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To Compare the Total Cholesterol and HDL-Cholesterol before and after Ultra-Centrifugation in Lipemic Samples

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Abstract

Objective: It is an everyday routine in laboratories to encounter interference in the analysis of lipids. These likely interferences (hemolysis, icterus and lipemia) are countered by asking physicians and patients to send fresh and properly collected samples. At the Aga Khan University (AKU) Laboratory, we receive 2-3 lipemic/turbid samples per day. Previously it was our departmental policy to advise these patients to go for lipoprotein electrophoresis, which though accurate was time consuming and not cost effective. We therefore studied ultra centrifugation/airfuge as an alternate method to clear lipid interference and provide accurate, reliable and cost effective results.

Methods: Daily 2-3 grossly turbid samples are identified on the lipid bench, 48 samples were received in 4 months (February to May 2004). These samples were analyzed for total cholesterol (TC) and High Density Lipoprotein -Cholesterol (HDL-C) before and after ultra centrifugation/airfuge.

Results: There was a positive correlation between the lipemia and the false high TC and HDL-C. The mean TC and HDL-C before ultra centrifugation were 263.06 mg/dl and 39.42 mg/dl respectively and after centrifugation these became 191.77 mg/dl and 33.06 mg/dl. P value showed a significant difference in both results.

Conclusion: This study suggests that the removal of turbidity by ultra centrifugation/airfuge is cost effective, less time consuming and provides accurate reliable results of TC and HDL-C in patients with lipemia interference (JPMA 55:239;2005).

Introduction

Lipids are hydrophobic (nonpolar), organic molecules insoluble in water, but soluble in organic solvents.¹ Because of their insolubility body lipids are generally found either in compartments, as in the case of membrane-associated lipids and droplets of triglycerols in adipocytes or transported throughout the body in association with protein as lipoprotein particles.² These particles include chylomicrons, very low density lipoproteins (LDL) and high-density lipoproteins (HDL) and act as a vehicle for transporting lipids in aqueous milieu of the circulatory system.¹

Lipid profile is frequently asked by the physicians, and includes total cholesterol (TC), high-density lipoprotein (LDL), low-density lipoprotein (LDL) and triglycerides (TG). The primary purpose of lipid profile testing is for coronary heart disease risk assessment and management of atherosclerotic disease.¹

A strong inverse relationship exists between High density lipoprotein Cholesterol (HDL-C) concentration with coronary heart disease (CHD) risk.^{3,4} The Framingham heart study indicates that modifying cholesterol intake and other risk factors actually reduces the incidence of cardiovascular diseases; these reports stress the need of knowing the accurate levels of cholesterol.⁵

HDL-C is considered as the single major factor for atherosclerosis and CHD risk assessment.⁶ HDL-C estimations show a significantly decreased level in obese cases as

compared to the non-obese controls.⁷

Hypertriglyceridemia with low levels of HDL are common abnormalities in diabetics.⁷ Triglyceride concentrations may reflect the accumulation of atherogenic lipoprotein in plasma,⁸ particularly when they cluster with increased LDL-C⁹ and a decreased HDL-C.¹⁰⁻¹² LDL-C is also used to guide the selection of treatment of CHD.¹³

According to National Cholesterol Education Program (NCEP) guidelines, more than 41% of American adults were estimated to have abnormally high screening values for cholesterol and to require further evaluation.^{14,15} TC/HDL ratio has been suggested to be the most important predictor of premature development of CHD and it should be included in any coronary risk screening profile. TC/HDL ratio of more than 4.5 generally requires intervention.^{6,16,17}

These recommendations place increased demands on total cholesterol, serum triglyceride and HDL-C measurements to be as accurate and reproducible as possible. In elderly patients, one of the Italian study suggests that homocysteine LP (a) and TC/HDL cholesterol should also be included for scoring the risk of CVD.¹³ Different substances may interfere in the analysis of analytes (hemolytic, bilirubin, and lipemia).¹ Lipemia interferes in the TC and HDL-C analysis and gives high false positive results.¹⁸ Previously it was our departmental policy to advise these patients to go for lipoprotein electrophoresis, which though accurate was

policy to advise these patients to go for lipoprotein electrophoresis, which though accurate was time consuming and not cost effective. We therefore studied ultra centrifugation/airfuge as an alternate method to clear lipid interference and provide accurate, reliable and cost effective results, more useful for the physicians' diagnosis and treatment of high-risk patients.

