levels 200mg/dl or higher[1]. With conventional analysis, non-HDL-C is able to quantify the total atherogenic burden by aggregate amount of “cholesterol” in all contributive particles. Non-HDL-C is a quick and simple mathematical calculation (Total Cholesterol–HDL-C), and can be calculated in the non-fasting state [17]. Non-HDL level imparts all the information provided by LDL-C along with additional information on the presence of atherogenic dyslipidemic particles without the need to measure TG levels [8]. Some have argued about the superiority of apoB over non-HDL-C and its introduction into routine clinical care [20] and others have favoured the use of non-HDL-C [27,28]. Non-HDL-C being a surrogate measure for apo B but uncertainty about its preference to apoB as a marker still exists [26,29]. Ramjee et al recently suggested that the benefits of non-HDL-C are manifold such as no additional cost, simple and easy calculation and well documented intervention effects[17].

The present study confirms that non-HDL-C is equivalent to apoB on the basis of ROC curve analysis (clinical discrimination is better evaluated by ROC curves and in the present study both apoB and non-HDL-C showed good discrimination, with an AUROC close to 0.8), which is in agreement with Stanley S.Levinson[30] who found little difference between apoB and non-HDL-C in discriminating CAD. Sondermeijer et al [31] also recently concluded that non-HDL-C and apoB

were comparable in their ability to predict risk of future CHD. Our study has some limitations. Inclusion of large number of patients in the study would have been more appropriate considering the diagnostic importance both for clinicians and the patients. More precise information and accuracy of this diagnostic marker would have been obtained if the study was followed up for at least 6 months. This would have been also beneficial for accessing treatment outcomes and further, correlation with angiographic findings would have made the study more thorough. In conclusion, our study has found that there is no difference between apoB and non-HDL-C in predicting cardiovascular risk. However, we would suggest the use of non-HDL-C as an initial screen for coronary risk particularly in Indian population. India being a developing country, people would not be burdened with additional cost of apoB estimation. Besides, non-HDL-C can easily be calculated from routine lipid panel in minimum time. In fact, Ramjee et al [17] has suggested non-HDL-C to be a marker of choice when compared to apoB.

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