# A Retrospective Analysis of Non-HDL Cholesterol: For Improved Cardiovascular Risk Assessment

**Sohail Rafi Khan[1], Ana Mukhtar[2], Altaf Ahmed[2]**


## Abstract

**Objective:** To assess Non-HDL cholesterol as an indicator of Cardiovascular risk (CVR) in addition to low density lipoprotein (LDL) cholesterol.

**Methods:** A retrospective study was conducted in the Department of Clinical Biochemistry, Indus Hospital Karachi from September 2007 to December 2012. After approval for conducting the research obtained from the Institutional Research Board a retrospective analysis of non-HDL cholesterol and LDL Cholesterol in 2115 lipid profiles was conducted, which had been analyzed on fasting serum or EDTA plasma by Randox Rx Imola and Daytona analyzers using Randox kits. Since this was a retrospective study that included all the lipid profiles presented in the lab from Sept 2007 to Dec 2012, so it did not require any sample size calculation.

**Results:** Statistical analysis performed on data was unpaired t-test using SPSS package Version 22. Analysis of 2115 lipid profiles shows that 1389 (66%) had Triglyceride levels above 1.7mmol/l of which 642 (46%) were females and 747 (54%) were males. Regarding males, in 77 out of 747 (10%) samples there was an elevated non-HDL cholesterol (greater than 3.4mmol/l) in the presence of a normal LDL cholesterol (less than 2.6 mmol/l.). In the other 22 out of 747 (2.9%) males, LDL-cholesterol was elevated in the presence of normal non-HDL cholesterol. In the females 66 out of 624 (10%) samples there was an elevated non-HDL cholesterol (greater than 3.4mmol/l) in the presence of a normal LDL-cholesterol (less than 2.6 mmol/l) and LDL-cholesterol was elevated in the presence of normal non-HDL cholesterol in 15 femalesout of 642 (2.3%).

**Conclusion:** Non-HDL cholesterol is also a necessary analysis for the true assessment of cardiac vascular risk in addition to LDL-cholesterol especially in the samples with elevated triglycerides samples. Therefore, it is recommended that non-HDL cholesterol be reported as part of lipid profile.


**Keywords:** Atherosclerosis, LDL-cholesterol, non-HDL cholesterol, lipid profile, cardiovascular risk (CVR).

**IRB:** Approved by the Ethical Review Committee of The Indus Hospital (IRD_IRB_2013_10 _001). Dated 31st October 2013.

(ASH & KMDC 20(2):97;2015).

## Introduction

Clinicians and researchers have used lipid profile to assess cardiovascular risk[1]. The measurement of LDL-cholesterol[1-5] has been the primary focus of attention, both as a diagnostic marker and as a target of therapy[2].

However recent studies have been reported in which the patients who succeeded in meeting their "target" LDL-cholesterol goals still developed complications from atherosclerotic vascular disease[6-8].

So it is clear that a narrow focus on LDL-cholesterol is not an advisable strategy for patient care[9] as there is now increasing evidence that there may be factors in addition to the level of LDL-cholesterol that may serve as better indicators of atherogenesis[4].

[1]Department of Biochemistry,
Karachi Medical and Dental College
[2]Department of Pathology
Indus Hospital, Karachi

**Correspondence: Dr Sohail Rafi Khan**
**Department of Biochemistry**
**Karachi Medical and Dental College**
**E-mail: sohailrafi56@gmail.cm**

In this regard it is important to identify the physicochemical event that precipitates atherosclerosis as being the entry of sterols into the arterial wall and their subsequent internalization by macrophages thereby creating foam cells[10].

There is increasing evidence that it is the Apolipoprotein B containing lipoproteins (β-lipoproteins) that are specifically responsible for the entry of cholesterol into the blood vessels causing atherosclerosis[10].

These sterols are driven into the arterial intima by a concentration gradient, therefore quantifying of all the circulating atherogenic lipoproteins (ß lipoproteins) may provide the best means of assessing cardiovascular risk and therapeutic goals[10,11].

Thus taking into account the role of ß lipoproteins in atherogenesis, there is need to focus on the levels of circulating Non-HDL cholesterol to assess cardiovascular risk inaddition to LDL-cholesterol.

