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

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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 1.7 mmol/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 3.4 mmol/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 3.4 mmol/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 females out 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).

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Introduction

Clinicians and researchers have used lipid profile to assess cardiovascular risk¹. The measurement of LDL-cholesterol¹⁻⁵ has been the primary focus of attention, both as a diagnostic marker and as a target of therapy². 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 disease⁶⁻⁸.

So it is clear that a narrow focus on LDL-cholesterol is not an advisable strategy for patient care⁹ 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 atherogenesis⁴.

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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\textsuperscript{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\textsuperscript{10}.

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

Thus taking into account the role of β lipoproteins in atherogenesis, there is need to focus on the levels of circulating Non-HDL cholesterol to assess cardiovascular risk in addition 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\textsuperscript{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 β-lipoproteins.

This may be particularly relevant in subjects with elevated levels of triglycerides and in diabetic patients\textsuperscript{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 vein of 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).
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.

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 our data shows that despite having normal LDL-cholesterol levels these subjects 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 assessing cardiovascular risk and in subjects with non-HDL cholesterol as the primary target when triglycerides are elevated\(^\text{15}\).

In addition it has been extensively reported that once triglyceride levels exceed 100 mg/dl, the more atherogenic dense LDL particles predominate and these particles because of their smaller size are more likely to penetrate the endothelial surface and initiate atherosclerosis\(^\text{15}\).

Previously workers have reported increased cardiovascular risk due to raised serum triglycerides and a raised triglyceride:HDL ratio\(^\text{16-18}\) 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 which show that 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\(^\text{11,19,20}\).

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 potentially atherogenic Apoprotein B containing lipoproteins (VLDL, IDL and Lpa) and non-HDL cholesterol estimation is particularly relevant 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 atherogenesis21.

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 diabetics22.

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 lipid profile 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 for apo B containing lipoproteins, providing a more reliable assessment of cardiovascular risk.

References


