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**RESEARCH ARTICLE**

# Non-high density lipoprotein cholesterol-risk predictor for coronary heart disease in Indian population

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## *Abstract*

**Background**: Although low-density lipoprotein cholesterol (LDL-C) is a well established atherogenic factor for coronary heart disease (CHD), it does not completely represent the risk associated with atherogenic lipoproteins in the presence of high triglyceride levels. Non high density lipoprotein cholesterol (non-HDL-C) is considered to be a measure of apolipoprotein B containing atherogenic lipoprotein and a better predictor for cardiovascular risk. The aim of this study was to evaluate the role of non-HDL-C as an atherosclerotic predictor and its association with the severity of coronary heart disease. **Methods**: The study consisted of 185(males/females)(aged 20-60 years, mean age 42.24$\pm$10.94) patients of CHD and 50 healthy controls. Out of 185 patients 75 were angiographically proven while the remaining 110 cases were diagnosed on the basis of clinical and echocardiographic findings. Based on the results of coronary angiography the 75 patients were further divided into three sub group according to severity of coronary artery disease (single, double and triple vessel disease). Fasting blood samples were assessed for Total cholesterol, HDL-cholesterol and Triglycerides. LDL-C was estimated by direct method and non-HDL-C was calculated. The levels of non-HDL-C in different grades of coronary vessel diseases were correlated and compared. **Results**: The non-HDL-C level in CHD group was significantly higher than in the control group ($p<0.001$). Further the levels of non-HDL cholesterol were lower in single (157.14$\pm$13.70 vs 179.23$\pm$22.52, 210.05$\pm$28.85 mg/dl; [$p<0.001$]) vs double and triple vessel respectively) and thus, directly correlating with severity of CHD. **Conclusion:** Serum non-HDL-C is closely associated with the development and severity of CHD.



## Introduction

Dyslipidemia is one of the major risk factor for development of coronary heart disease (CHD). Low density lipoprotein cholesterol (LDL) is constantly regarded as the key factor for atherosclerosis and a primary target of lipid lowering therapy for cardio vascular diseases[8].

Recently it has been found that non-HDL-C has a distinct advantage over LDL-C in predicting coronary heart disease. Non-HDL-C is equally accurate when measured on a fasting or non fasting lipid panel as neither total cholesterol nor HDL-C change significantly after meals[19]. Moreover, the impact of elevated triglyceride levels in the calculation of LDL-C with the Friedewald formula suggests that non-HDL-C is beneficial in determining the risk of atherosclerosis and CVD in patients with hypertriglyceridemia[24].It was shown that LDL-C was erroneously estimated with approximately 17% and 25% error at serum triglyceride concentration from 151-200 mg/dl and 201-300 mg/dl respectively[10].

Although LDL-C is an important risk factor for CHD, more than 50% of all cardiovascular events occur in patients who have "normal" LDL-C levels[3]. Non-HDL-C has been suggested as a good predictor of initial CAD and is independent of the levels of LDL[17].

Non-HDL-C is calculated as the difference between total cholesterol(TC) and high density lipoprotein cholesterol(HDL-C) and thus, represents cholesterol carried on all proatherogenic apo-B-containing particles[VLDL-C,IDL-C,LDL-C as well as chylomicron remnants and lipoprotein(a)][23].Adult Treatment Panel (ATP III) guidelines of the US National Cholesterol Education Program (NCEP) recommend non-HDL-C as a secondary target of therapy in person with triglycerides at least 200 mg/dl. The target for non-HDL-C is 30mg/dl higher than the target for LDL-C[2].

Both European and American Cardiological Societies emphasize the importance of this parameter for assessing the risk of atherosclerosis and coronary heart disease[14]. However, little attention is being paid to the use of non-HDL-C in India.

Recently the role of non-HDL-C has been highlighted in predicting and reducing CVD risk in patients of dyslipidemia treated pharmacologically[23].Of all the lipid parameters, only non-HDL-C showed a significant association with the process of atherogenesis[21]. The Bogalusa Heart Study showed a possible relationship between the values of non-HDL-C in childhood and risk of cardiovascular diseases in adulthood[9].

In the past a number of studies have investigated the relationship between non-HDL-C and the risk of CHD[11,20,18]. In fact, Jamal S.Rana et al suggested it to be better indicator than LDL-C in predicting cardiovascular events and has been shown to predict CHD similar to apolipoprotein B levels[12]. However, very limited data is available worldwide correlating non-HDL-C with the severity of coronary heart disease, more so in India. In view of this, we have investigated the role and correlation of non-HDL-C with severity of coronary heart disease.