Material and Methods

This descriptive study was carried out in section of Chemical Pathology at Aga Khan University Hospital. The study included 48 lipemia samples received in the laboratory during a period of 4 months (February - May 2004) for the analysis of lipid profile.

All the samples for the present study were collected after 10-12 hours fasting. Venous blood (4-5ml) was collected in an evacuated B.D. vacutainer tube with gel and clot activator. Grossly lipemic samples were identified in the samples received for the analysis of lipid profile. Routinely the nonlipemic samples were centrifuged at 3000 rpm for 3 minutes to separate the serum and then analyzed on Hitachi 912, which is a fully automated, discrete, computerized analyzer which performs potentiometric and photometric assays. It is composed of two hardware units: a) analytic and b) control unit. The characteristic features are a) automated maintenance functions including pipes, b) 360 test/hour throughput c) automatic calibration performance followed by quality control.¹⁹ Methodology was Homogenous enzymatic calorimetric, using the reagents, calibrator and standards of ROCHE

For this study, we separated the serum after centrifugation (3000 rpm for 3-5 minutes) into two tubes, one tube was retained for direct analysis without ultra centrifugation and the second tube was sent to processing bench for ultra centrifugation by using BECKMAN airfuge. The table top airfuge is a miniature air turbine, which is an exceptionally safe instrument used to clarify lipemic serum. Its rotors are designed to meet specific needs and are made of anodized aluminum held in a place by pressure differential, created during centrifugation. This fixed angle rotors holds up six microlitre size tubes. Its maximum speed is 95000 rpm at 207 kPa air pressure. These rotors are ideal for the efficient sedimentation of small sample volume in short time. We spun our lipemic serum for 10 minute at a speed of 90,000 rpm.²⁰

Both, total cholesterol and HDL-C on these samples (neat and airfuged) was analyzed simultaneously on Hitachi 912 using the reagents, calibrator and standards of ROCHE. In addition our laboratory met the regular national and international quality control requirement for the analysis of total cholesterol and HDL-C.

Statistical Analysis

Mean and standard deviation were used to analyze the data by SPSS version 11.5. To assess the significant difference a p-value of 0.01 was taken as significant. The paired "t" test was applied to calculate significant difference between with and without ultracentrifugation.

Results

A total of 48 lipemic/turbid samples were received for analysis of lipid profile in the present study over a period of four months (February-May 2004). All 48 samples were analyzed for TC and HDL-C before and after the ultra centrifugation. Before the ultra centrifugation TC ranged from 160 mg/dl to 486 mg/dl with a mean of 263.06±87.17 mg/dl and HDL-C ranged from 15 mg/dl to 63 mg/dl with a mean of 39.42±10.45 mg/dl and the p-value was 0.0022. While after the ultra centrifugation TC ranged from 92 mg/dl to 467 mg /dl with a mean of 191.77±65.45 mg/dl and HDL-C ranged from 13 mg/dl to 61 mg/dl with a mean 33.06±9.38 mg/dl (Table).

Table. Comparison of TC and HDL-C before and after ultra centrifugation in Lipemic/turbid samples.

TC and HDL-C were significantly different before and after the ultra-centrifugation of lipemic samples.

	Before ultracentrifugation	After ultracentrifugation	P value
Total Samples	48	48	0.01
HDL-C	39.42±10.45	33.06±9.38	0.0022
Total Cholesterol	263.06±87.17	191.77±65.45	0.000017

Mean of TC and HDL-C before and after the ultra centrifugation/airfuge with the P- Value is less than 0.01.