The risk of developing atherosclerosis has been well documented to be associated with increased levels of LDL-cholesterol, however recently there is increasing evidence that Non-HDL cholesterol may be a better indicator of atherosclerotic risk than LDL-cholesterol[12-14]. Non-HDL cholesterol is determined by subtracting HDL-cholesterol from the total serum cholesterol (total cholesterol - HDL-cholesterol), it means that Non-HDL cholesterol is sum of all the ß-lipoproteins.

This may be particularly relevant in subjects with elevated levels of triglycerides and in diabetic patients[12].

This retrospective analysis was carried out with objective of determining the usefulness of reporting of Non-HDL cholesterol in the lipid profile as a more complete means of assessing cardiovascular risk.

**Subjects and Methods**

A retrospective cross sectional analysis of lipid profile data from 2115 patients of both genders reported by Indus Hospital laboratory from September 2007 to December 2012 was done.From these lipid profiles of both genders those with triglycerides above 1.7 mmol/l (n= 1389) were further analysed on the basis of Non-HDL cholesterol.

Since this study was a retrospective analysis of lipid profiles analyzed at Indus Hospital over a 5-year period the sample size was not calculated. Non-HDL cholesterol was estimated by the formula: Total cholesterol - HDL-cholesterol (TC-HDLC). No exclusion criteria were applied to samples and they were all analyzed as follows.

Fasting serum samples taken for lipid profiles were collected in vacutainer tubes (BD, NJ USA) from antecubital veinof adult male and female patients (above 18 years of age) attending OPD clinics at Indus Hospital and allowed to clot for 5 minutes,centrifuged at 3000G for 5 minutes then subsequently analysed on the same day on Randox analysers Rx Imola and Daytona.

The Randox kits were used for the analysis of the following lipid profile parameters i.e. total serum cholesterol (TC) was measured by enzymatic endpoint CHOD-PAP method, total serum triglycerides (TG) was measured by enzymatic Glycerol Phosphate Oxidase / Peroxidase, serum low density cholesterol LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) was measured by direct homogenous assay under strict internal quality control (IQC) procedures. The assay coefficient of variation for these parameters were found to have the following results total cholesterol (1.8%), total triglycerides (3.3%), HDL-cholesterol (2.3%), LDL-cholesterol (2.3%).

Statistical analysis performed was independent t-test using SPSS package Version 22. T-test was applied to the lipid parameters of two groups under study i) High non-HDL cholesterol ii) High LDL cholesterol.

Significant differences ($p<0.05$) were found in the lipid parameters of the two mentioned groups except in the HDL cholesterol level (Table 1).

