Non-HDL as a Valid Surrogate Marker of Small Dense LDL in a Young Indian Population

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Abstract Small dense (sd) LDL is a significant independent risk factor for premature coronary artery disease (CAD). Unfortunately, its estimation is not popular, due to the limited availability of specialized equipment, high cost and time-consuming technique. Non-HDL is a calculated, single index measure of all atherogenic apolipoprotein-B containing lipoproteins. This study aimed at identifying non-HDL as a superior surrogate marker of sdLDL cholesterol in a young Indian population. 161 healthy subjects < 45 years were tested for lipid profile, apolipoproteins A1 and B, and sdLDL particle size. sdLDL particles showed negative correlation with non-HDL ($r = -0.283$, $p < 0.001$), LDL ($r = -0.195$, $p = 0.013$) and apoB/apo A1 ($r = -0.175$, $p = 0.026$), the significance being greatest with non-HDL. ROC showed AUC for non-HDL, LDL and apoB/apo A1 as 0.704, 0.686, and 0.596 respectively. For LDL < 130 mg/dL, sdLDL showed a more significant negative correlation with non-HDL ($r = -0.291$, $p < 0.001$) as compared with apoB/apoA1 ($r = -0.172$, $p = 0.037$). For triglycerides < 200 mg/dL, sdLDL particle size showed higher significant negative correlation with non-HDL ($r = -0.213$, $p = 0.015$) than with LDL ($r = -0.176$, $p = 0.045$) while for triglycerides between 200 and 400 mg/dL, significant negative correlation was observed only with non-HDL ($r = -0.372$,

$p = 0.043$). Hence, our study suggested that non-HDL is a superior surrogate marker of sdLDL particle size as compared to LDL and apoB/A1 ratio in a young healthy Indian population and should be used for optimum assessment of dyslipidemias and CAD risk.

Keywords Non-HDL · Small dense LDL · Apolipoproteins · Risk factors · Premature coronary artery disease

Introduction

There has been an explosive increase in premature coronary artery disease (CAD) in Indians which affects a population of ≤ 45 years of age and follows a highly malignant course. Traditional lipid tests like cholesterol and LDL are established risk factors, however, their levels are not always elevated in CAD. Therefore, the challenge facing us is early identification of “at risk individuals” to reduce the morbidity and mortality associated with premature CAD.

One of the major risk factors for CAD is elevated LDL, which is also the primary target of lipid lowering therapy [1]. LDL is divided into heterogenous subclasses which differ in their atherogenic potential. Small dense (sd) LDL are highly significant risk biomarkers for premature CAD and their atherogenic effect is due to the following mechanisms: remain longer in the plasma, do not bind very well to the LDL receptors, bind well with the scavenger receptors, show increased susceptibility to oxidation and enter the arterial wall easily. Also, sdLDL may promote endothelial dysfunction, induce plasminogen activator inhibitor-1 (PAI-1) and thromboxane secretion in endothelial cells, and lead to calcium increase in the

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arterial smooth muscles. Hence, patients with a higher sdLDL fraction are more prone to the severer form of CAD [2]. Numerous other studies have also observed that sdLDL can predict CAD occurrence independent of traditional lipid parameters [3–5].

Unfortunately, estimation of sdLDL has not gained wide acceptance in diagnostic use due to the limited availability of the specialized equipment used for its estimation, high cost and time consuming technique. Therefore, it would be very useful for clinicians and highly cost effective for patients, if, from the routinely measured lipid profile tests, we could identify the parameter whose levels would best reflect sdLDL levels in blood.

Non-HDL, calculated as total cholesterol minus HDL cholesterol, is a single index measure of all the atherogenic lipoprotein B containing fractions i.e. LDL, VLDL, IDL and Lp(a). As the current consensus is that all the triglyceride rich lipoproteins are atherogenic and since non-HDL represents the cholesterol content of these atherogenic lipoproteins, ATP III designated non-HDL cholesterol as “atherogenic cholesterol” and identified it as a secondary target of therapy after LDL [6].