## Methods

From November 2011 to August 2013, 185 coronary heart disease patients (aged 20-60,mean 42.24$\pm$10.94 years), examined and treated at Advanced Cardiac Centre, PGIMER, Chandigarh and ACE Heart and Vascular Institute, Mohali were selected for the present study.50 healthy individuals served as controls. The institutional Ethical Committee approved the study and informed consent was obtained from the patients. The patient's demographic profile, socioeconomic status, personal habits and disease risk factor histories were recorded.

### Inclusion criteria

Coronary heart disease patients (aged 20-60, mean age 42.24$\pm$10.94 years) of either sex with a history of acute chest pain, non ST-segment elevation, unstable and stable angina were included for this study. Coronary angiography was performed by on-site qualified interventional cardiologists. Based on the results of coronary angiography the 75 patients were further divided into three sub group according to severity of coronary artery disease (single, double and triple vessel disease).Healthy individuals free of any symptoms of coronary heart disease were taken as controls.

### Exclusion criteria

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. Tobacco and alcohol abusers were also excluded. The same exclusion criteria were also applied for the selection of controls.

### Analysis of blood lipids

Fasting venous blood samples were collected and analyzed on VITROS 250 analyzer by Ortho Clinical Diagnostics (Johnson & Johnson's) for total cholesterol, triglyceride, HDL-C by dry slide technology (Reflectance spectrophotometry) and LDL-C by using direct assay. Non-HDL-C was calculated. LDL-C was also calculated by Friedewald formula. For serum lipid reference level, National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) guideline was referred. Dyslipidemia was defined by presence of one or more abnormal serum lipid concentration.

### Statistical Analysis

Results were presented as mean ± Standard deviation (SD) and with 95% confidence intervals. The independent't' test was used to compare the means between the study and control group and one way ANOVA was used to analyze the difference within the group(single vessel, double vessel and triple vessel). Pearson correlation coefficient(r) was computed to find the correlation between non-HDL-C with different lipid parameters. Area under receiver operating characteristic curve was calculated to assess the utility and to compare the predictive value of non-HDL-C and LDL-C and further independent't' test was used for the comparison of non-HDL-C, direct LDL and calculated LDL with

varying (<200 and >200 mg/dl) levels of triglyceride. Significance level was considered at $p<0.05$. Statistical analysis was performed using SPSS 16.0 version.

## Results

Among the 235 subjects participating in the study males constituted 68.9% of the total sample population compared with females constituting 31.1%.The age range of the total sample population was 20-60 years (mean age 42.24±10.94). Subjects were divided in two groups, control (n=50) and the CHD group (n=185). Based on the results of coronary angiography the 75 patients were further divided into three subgroups (single, double and triple vessel disease) according to severity of coronary artery disease. 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**).

**Table-1: Demographic characteristics of CHD patients and controls**

| | | Test n (%) | Control n (%) |
|---|---|---|---|
| Cases | | 185 | 50 |
| Age | | 44.66±9.70 | 33.26±10.66 |
| Male | | 136(74) | 26(52) |
| BMI | | 26.15±3.90 | 23.84±4.44 |
| Young | ≤40 | 64(35) | 41(82) |
| Elderly | >40 | 121(65) | 9(18) |
| Urban | | 137(74) | 42(84) |
| Rural | | 48(26) | 8(6) |
| Life Style | Act | 13(7) | 6(12) |
| | Mod | 41(22) | 33(66) |
| | Sed | 131(71) | 11(22) |
| Diet | V | 76(41) | 23(46) |
| | NV | 109(59) | 27(54) |
| HTN | | 111(60) | Nil |
| F/H DM | | 87(47) | 28(56) |
| F/H CHD | | 104(56) | 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 non-HDL-C) levels were measured for all subjects and the results are also summarized in **Table 2**. Mean Non-HDL-C concentrations were 107.23±27.64 and 167.54±46.36 for control and CHD group, respectively. Compared with the control group, the total Cholesterol, LDL-C, Triglycerides and non-HDL-C in the CHD group was significantly increased respectively ($p<0.001$, $p<0.001$, $p<0.001$, $p< 0.001$) and HDL-C in the CHD group was significantly decreased ($p<0.01$).

