Serum Triglycerides and Non-High Density Cholesterol: Important Indicators for Cardiovascular Risk Assessment

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Abstract

This cross sectional study was carried out from September 2007 to December 2012 at the Department of Clinical Biochemistry, The Indus Hospital, Karachi. Analysis of 2115 lipid profiles showed that 1389 (66%) patients had triglyceride levels above 1.7 mmol/l. Of these, 10.3% of the samples showed high non-HDL-C with normal LDL-C while 2.6% showed high LDL-C with normal non-HDL-C. Non-HDL cholesterol is an important lipid profile parameter that could serve as an additional screening tool along with LDL-C for complete cardiac vascular risk profile assessment particularly in high risk patients. This together with TG/HDL ratio, an indicator of small dense LDL, could help clinicians in rigorous patient management. Therefore, it is recommended that non-HDL cholesterol be added as part of lipid profile parameter and TG/HDL ratio be recognized as a possible important indicator of cardiovascular risk.

Key words: Atherosclerosis, LDL-Cholesterol, Triglycerides, Non-HDL cholesterol, TG/HDL ratio Lipid profile, cardiovascular risk (CVR).

I. INTRODUCTION

Cardiovascular disease (CVD) is a leading global cause of mortality with World Health Organization (WHO) reporting 17.5 million deaths in 2012. In Pakistan, CVD poses a major burden of morbidity and mortality, with mortality rates ranging from 30-40% annually.

Most often, CVD is manifested in the form of coronary heart disease (CHD), myocardial infarction and stroke. A number of host factors have been associated with risk of CVD including hypertension, smoking, high blood cholesterol, diabetes etc. Of these, high blood cholesterol level is considered as one of the major determinants to assess the risk of CVD. According to the National Health Survey of Pakistan (NHSP), approximately 20% of Pakistani population suffers from high cholesterol levels indicating the CVD risk burden in Pakistan.

Worldwide, clinicians utilize lipid profile testing to assess cardiovascular risk in patients. A standard fasting lipid profile includes measurement of Total serum cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Total serum Triglycerides. Of these, LDL is widely recognized as a diagnostic and therapeutic marker against CVD. Briefly, LDL is a lipoprotein that transfers cholesterol, phospholipids and triglycerides from different parts of the body into the cells. Increasing concentrations of LDL particles are therefore strongly associated with atherosclerosis within the walls of arteries over time leading to sudden plaque ruptures and clot release. This results in narrowing or closing of artery opening leading to cardiovascular disease, stroke and other vascular complications. In addition to LDL, a number of apolipoprotein B (apoB) rich lipoprotein i.e very-low-density lipoprotein (VLDL) and Intermediate-density lipoproteins (IDL) collectively known as non-HDL-C, are also considered atherogenic. Briefly, non-HDL-C (unlike LDL-C) represents the cholesterol content present in all the atherogenic lipoproteins.
In recent years, a number of studies have commented on the utility and limitations of LDL levels as diagnostic CVD marker. The major limitation documented was observation of atherosclerotic complications in patients even after they had reached their targets of acceptable LDL-C goals.\(^5\)\(^,\)\(^7\)\(^,\)\(^8\) This indicates that atherogenic agents other than LDL-C may have a significant role in posing as a residual risk factor. Therefore, assessments of residual factors i.e measurement of alternate atherogenic particle concentration beyond LDL have been recommended.\(^9\)\(^,\)\(^10\) Clinically, this would mean measurement of non-HDL Cholesterol levels as this would give information on all cholesterol present in potentially atherogenic lipoprotein particles (VLDL, intermediate density lipoprotein and lipoprotein).

Serum Triglyceride have long been a neglected part of the lipid profile panel of tests although the several studies have been reported on the association of serum triglyceride levels and coronary heart disease.\(^28\)\(^,\)\(^29\)\(^,\)\(^30\) The association between serum triglycerides and coronary heart disease is emphasized by Non-HDL cholesterol and HDL Cholesterol.\(^10\)\(^,\)\(^12\)\(^-\)\(^13\) Thus, increased value of TG/HDL ratio is indicative of increased presence of small, dense LDL particles.

A number of studies have documented that Non HDL-C can serve as an important predictor of initial coronary heart disease (CHD), recurrent episodes of angina pectoris and non fatal myocardial infarction (MI). Therefore, determining non-HDL-C with LDL-C would provide a better understanding of patient profile/risk as it would provide a complete assessment of all atherogenic agents.\(^11\)\(^,\)\(^12\) Though recommended, Non-HDL-C is as yet, not a part of standard lipid profile test and is therefore not reported to clinicians. If added, it would allow clinicians to manage patients accordingly. The main advantage of including non-HDL-C as a CVD screening tool is that it does not require additional testing and can be measured simply by subtracting Total Cholesterol with HDL-C thus giving the sum of all atherogenic β-lipoproteins.