Non-HDL measurement has many advantages. It can be easily calculated from the routinely performed lipid profile tests without incurring any additional cost to the patient. It is inexpensive and reliable even when measured in the non fasting state unlike LDL which is not very reliable when measured in the presence of post-prandial hypertriglyceridemia [6, 7]. Non-HDL is already an accepted surrogate marker for apolipoprotein (apo) B and would offer an added advantage if it could be a predictor of the atherogenicity of sdLDL as well.

So, considering these aspects, our study aimed to identify, from the routinely measured lipid profile tests, a valid surrogate marker reflective of the atherogenicity of small dense LDL, in a young Indian population with triglyceride levels ≤ 400 mg/dL.

Materials and Methods

This study, approved by the ethics committee of the hospital, was conducted on 161 subjects attending the Cardiology Department and who fulfilled the inclusion criteria. Informed consent was taken from all the subjects. The study group consisted of subjects with a normal coronary angiogram or minimum disease, i.e., $< 25\%$ on coronary angiography. Subjects with the following conditions were excluded from the study: history of acute/chronic infections, history of acute/chronic inflammations, neoplasms, previous history of myocardial infarction, previous history of coronary artery intervention and intake of lipid lowering

drugs. Fasting blood samples were collected, centrifuged, and stored at the recommended temperature until assayed.

LDL Subfraction Analysis

LDL subfractions were analyzed by polyacrylamide gel tube electrophoresis (Lipoprint LDL system, Quantimetrix). The samples were categorized as pattern A or B based on mean LDL particle size (Fig. 1a, b). LDL

a

b

Fig. 1 a, b: LDL subtypes 1 and 2 represent large, buoyant LDL, while subtypes 3–7 represent small, dense LDL. LDL particles with a mean diameter of > 268 Å were defined as large, buoyant LDL particles or “pattern A”. LDL particles of diameter < 265 Å were defined as “Pattern B” or the “small dense LDL” while particles of size between 265 and 268 Å were considered as “Intermediate pattern”. In this study, the sdLDL and the intermediate LDL were pooled in the analysis to reflect the overall sdLDL. **a** Example of large, buoyant LDL (Pattern A). **b** Example of predominantly small dense LDL (Pattern B)

particles with a mean diameter of $> 268 \text{ \AA}$ were defined as large, buoyant LDL particles or “pattern A”. LDL particles of diameter $< 265 \text{ \AA}$ were defined as “Pattern B” or the “small dense LDL” while particles of size between 265 and $268 \text{ \AA}$ were considered as “Intermediate pattern”. The sdLDL and the intermediate LDL were pooled in the analysis to reflect the overall sdLDL.

Estimation of Lipid Profile

Analysis of lipid profile included serum cholesterol, HDL-C, LDL-C, triglycerides and apolipoprotein A1 and B. These parameters were analyzed on a fully automated clinical chemistry analyzer (Beckman Coulter Unicel DXC-860). Cholesterol and triglycerides were estimated by the enzymatic methods and LDL and HDL estimated by the direct elimination methods. Apolipoprotein A1 and B were measured by immunodiffusion. Non-HDL was calculated as total cholesterol minus HDL.

Statistical Analysis

Statistical analysis was performed by the SPSS program for Windows, version 17.0 (SPSS, Chicago, Illinois). Continuous variables are presented as mean $\pm$ SD, and categorical variables are presented as absolute numbers and percentage. Data was checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired *t* test, whereas the Mann–Whitney *U* test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the Chi square test or Fisher’s exact test. Spearman correlation was calculated for sdLDL with non-HDL, LDL and ApoB/ApoA. A receiver operating characteristics (ROC) analysis was calculated to determine the area under the curve and the sensitivity and specificity were calculated to analyze the diagnostic value of all these markers. For all statistical tests, a *p* value less than 0.05 was taken to indicate a significant difference.

Results

Table 1 lists the comparison of the lipid tests between men and women. There was no significant difference between men and women with respect to the levels of cholesterol, LDL and triglycerides, however, HDL was significantly lower in men. The sdLDL particle size was lower in males as compared to females but this difference was not significant. Non-HDL and the ratio of apoB/apoA1 were both significantly higher in men as compared to women.