**Table-2: Blood Lipid assay in CHD patients and controls**

| Risk Parameter | Group | N | Mean | Std. Deviation | 95% Confidence Interval | p |
|---|---|---|---|---|---|---|
| TC | C | 50 | 152.54 | 29.62 | 144.12 - 160.96 | <0.001 |
| | T | 185 | 209.91 | 48.22 | 202.91 - 216.90 | |
| LDL-C | C | 50 | 91.00 | 25.30 | 83.81 - 98.19 | <0.001 |
| | T | 185 | 142.98 | 44.01 | 136.60 - 149.36 | |
| HDL-C | C | 50 | 45.30 | 6.37 | 43.50 - 47.11 | <0.01 |
| | T | 185 | 41.34 | 8.70 | 40.08 - 42.60 | |
| TG | C | 50 | 97.78 | 30.05 | 89.24 - 106.32 | <0.001 |
| | T | 185 | 197.18 | 84.86 | 184.87 - 209.49 | |
| Non-HDL-C | C | 50 | 107.23 | 27.65 | 99.38 - 115.09 | <0.001 |
| | T | 185 | 167.54 | 46.37 | 160.82 - 174.27 | |

All values in mg/dl, p-value<0.001=***, p-value<0.01=**
CHD, coronary heart disease; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; Non-HDL-C, non-high density lipoprotein cholesterol

**Correlation of Non-HDL-C with severity of Coronary heart disease**

Comparison of Non-HDL-C was done with the different levels of severity of coronary heart disease. Increase in the levels of Non-HDL-C was found with increasing severity of disease and this positive correlation was found to be statistically significant (**Figure 1**).

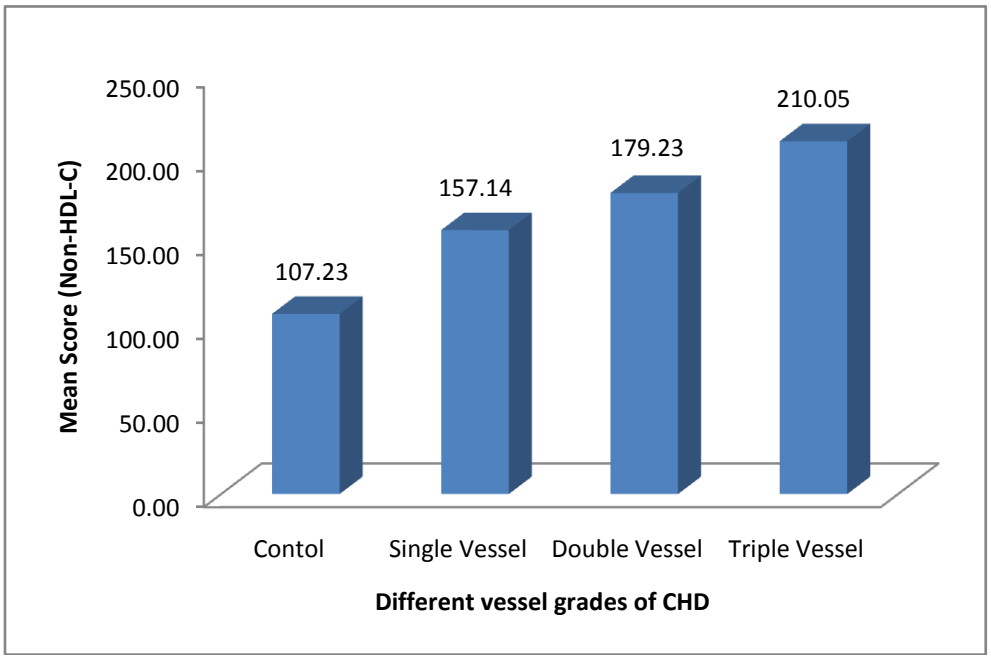


**Figure1. Non-HDL-C in control and angiographically proven CHD patients with different vessel grades**

**Correlation of Non-HDL-C with other lipid parameters**

Non-HDL-C was correlated with other biochemical parameters and statistically significant correlation was found between non-HDL-C and total cholesterol, LDL-C and Triglycerides (**Table 4)**.