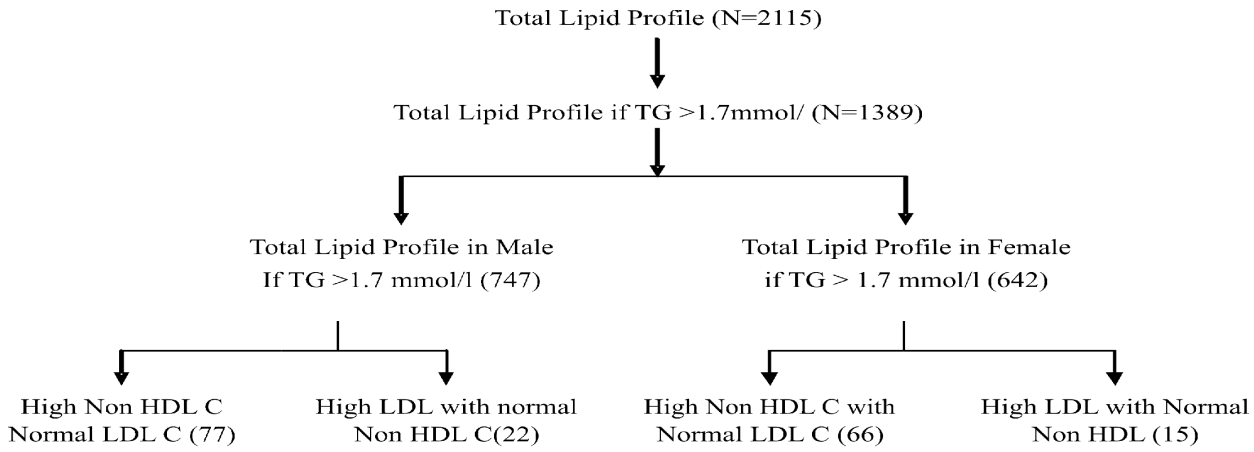


**Fig. 1.** Lipid profile in patients involoved in study

**Table 1.** Analysis of Lipid and Lipoprotein parameters in terms of having elevated non-HDL cholesterol or elevated LDL cholesterol

| Variable | High Non HDL C with normal LDL C (Male:77) (Mean ± SD) | High LDL C with Normal non HDL C (Male:22) (Mean ± SD) | t-test P-value | High Non HDL C with normal LDL C (Female: 66) (Mean ± SD) | High LDL C with Normal non HDL C ( Female :15) (Mean ± SD) | t-test P-value |
|---|---|---|---|---|---|---|
| Non HDL C mmol/l | 3.86 ± 0.61 | 3.15 ± 0.17 | 0.001** | 3.91 ± 0.98 | 2.82 ± 0.61 | 0.001* |
| LDL C mmol/l | 2.19 ± 0.38 | 2.78 ± 0.17 | 0.001** | 2.18 ± 0.33 | 2.82 ± 0.22 | 0.001* |
| Triglyceride mmol/l | 3.62 ± 1.88 | 2.37 ± 0.81 | 0.003** | 3.58 ± 1.98 | 2.25 ± 0.85 | 0.012* |
| HDL mmol/l | 0.82 ± 0.18 | 0.81 ± 0.13 | 0.735(N.S.) | 0.88 ± 0.17 | 1.15 ± 0.50 | 0.001* |
| Triglyceride/HDL Ratio | 4.41 ± 2.8 | 2.92 ± 0.85 | 0.015* | 5.20 ± 2.11 | 1.95 ± 0.79 | 0.019* |

t-test was used to compare the listed parameters from the high Non-HDL cholesterol and high LDLcholesterol groups.

*Significance = $p<0.05$

** Significance = $p<0.001$ ,

N.S. = Not significant

To convert cholesterol values from mmol/l to mg/dl multiply by 38.67

To convert triglyceride valuesfrom mmol/l to mg/dl multiply by 88.67

The important point to note is that the Non-HDL cholesterol becomes significant for both males and females with high triglycerides (Fig.1) when evaluating cardiovascular risk.

## Results

In this retrospective analysis of 2115 lipid profiles it was found that 1389 (66%) had triglyceride levels above 1.7 mmol/l of which 642 (46%) were females and 747 (54%) were males (Fig. 1).

In the males 77 out of 747 (10%) samples there was a elevated non-HDL cholesterol in the presence of a normal LDL cholesterol. While LDL-cholesterol was elevated in the presence of normal non-HDL cholesterol in 22 males out of 747 (3%) (Fig.1).

Similarly in the females 66 out of 642 (10%) samples (were found to be having elevated non-HDL cholesterol in the presence of a normal LDL-cholesterol. While 15 of the 642 females had elevated LDL-cholesterol in the presence of normal non-HDL

cholesterol (2.3%).Further analysis of lipid and lipoprotein parameters in terms of having elevated Non-HDL cholesterol or elevated LDL-cholesterol is given in (Table 1).

**Discussion**

The study emphasizes the need for estimating non-HDL cholesterol when assessing cardiovascular risk. The determination of LDL-cholesterol alone does not give the required assessment and there is a risk of misplacement of cardiovascular risk category particularly when triglyceride levels are raised.

The results show that in the elevated non-HDL cholesterol patients the LDL cholesterol is significantly lower but it should be noted that because the higher TG:HDL ratio in this group the LDL is more atherogenic[15].

Since it is possible that for two individuals to have the same LDL-cholesterol level and be at different levels of cardiovascular risk because of the differences in LDL particle number. The point to appreciate is that LDL-cholesterol indicates the cholesterol within the LDL particle which gives little information about the number of particles therefore may not be a true indicator of cardiovascular risk.