Discussion

Difficulty in obtaining accurate laboratory measurement of plasma lipid values is not a new concern.²¹ Many substances interfere in the analysis of different lipids. Interference by endogenous and exogenous substances with assays for clinical analytes is a common problem in 'laboratory medicine'. The effect of a substance present in the sample that alters the correct value of the result is usually expressed as concentration or activity, for an analyte. There are four major endogenous compounds that consistently interfere with laboratory result: hemoglobin, bilirubin, lipids and para proteins.¹

Many a times clinicians call the laboratory with concerns about inconsistent results. One of the common detection system for these inconsistent results is to compare

the patient's current result with a previous one i.e. Delta check.^{22,23} A second approach is to analyze the sample with different methods. If the result for the two methods is different, this suggests that interference is present. If the results are similar for the two methods either no interference is present or the interference by the interferent is essentially the same for the two methods. A third approach is to add serially high concentration of the putative interferent to aliquots of the same material.^{22,23}

Routinely we experience that the sample received for lipid profile analysis are after 10-12 hours fasting, but some of them appear milky due to lipemia even after this period. Lipemia is the result of circulating chylomicrons. A creamy layer floating at the top signals the presence of chylomicrons. While elevated levels of LDL-C do not cause turbidity, they may cause an orange discoloration of the sample due to carotenoids bound to LDL particles.¹

Chylomicrons are basically large lipoproteins complexes that are made by the gut and serve an important function in the transport of fats, mainly dietary TG. These are water soluble and are mixtures of triglycerides (82%) protein (2% as apoprotein), cholesterol (9% mainly as ester) and phospholipids (7%). Due to the presence of this large lipid-protein in more than desirable extent, the plasma becomes lactescent (turbid).²⁴

Chylomicrons cause interference by turbidity or light scattering and volume displacement.²² As a pathologist, it is our duty to find out new methods and update our analysis to provide on time, accurate, and reliable results. We have to search for easier and less time consuming methodologies to analyze the TC and HDL-C. One way to determine the LDL-C is to calculate LDL-C from TC and HDL-C by the help of Friedewald's equation.

There are different ways to reduce the interference of chylomicrons, one way is, to get the sample after 10-12 hours fasting because no chylomicron are generally found in the blood of healthy person after 10-12 hours fasting.²⁴ Another way is to ask for a lipoprotein electrophoresis for these lipemic serums because in electrophoresis a prominent band is seen at the origin.¹ Previously gel electrophoresis was recommended for these patients. Unfortunately the gel electrophoresis method is time consuming, expensive and limited requests of lipoprotein electrophoresis are made by the physicians.

Recent advances suggest that an easy and cost effective method is ultra centrifugation. After the ultra centrifugation process the chylomicrons comes in supernatant, which can be easily separated, from the sample.¹ Unfortunately we do not have local data on the role of ultra centrifugation on lipemia.

Search for reliable biochemical marker for CHD has been difficult because of complexity of the disease process. CHD is a multifactorial disease where lipoprotein represent one of the primary risk factors.³ Accurate decision regarding classification of CHD risk and treatment of above normal LDL-C concentration depends upon reliable TC measurements.⁴ Total cholesterol, HDL-C, LDL-C and TG are recommended in the initial evaluation for classification of patients into highly desirable, desirable, borderline, high and very high risk for CHD.¹³

Castelli et al have developed a relative risk score for CHD based on the total cholesterol/high density lipoprotein (TC/HDL-C) ratio and ratio more than 4.5 generally required intervention.^{17,21} A low serum level of HDL-C appears to be an important risk factor of CHD, particularly in a population whose serum level of total cholesterol is high.²⁵

This study shows that before ultra centrifugation, the result of HDL and TC was higher as compared to after ultra centrifugation. This signifies that lipemia interferes with the analysis of TC and HDL-C.

It is recommended that all milky (lipemia/turbid) samples should be ultra centrifuged and the report issued with comments like "After ultra centrifugation due to gross turbidity/lipemia." This increases the reliability and accuracy of the results and avoids lipoprotein electrophoresis.

The study concluded that ultracentrifugation is a cost-effective, reliable and less time consuming technique to remove chylomicrosis.

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