Figures 2, 3 and 4 shows scatter plots for comparison of small dense LDL particle size with non-HDL, LDL and

Table 1 Biochemical characteristics of the study group

	Men (n = 95) Mean $\pm$ SD	Women (n = 66) Mean $\pm$ SD	<i>p</i> Value
Age (years)	38.86 $\pm$ 6.98	42.15 $\pm$ 5.28	0.001
Cholesterol (mg/dL)	163.08 $\pm$ 34.97	154.94 $\pm$ 37.21	0.159
HDL (mg/dL)	33.18 $\pm$ 9.92	38.18 $\pm$ 11.12	0.003
LDL (mg/dL)	92.44 $\pm$ 24.14	87.48 $\pm$ 28.98	0.240
TG (mg/dL)	155.48 $\pm$ 66.01	134.73 $\pm$ 56.148	0.039
Apo A1 (mg/dL)	109 $\pm$ 27.97	113.38 $\pm$ 23.17	0.297
Apo B (mg/dL)	84.48 $\pm$ 21.04	77.09 $\pm$ 23.83	0.039
ApoB/ApoA1	0.80 $\pm$ 0.22	0.70 $\pm$ 0.24	0.006
Lp(a) (mg/dL)	30.22 $\pm$ 27.71	37.65 $\pm$ 33.94	0.129
sdLDL ($\text{\AA}$)	266.26 $\pm$ 11.21	268.72 $\pm$ 6.03	0.099
Non-HDL (mg/dL)	129.91 $\pm$ 31.55	116.76 $\pm$ 32.46	0.011

Variables are given as mean $\pm$ standard deviation

HDL high density lipoprotein cholesterol; *LDL* low density lipoprotein cholesterol; *TG* triglycerides; *ApoA1* apolipoprotein A1; *Apo B* apolipoprotein B; *Lp(a)*: lipoprotein (a); *sdLDL* small dense LDL; *Non-HDL* non-high density lipoprotein cholesterol

Fig. 2 Scatter plot for correlation of sdLDL with non-HDL

apoB/apoA1 respectively in the study subjects. The correlation coefficient of non-HDL for sdLDL particle size ($r = -0.283$) was significantly higher than with LDL ($r = -0.195$) and apoB/apo A1 ($r = -0.175$).

Figure 5 illustrates the Receiver Operator Characteristic (ROC) curves to evaluate the discriminatory ability of non-HDL, LDL and apoB/apo A1 for predicting sdLDL. The area under the curve (AUC), for non-HDL, LDL and apoB/apo A1, were 0.704, 0.686, and 0.596 respectively. This figure showed that non-HDL had a significantly higher predictive value than LDL and apoB/apo A1 for sdLDL

Fig. 3 Scatter plot for correlation of sdLDL with LDL

Fig. 4 Scatter plot for correlation of sdLDL with apo B/apo A1

particle size. The optimum cut-off point of non-HDL, with a sensitivity and specificity of 72.5 and 62.0% respectively was 124.5 mg/dL.

Figure 6a, b show scatter plots for comparison of sdLDL particle size with non-HDL and apo B/apo A1 when the study group was stratified by LDL. The correlation coefficient of non-HDL for sdLDL particle size ($r = -0.291$) was significantly higher than with apo B/apo A1 ($r = -0.172$) when stratified with $LDL \leq 130$ mg/dL ($n = 148$). However when stratified with $LDL > 130$ mg/dL (Fig. 7a, b), neither non-HDL nor apo B/apo A1 showed any significant correlation ($r = 0.162$ and $r = -0.071$

Fig. 5 The ROC curves of non-HDL, LDL and apo B/apo A1 for predicting sdLDL particle size ($< 265 \text{ Å}$). The AUC for non-HDL, LDL and apo B/apo A1 are 0.704, 0.686, and 0.596 respectively

respectively) with sdLDL particle size. This was probably because of the very small sample size ($n = 13$) and therefore requires further investigation in a larger population.