Therefore a more reliable assessment of cardiovascular risk is to estimate the number of atherogenic particles (i.e. Apo B containing) in serum for this reason non-HDL cholesterol has been proposed as a better predictor of cardiovascular disease than LDL-cholesterol.

The analyses of our results by unpaired t-test using SPSS version 22 show (Fig. 1) this is particularly relevant for dyslipidaemic subjects (e.g. triglycerides in excess of 1.7 mmol/l). In this group ourdata shows that despite having normal LDL -cholesterol levels thesesubjects have increased levels of Non-HDL cholesterol (males 77 in 747 and females 66 in 624) (Fig. 1).

The National Cholesterol Education Programme (NCEP) has identified both LDL-cholesterol and non-HDL-cholesterol as the therapeutic targets when assessingcardiovascular risk and insubjects with non-HDL cholesterol as the primary target when triglycerides are elevated[15].

In addition it has been extensively reported that once triglyceride levels exceed 100 mg/dl, the more atherogenics mall dense LDL particles predominate and these particles because of their smaller size are more likely to penetrate the endothelial surface and initiate atherosclerosis[15].

Previously workers have reported increased cardiovascular risk due to raised serum triglycerides and a raised triglyceride:HDL ratio[16-18] the raised TG:HDL ratio is indicative of small dense LDL particles which are suspected of being more atherogenic than normal LDL.

In our analysis of lipid profiles the dyslipidaemic groups both male and female (TG > 1.7mmol/l) had elevated non-HDL cholesterol had significantly lower LDL-cholesterol but it should be remembered that despite having similar LDL-cholesterol levels the LDL particles have more atherogenic potential because they are small and dense because significantly higher TG:HDL ratio.

This perspective is further enforced by the initial results whichshow that the in the raised triglycerides group there is elevation of TG:HDL ratio which is also an indicator of the presence of small dense LDL particles.

Several studies have reported that many patients who achieve their LDL-cholesterol targets still develop complications from atherosclerosis. Consequently we have to investigate alternate measurement for providing us with better diagnostic reliability ranging from non-HDL cholesterol, LDL particle number, to Apo B levels[11,19,20].

The reasons that make non-HDL a useful addition to the existing lipid profile may be summarized as it requires only two analysis Total Cholesterol and HDL cholesterol (non-HDLC (mg/dL) = TC - HDLC), the non-HDL analysis does not require a fasting sample, it does not add to the cost

of lipid profile analysis no additional analysis required, non HDL cholesterol may be a better indicator of CVR because it gives a more complete assessment of the the potentially atherogenic Apoprotein B containing lipoproteins (VLDL, IDL and Lpa) and non-HDL cholesterol estimation is particularly relevant in in patients with elevated triglycerides the estimation of LDL-cholesterol alone does not give the true CVR because a simple estimation of LDL does not give a complete assessment of the apolipoprotein B containing lipoproteins.

Additionally non-HDL cholesterol has been shown to correlate with clinical outcome this has been shown in studies that have investigated the role of lipid profile parameters on coronary artery calcification (CAC) as an early marker of subclinical atherosclerosis. Only Non-cholesterol showed a significant association with the process of atherogenesis[21].

Utility of non-HDL cholesterol in the diagnosis of CVD was confirmed in numerous clinical trials which have compared the diagnostic value of non-HDL cholesterol as a prognostic factor of acute coronary events and myocardial infarction among healthy subjects and diabetics[22].

A strong association has been documented between non-HDL cholesterol and the intima media thickness (IMT) in carotid artery increasing IMT being noted with increasing Coronary heart disease and increasing non-HDL cholesterol concentrations [21,23-25]. We would strongly recommend that non-HDL cholesterol be added to the routine reporting of lipidprofile as it incurs no additional cost and adds greatly to the diagnostic reliability.

One limitation of our study is that it is a retrospective analysis therefore we did not determine the sample size prior to the study this will be addressed in follow up studies which will be prospective and attempt to relate non-HDL cholesterol to arterial changes.