One of the objectives of the study was to investigate the association of serum Triglycerides with Non-HDL cholesterol in the Pakistani population and its linkage to the presence of small, dense LDL as indicated by the increased TG/HDL ratio which is suggestive of increased cardiovascular risk. In Pakistan, there is paucity of data on this aspect.\(^13\)\(^,\)\(^14\) Therefore, to fulfill this knowledge gap, a retrospective, observational study was designed to assess non-HDL-C levels in patients focusing on its association with serum Triglycerides and small dense LDL. The main objective of this study is to provide evidence on the utility of non-HDL C testing and to assess its significance as a complete means of cardiovascular risk with reference to serum triglycerides.

### II. MATERIALS AND METHODS

#### Study Setting:

The study was conducted by the Department of clinical Biochemistry at The Indus Hospital, a 150 bedded multi-disciplinary tertiary care hospital. Medical records from September 2007 to December 2012 were analyzed for the study.

#### Study Design and Sampling

A retrospective, cross sectional study was designed to determine the non-HDL-C level of patients in whom lipid profile was conducted. Medical record of 2,115 patients, irrespective of gender and age were included in the study. Non-HDL-C was calculated and statistically analyzed in all patients reported to have high triglyceride levels (1.7mmol/l). Non-HDL-C was estimated by subtracting Total Cholesterol with HDL-C (Total Cholesterol- HDL Cholesterol). No exclusion criteria were applied for the analysis. The ratio TG/HDL ratio was calculated by dividing the Triglyceride (mg/dl) by the HDL-cholesterol (mg/dl).

Briefly, diagnostic lipid profile testing was performed by collecting serum samples from patients attending The Indus Hospital OPD. The samples was allowed to clot for 5 mins, centrifuged at 3000g for 10 mins and analyzed on Randox analyzers (Rx Imola and Daytona) as per manufacturer’s instruction. The following lipid profile parameters were tested on these analyzers i.e. Total serum Cholesterol (TC) measured by enzymatic endpoint CHOD-PAP method, Total serum Triglycerides (TG) measured by enzymatic Glycerol Phosphate Oxidase / Peroxidase , serum Low Density Lipoproteins cholesterol (LDL-C) and HDL-cholesterol (HDL-C) measured by direct homogenous assay. The assay...
coefficient of variation TC, TG, HDL-C and LDL-C was 1.8 %, 3.3 %, 2.3 %, 2.3% respectively.

The study was approved by the Institutional Review Board of The Indus Hospital (IRB_IRB_2013_001).

**Statistical analysis**

Data was entered and analyzed using SPPS (v.22). Comparison of lipid parameters of groups having high non-HDL-C and high LDL-C was analyzed using unpaired t-test. Statistical significance was defined as p<0.05.

### III. RESULTS

A total of 2115 total lipid profile results were retrospectively retrieved through medical record system of The Indus Hospital. Results showed that out of 2115, 66% (1389) of the patients had high non-HDL-C and high LDL-C was analyzed using unpaired t-test. Statistical significance was defined as p<0.05.

The data of 1389 patients was further stratified into two groups i.e. Group A: elevated non HDL-C with normal LDL-C and Group B: elevated LDL-C with normal non HDL-C. Triglyceride levels are compared between the two groups and it was observed that difference between the two groups was statistically significant with a p value of <0.0001 (Table. 1). Analysis revealed that high non-HDL-C was observed in 10.2% (143/1389) of the samples while elevated LDL revealed that high non-HDL-C was analyzed using unpaired t-test. Statistical significance was defined as p<0.05.

**Table 01. Triglyceride levels and Non-HDL-C**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Triglyceride (mmol/l) Mean ± SD</th>
<th>P-value</th>
<th>HDL (mmol/l) Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-HDL-C</td>
<td>3.62 ± 1.88</td>
<td>0.003&lt;</td>
<td>0.82 ± 0.18</td>
<td>0.33&lt;</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.37 ± 0.81</td>
<td>0.735&lt;</td>
<td>0.81 ± 0.13</td>
<td>0.98&lt;</td>
</tr>
<tr>
<td>Non-HDL-C (Female)</td>
<td>3.58 ± 1.98</td>
<td>0.012&lt;</td>
<td>0.88 ± 0.17</td>
<td>0.019&lt;</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.25 ± 0.85</td>
<td>0.000&lt;</td>
<td>1.15 ± 0.50</td>
<td>0.000&lt;</td>
</tr>
<tr>
<td>Non-HDL-C (Male)</td>
<td>4.41 ± 2.8</td>
<td>0.012&lt;</td>
<td>2.92 ± 0.85</td>
<td>0.33&lt;</td>
</tr>
<tr>
<td>LDL-C</td>
<td>5.2 ± 2.11</td>
<td>0.019&lt;</td>
<td>1.95 ± 0.79</td>
<td>0.000&lt;</td>
</tr>
</tbody>
</table>

*Significance = P < 0.05

Total serum Triglycerides and the TG/HDL ratio were significantly higher in the high Non–HDL groups for both males and females.