Figures 8 and 9 show the scatter plots for comparison of sdLDL particle size with non-HDL, LDL and apo B/apo A1 when the subjects were stratified by triglyceride levels. With triglyceride levels less than 200 mg/dL ($n = 131$), (Fig. 8a–c), the correlation coefficient of sdLDL particle size with non-HDL ($r = -0.213$) was significantly higher than the correlation coefficient with LDL ($r = -0.176$) as well as with apo B/apo A1 ($r = -0.153$). The correlation coefficient of sdLDL particle size (Fig. 9a–c) was also significantly higher when compared with non-HDL ($r = -0.372$) as compared with LDL ($r = -0.316$) and apo B/apo A1 ($r = -0.215$) for TG levels between 200 and 400 mg/dL ($n = 30$).

Hence, from the results of our study we conclude that, of the three parameters studied i.e. non-HDL, LDL and apo B/apo A1, non-HDL appeared to be the most useful surrogate marker for sdLDL particle size when the triglyceride values were ≤ 400 mg/dL.

Discussion

This study demonstrated a significant negative correlation of sdLDL particle size with non-HDL which was stronger than the correlation with LDL as well as with apo B/apo A1. It is well known that non-HDL acts as an indirect marker of elevated LDL, and to negate any effect of raised LDL on the sdLDL particle size and non-HDL, we stratified the study group on the basis of LDL levels. A

Fig. 6 a, b Scatter plot for correlation of sdLDL with non-HDL and apo B/apo A1 respectively when subjects were stratified with $LDL \leq 130$

significant negative correlation of sdLDL particle size was observed with non-HDL when LDL levels were < 130 mg/dL. However, no correlation was observed in LDL values > 130 mg/dL which was probably because of the very small sample size. Since there is a distinct possibility of non-HDL increasing in hypertriglyceridemia [8], we also compared the relationship of the sdLDL particle size with LDL and apo B/apoA1 ratio in subjects stratified by TG levels. The correlation of sdLDL particle size was found to be highest with non-HDL for triglyceride values < 200 mg/dL as well as with triglyceride values

Fig. 7 a, b Scatter plot for correlation of sdLDL with non-HDL and apo B/apo A1 respectively when subjects were stratified with $LDL > 130$

between 200 and 400 mg/dL. Hence, the conclusion drawn from this study was that non-HDL is a better surrogate marker of sdLDL than other established markers like LDL and apo B/apo A1 in a healthy Indian population of ≤ 45 years and with triglycerides levels up to 400 mg/dL. However for $LDL > 130$ mg/dL, further studies are required in a larger population as our sample size for this group was very small.

Non-HDL represents the atherogenic potential of all the apolipoprotein B containing lipoproteins which does not

Fig. 8 a–c Scatter plot for correlation of sdLDL with non-HDL, LDL and apo B/apo A1 respectively when subjects were stratified with TG ≤ 200

get reflected by estimating LDL alone and hence, the NCEP adult treatment panel III (ATP III) had addressed the relationship between non-HDL, LDL and atherogenic risk as early as 2001 [9]. Further, ATP III guidelines have designated non-HDL as a secondary target for lipid lowering therapy after achieving target LDL levels and, since non-HDL levels significantly correlate with apo B levels, both have been proposed by various studies as significant markers reflecting the progression of the disease [10–13]. Therefore, as suggested by Robinson, “ultimately, it may

be desirable to move toward non-HDL-C as the primary target of therapy once non-HDL has been routinely incorporated into clinical practice” [13]. There is also considerable evidence that apo B containing lipoproteins promote atherogenesis at the arterial wall and Doi H et al. [14] in their study had observed that VLDL and TG rich VLDL remnant particles lead to endothelial dysfunction. Also, fasting and post-prandial plasma isolated triglyceride rich lipoprotein induce foam cell formation and are significant contributors to the progression of CAD [15, 16]. Further, a

Fig. 9 a–c Scatter plot for correlation of sdLDL with non-HDL, LDL and apo B/apo A1 respectively when subjects were stratified with TG = 200–400 mg/dL

prothrombotic state is triggered by these lipoproteins [17] and another study, the Lipid Research Clinics Program Follow up Study, has shown that the risk of cardiovascular mortality increased more than twofold in subjects without initial cardiovascular disease with the highest non-HDL-C levels [18]. Therefore, assessment of non-HDL in routine laboratory reports was recommended in a commentary by Lavie et al. [19] who felt that the calculation of non-HDL may be more accurate than the one for LDL especially when the triglycerides are moderately elevated. However, currently, not all laboratory reports mention the non-HDL

value along with the lipid profile. Also, its interpretation and goals have not been very popular with the clinicians so far.