## Conclusion

Our study shows that subjects with raised triglycerides may have normal LDL-cholesterol but elevated non-HDL cholesterol so their true cardiovascular risk may be assessed by taking into account LDL-cholesterol and non-HDL cholesterol. The estimation of non-HDL cholesterol is a simple, cost effective procedure measure and serves as a surrogate marker forapo B containing lipoproteins, providing a more reliable assessment of cardiovascular risk.

## References


1. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150-60.
2. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18,499-502.
3. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogenous assays versus calculation. Clin Chem 2002;48:236-54.
4. Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis.; Circulation 2004;109,112-7.
5. Holvoet P, Mertens A,Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronar artery disease. Arterioscler Thromb Vasc Biol 2001;21:844-8.
6. Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. Clin Chem 1995;41:1414-20.
7. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227-39.
8. McQueen MJ, Hawken S, Wang X, Qunpuu S, Sniderman A, Probstfield J, et al. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. Lancet 2008;372:224-33.

9. Menown B, Murtagh G, Maher V, Cooney MT, Graham IM, Tomkin G. Dyslipidaemia therapy update: the importance of full lipid profile assessment. Adv Ther 2009;26:711-8.

10. Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 2007;116:1832-44.

11. Master SR, Rader DJ. Beyond LDL cholesterol in assessing cardiovascular risk apo B or LDL-P? Clin Chem 2013;59:723-5.

12. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

13. van Deventer HE, Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, et al. Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. Clin Chem 2011;57:490-501.

14. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, et al. Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the strong heart study. Diabetes Care 2003;26:16-23.

15. Boizel R, Benhamou PY, Lardy B, Laporte F, Foulon T, Halimi S. Ratio of Triglycerides to HDL cholesterol is an indicator of LDL particle size in patients with type2 diabetes and normal HDL cholesterol levels. Diabetes Care 2000;23:1679-85.

16. Blaha MJ, Blumenthal RS, Brinton EA, Jacobson TA. National Lipid Association Taskforce on Non-HDL Cholesterol. The importance of non-HDL cholesterol reporting in lipid management. J Clin Lipidol 2008;2:267-73.

17. Miller M, Stone NJ, Ballantyne C, Bittner V, Crigui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292-333.

18. Bittner V, Johnson BD, Zineh I, Rogers WJ, Vido D, Marroguin OC, et al. The triglyceride/high-density lipoprotein cholesterol ratio predicts all-cause mortality in women with suspected myocardial ischemia: a report from the Women's Ischemia Syndrome Evaluation (WISE). Am Heart J 2009;157,548-55.

19. Rana JS, Boekholdt SM, Kastelein JJ and Shah PK. The role of non-HDL cholesterol in risk stratification for coronary artery disease. Curr Atheroscler Rep 2012;14:130-4.

20. Sonmez A, Nikolic D, Dogru T, Ercin CN, Genc H, Cesur M. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J Clin Lipidol 2015;9:576-82.

21. Orakzai SH, Nasir K, Blaha M, Blumenthal RS, Raggi P. Non-HDL cholesterol is strongly associated with coronary artery calcification in asymptomatic individuals. Atherosclerosis 2009;202:289-95.

22. Liu J, Sempos C, Donahue RP, Dorn J, Trevisan M, Grundy SM. Joint distribution of non-HDL and LDL cholesterol and coronary heartdisease risk prediction among individuals with and without diabetes. Diabetes Care 2005;28:1916-21.

23. Kawamoto R, Oka Y, Tomita H, Kodama A. Non-HDL cholesterol as a predictor of carotid atherosclerosis in theelderly. J Atheroscler Thromb 2005;12:143-8.

24. Aoki T, Yagi H, Sumino H, Tsunekawa K, Araki O, Kimura T. Makoto N, et al. Relationship small dense low-density lipoprotein cholesterol concentrations measured by homogenous assay in Japanese subjects. Clinica Chimica Acta 2015;442:110-4.

25. Yang C, Sun Z, Li Y, Ai J, Sun Q, Tian Y. The correlation between serum lipid profile with carotid intima-media thickness and plaque. BMC card 2014;14:181.