**Table-4 Correlation between Non-HDL-C and Lipid Parameters**

| | | TC | LDL | HDL | TG |
|---|---|---|---|---|---|
| Non-HDL-C | Pearson Correlation (r) | 0.965 | 0.955 | 0.139 | 0.350 |
| | p | <0.001 | <0.001 | NS | <0.001 |

p-value <0.001=***, NS=non significant

Non-HDL-C, non-high density lipoprotein cholesterol: TC, total cholesterol: HDL, high density lipoprotein cholesterol: TG, triglyceride

**Comparison of predictive value of Non-HDL-C and LDL-C**

To compare the predictive value of non-HDL-C and LDL-C ROC curve analysis was done. AUROC was >0.8 for both suggesting that both of these parameters can be used to evaluate risk of CHD in Indian population and further if we compare the AUROC between the two then it was more for non-HDL-C (0.872) as compared to LDL-C (0.854). The ROC curve is shown in **Figure 2**.

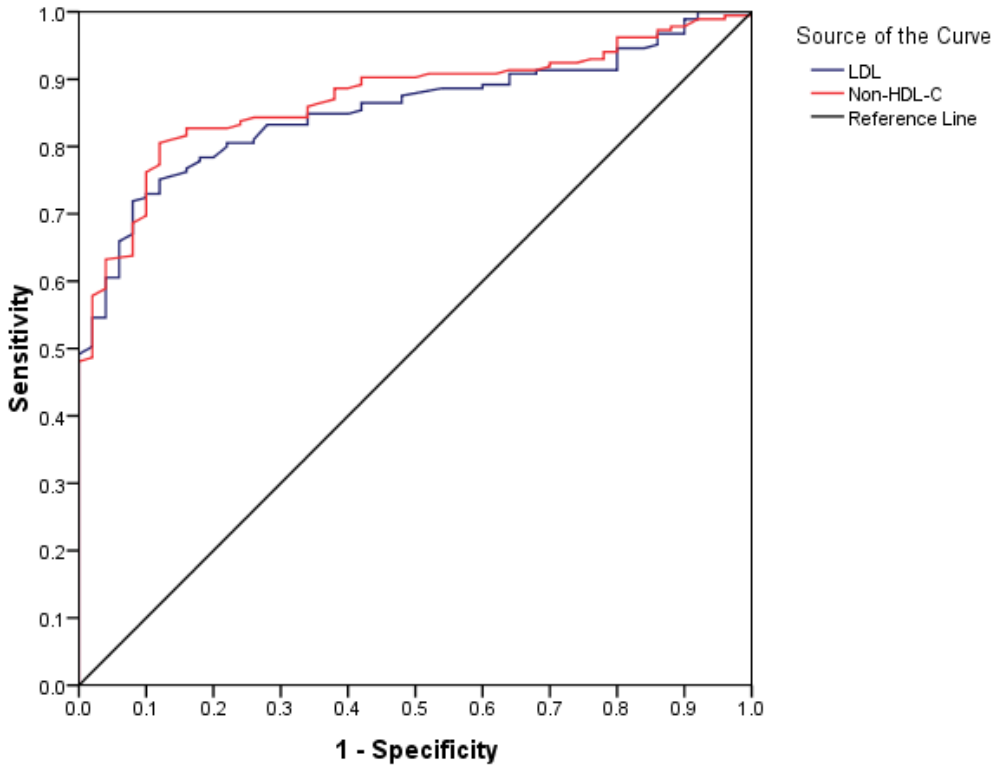


**Figure2. Receiver Operating Characteristic curve for LDL-C and Non-HDL-C in CHD patients. Area under the curve is greater for Non-HDL-C than for LDL-C.**

**Comparison of Non-HDL-C, Direct LDL Cholesterol and Calculated LDL-C in Hypertriglyceridemic group A (Triglyceride<200mg/dl) and group B (Triglyceride>200mg/dl)**

To compare the predictive value of non-HDL-C, direct and calculated LDL-Cholesterol in hypertriglyceridemia, test subjects were divided into 2 groups; group A (Triglyceride<200 mg/dl) and group B (Triglyceride>200)**.** Non-HDL-C showed statistically more significant difference in both the groups as compared to direct LDL while non significant results were found for calculated LDL. (**Table 6**)