Targeting non-HDL has significant implications in clinical practice. A study in 2009 showed that lowering non-HDL would predict reduction of CHD risk in a 1:1 relationship and that non-HDL was twice as good as LDL for risk prediction [20]. In her editorial comment, JG Robinson has observed that “non-HDL should be routinely used as a target of therapy to reduce cardiovascular risk” [21]. Also some studies have proposed that non-fasting

triglycerides are better predictors of cardiovascular risk than fasting levels [22]. However due to lack of standardization, it is not always feasible to quantitate hypertriglyceridemia limits. On the other hand, post-prandial chylomicronemia minimally influences coronary heart disease risk which further increases the advantage of using non-HDL as a target of therapy [23].

The atherogenic nature of sdLDL is of particular significance to us because of the high incidence of premature CAD in Indians. Its small size helps it to penetrate the arterial walls very easily. Also, their longer circulation time increases the probability of atherogenic modifications while their pro-inflammatory properties make them prone to aggregation and formation of complexes, further increasing their atherogenicity [24, 25]. Small dense LDL is associated with a threefold higher risk of myocardial infarction and its predominance forms a major component of an atherogenic lipoprotein phenotype. We had shown in our previous study that sdLDL particle size and cystatin C were significantly and independently associated with the presence of CAD events in young Indian patients with normal kidney function. sdLDL had emerged as a more sensitive predictor of CAD than cystatin C and was also significantly associated with the severity of CAD [2]. Similarly, sdLDL was confirmed as a strong and independent predictor of CHD in the first 7 years of follow-up [26] and Koba et al. [27] in their study linked the progression of CHD to the concentration of sdLDL. Another study by M Ai et al. [28] observed that sdLDL are better markers for CHD risk assessment and a number of other studies have also documented the role of sdLDL in predicting CAD risk as well as its role in the pathogenesis of atherosclerosis [29–31]. Unfortunately, their measurement is tedious and expensive and therefore not easily available in most labs.

Our study showed a significant negative correlation between sdLDL and apo B/apo A1 though this correlation was not as strong as with non-HDL and LDL. This was in accordance with other similar studies which showed that apo B has the potential to predict CAD risk as well as non-HDL since it is a key component of the atherogenic lipoproteins [13, 18]. In fact, they may predict risk better than even LDL in statin treated patients [10]. Another study in 2014 suggested that apo B should be added to the lipid profile of patients at risk of CAD [32]. However, this raises the question of justifying the need to assay yet another lipoprotein parameter when a simple calculation can clearly help optimize risk reduction. So though apo B can be acknowledged as correlating relatively strongly with non-HDL, non-HDL cholesterol has gained wider acceptance due to its easy availability and reliability.

Therefore the current scenario is that, despite LDL being a strong risk factor, it is not always elevated in CAD [33] and estimation of sdLDL, which is more atherogenic than

the larger LDL fractions, is not always feasible in most laboratories. Hence, we hope our study will highlight the usefulness of non-HDL cholesterol as an inexpensive yet reliable lipid marker for not only evaluating the atherogenicity associated with the apo-B containing lipoproteins, but also emphasize its importance as a superior predictor of small dense LDL particle size.

There are certain limitations in our study as it included only a cross section of the population. Also the sample size with LDL more than 130 mg/dL was very small and hence this study needs to be done in a larger population to understand clearly the effect of raised LDL on non-HDL. Detailed information of the effect of age, ethnicity as well as risk factors like hypertension, diets etc. were not included.

Conclusion

Non-HDL is a highly significant surrogate marker for small dense LDL in young Indians. The current status of non-HDL is as a secondary goal for all patients with triglycerides between 200 and 499 mg/dL. Given the easy availability and reliability of non-HDL estimation, it makes greater economic sense to combine all the atherogenic lipoproteins in a single calculation. Hence, the addition of non-HDL in routine lipid profile reports will allow optimum assessment of dyslipidemias as well as premature CAD risk and may even end up being recommended as a primary target in the forthcoming NCEP-ATP guidelines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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