### IV. DISCUSSION:

The significance of serum Triglycerides and non-HDL-C as a parameters to assess CVD risk in Pakistani population was evaluated in this study. It is evident from the results that when Triglycerides are raised non-HDL-C levels with normal LDL-C were significantly higher (p value <0.0001). These results are consistent with previously published literature from various countries indicating that non-HDL-C is an important lipid profile parameter and needs to be determined in patients for risk assessment.15-17

It has been documented in studies that LDL-C may not be sufficient for risk profiling of patients as elevated LDL-C levels indicates cholesterol within the LDL particle.18-20 However, the variation in size, density, and composition of the LDL-C particle governs its properties. The use of gradient gel electrophoresis has demonstrated the existence of two distinct LDL-C phenotypes. The larger, less dense particles are known as pattern A and the smaller, denser particles are known as pattern B, this being dominant when both Triglyceride levels are increased and the Triglyceride /HDL ratio is raised . Studies have shown that these small, dense LDL-C particles are capable of efficient transport into the arteries due to their size and thus pose a higher risk of CHD.21 This is indicated by the increased ratio of TG/HDL that is found in the raised Non-HDL cholesterol groups in both males and females.

Therefore, patients with same or lower LDL-C can be at a variable risk profile depending on the type of predominant LDL particles in serum. In contrast, non-HDL-C gives the total amount of all atherogenic particles present in patient serum. Therefore, reporting of both non-HDL-C in conjunction with LDL-C would help clinicians...
in determining the complete CVD risk profile leading to better patient management.

The results of this study are especially important with respect to dyslipidemic patients i.e. those showing triglyceride levels above 150mg/dl. It was observed that in such patients, the LDL-C was found to be normal while the non-HDL-C was elevated. This finding is further supported by published literature which reports that excess triglyceride in serum (>100mg/dl) can cause the smaller atherogenic particles (IDL, VLDL, Lp (a)) to predominate and penetrate endothelial surface more efficiently than LDL (due to their small size), thus initiating atherosclerosis.22-24 Thus, the data from our study supports the utility of determining non-HDL-C in dyslipidemic patients. Management of such high risk patients would be significantly influenced if only LDL-C is considered as a marker of cardiovascular risk and prone to misinterpretation.

Clinical studies investigating the role of lipid profile parameters including non-HDL-C, as early markers of atherosclerosis and coronary artery calcification (CAC) have reported significant association of non-HDL-C with atherogenesis process.25-28 Furthermore, strong association has also been documented between non-HDL-C and the intima media thickness (IMT) in carotid artery. Increasing IMT with coronary heart disease was documented in patients with elevated non-HDL-C concentrations.29, 30

The results from our study provide baseline data and evidence on the significance of non-HDL-C assessment from Pakistan. The strength of our study is the large sample size used to draw the conclusions. There are several limitations of present study we faced which are: First, This study is the retrospective design which does not allow clinical assessment of patients. Second, it is a single centre study, multi centre studies could be the representative for all tertiary care hospitals to smaller hospitals. Third, our study was conducted irrespective to the age as compared to previous studies, elderly age subjects are prone to the risk of cardiovascular diseases. Fourth, we had no idea regarding the proportion of non-fasting lipid profiles as it is a retrospective study. However, a fasting blood sample is not necessary for a reliable measurement of total cholesterol and HDL-cholesterol. Fifth, we have no information about participant in our study regarding the usage of lipid profile. Sixth; we had no record of participants who had any current or previous history of cardiovascular disease. Such differences could affect the results; therefore, it is recommended that larger prospective studies along with clinical data from various areas of Pakistan would serve to support the plausibility of non-HDL-C as a lipid profile parameter.

In summary, we would like to emphasize on utilizing non-HDL-C as a CVD risk-screening tool. Firstly, it is easy to calculate i.e. simple subtraction of TG with HDL-C. Therefore, it does not add to the cost of lipid profile. Secondly, it is a better indicator of CVD as it gives a thorough assessment of all the potentially atherogenic Apoprotein B containing lipoproteins (VLDL, IDL and Lpa). This is particularly relevant in patients with elevated triglycerides as the estimation of LDL-C alone does not provide information on other atherogenic particles that may have more efficient mechanism of initiating atherosclerosis than LDL-C. In summary, based on the available data, the use of a simple non-HDL –C calculation in a lipid profile testing complemented by determination of the new risk factors, will allow a better assessment of the CVD risk.

V. Conclusion:

The study has highlighted the importance of measurement of Triglycerides and non-HDL-C as a prognostic factor for coronary heart disease additionally the ratio of TG/HDL should also be monitored. The estimation of Non-HDL cholesterol is a simple, cost effective procedure measure and serves as a surrogate marker for apoB containing lipoproteins therefore providing a more reliable assessment of cardiovascular risk. This assessment may be especially important for the Pakistani and South Asian population.

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