**Table 6**: **Comparison of Non-HDL-C, Direct LDL and Calculated LDL in Hypertriglyceridemic group A (Triglyceride<200mg/dl) and group B (Triglyceride>200mg/dl)**

| Parameters | Groups | N | Mean | Std. Deviation | p-value |
|---|---|---|---|---|---|
| Calculated LDL | A | 108 | 128.99 | 44.57 | NS |
| | B | 77 | 129.32 | 41.92 | |
| Direct LDL | A | 108 | 136.87 | 45.06 | <0.05 |
| | B | 77 | 151.55 | 41.25 | |
| Non-HDL-C | A | 108 | 155.61 | 47.33 | <0.001 |
| | B | 77 | 184.28 | 39.56 | |

p-value <0.001=***, p-value<0.05=*, NS; non significant

LDL, low density lipoprotein cholesterol; Non-HDL-C, non-high density lipoprotein cholesterol

## Discussion

Cardiovascular disease is the leading cause of death in both the developed and the developing countries[15].Therefore, the early identification of individuals at increased risk is of pivotal importance in order to modify the factors contributing to this high risk profile.

Elevated LDL-C remains the primary focus for cardiovascular risk assessment till now, but ample supportive research indicates that LDL-C alone is not an optimal marker of cardiovascular risk assessment and its targeted treatment. Furthermore, other triglycerides rich lipoproteins are also atherogenic, including VLDL remnants and intermediate density lipoprotein (IDL). Of late it has been seen that cases who succeeded in meeting their target LDL-C but still developed complications from atherosclerotic vascular disease and suffered cardiovascular events. These patients thereby bear the burden of having residual cardiovascular risk not identified by conventional risk markers. Hence, there is an increased need to have alternate, more comprehensive and accurate measurements of atherogenic particle concentrations beyond LDL-C. The non-HDL-C appears to be superior to LDL-C in its ability to predict cardiovascular events. Importantly, measurement of non-HDL-C incurs no additional diagnostic cost, as it can be calculated by a simple mathematical equation.

There is paucity of information on the importance of non-high density lipoprotein cholesterol (non-HDL-C) and its correlation with severity of coronary heart disease in India.

Few studies have demonstrated the predictive value of non-HDL-C and its superiority over LDL-C in causing cardiovascular diseases[16,13]. Arsenault et al found that non-HDL-C could still predict the CHD risk even at a low LDL-C level[1]. In another study, non-HDL-C was suggested to be a good predictor of initial CAD and independent of the levels of LDL[17]. Sniderman A et al[25] and Rallidis LS et al[22] have indicated that non-HDL-C is a better indicator for cardiovascular risk assessment than LDL-C.

A Meta analysis of relationship between non-HDL-C reduction and CHD risk reported that the non-HDL-C is an important target of therapy for CHD prevention[23].The lipid research clinics (LRC) Program follow up study found that highest quartile of non-HDL-C predicted CHD events and all-cause mortality while that of LDL-Cholesterol failed to do so in either sex[5].

Non-HDL-C has been shown to be a better predictor of marker of risk in both primary and secondary prevention studies. In a recent analysis of data combined from 68 studies, non-HDL-C was found to be the best predictor among all cholesterol measures, both for CAD events and for strokes[7].

Our study confirms many observations cited above that non-HDL-C is better indicator than LDL-C in predicting coronary heart disease. A good correlation of non-HDL-C with total as well as LDL Cholesterol was found in the study. This shows that value of non-HDL-C also reflects the value of total cholesterol and LDL Cholesterol. ROC curve analysis also confirmed the superiority of non-HDL-C over LDL-C. Furthermore non-HDL-C was also found to be a better risk predictor in hypertriglyceridemic patients than LDL.

Our study showed a good correlation of non-HDL-C with the severity of coronary heart disease (CHD) as has been reported by Dazhi Ke et al[6].

In view of its excellent role in predicting the cardiovascular risk we suggest the inclusion of non-HDL-C in future routine lipid profile from all the laboratories in India as has been recommended by American Diabetes Association (ADA), American College of Cardiology (ACC), and National Lipid Association[3,4].

## Limitations

Considering the diagnostic importance both for the clinicians and the patients, larger sample size in the study would have been appropriate for providing more precise information and accuracy of the non-HDL-C as a predictive marker of coronary heart disease. Follow up study for at least 6 months would have been beneficial for assessing the treatment outcomes.

## Conclusions

Thus, we found non-HDL-C to be having not just equivalent but marginally better predictive value than LDL-C for atherogenesis. Being a calculated parameter, it incurs no additional cost and is more comfortable not requiring fasting sample. Further, the role of non-HDL-C is distinguished in few patients where LDL-C levels were not expected to be accurate (triglyceride level>200mg/dl). Non-HDL-C hence should be included in every routine lipid profile panel.

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