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Mini-Review

Lipid Profile: A Review of its Basics

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Abstract

Lipid profile (LP) is one of the most commonly performed laboratory investigation globally whose proper performance and interpretation has significant ramifications on the health of an individual. This mini-review looks at the lipid profile test inside out – its importance, role of non-HDL cholesterol, candidates for testing, sample collection technique, preanalytical and analytical errors and limits of normality. Clinicians ordering the test must keep these intricacies in mind while interpreting the results of the investigation and modifying treatment.

Keywords: Lipid profile, cholesterol, non-HDL cholesterol, Friedewald's equation

Introduction

Lipid profile is one of the most commonly done biochemical tests in day-to-day clinical practice. It represents a combination of tests ordered for the assessment of disorders of lipid metabolism and associated complications, particularly atherosclerotic cardiovascular disease (ASCVD). The burden of ischemic heart disease and cerebrovascular diseases is immense, and they contribute approximately to 84.5% of cardiovascular deaths and 24.2% of death from all causes.[1] These figures will continue to increase owing to a sedentary lifestyle and fat-rich food consumption. An LP entails total cholesterol and its fractions viz. low-density lipoprotein (LDL), high-density lipoproteins (HDL) and very low-density lipoprotein (VLDL), and triglycerides (TG).

Cholesterol (3-hydroxy-5,6-cholestene) is a sterol compound containing a cyclopentanoperhydro-phenanthrene (CPP) skeleton which serves different physiological functions, e.g., synthesis of fat-soluble vitamins, bile acids and hormones. However, in altered concentrations, they have pathological implications. LDL has a positive correlation with the risk of ASCVD as seen in the landmark Framingham Heart Study, so is the case with VLDL and TG. On the other hand, HDL has a protective action and prevent ASCVD.[2]

Non-High Density Lipoprotein Cholesterol

Non–HDL cholesterol is simply defined as the difference between total and HDL cholesterol and constitutes all the potentially proatherogenic apolipoprotein B (apoB)-containing particles [primarily VLDL, intermediate-density lipoprotein (IDL), and LDL as well as chylomicron remnants and lipoprotein(a)].[3] Its measurement has an advantage over direct LDL measurement as a more comprehensive ASCVD risk marker as the latter excludes the cholesterol of TGRLs, which are proatherogenic.

Thus, for patients with diabetes having mixed dyslipidemia, calculated LDL cholesterol might not be an adequate index of overall lipid-associated risk with non-HDL providing additional information over LDL cholesterol alone. Owing to its

simple calculation, the non–HDL cholesterol level is easily available to the physician with every lipid profile report without any additional cost. Since it excludes the measurement of TG, it avoids the possible downside of TG as a marker of CHD risk and directly reflects the cholesterol content of all particles that may be proatherogenic. Furthermore, its derivation does not need an LP to be done in the fasting state, and it avoids the possible imprecision related with the intra-individual variability of triglyceride measurements. In the Strong Heart Study, a long-term follow-up of patients with diabetic dyslipidemia, non-HDL cholesterol was a stronger predictor of cardiovascular disease than other lipid parameters, e.g., total/HDL cholesterol used earlier.[3,4]

When Should we Order a Lipid Profile Test?

Notwithstanding the subtle differences in different clinical practice guidelines, the fundamental objectives remain the same for LP testing, i.e., to prevent ASCVD in healthy subjects (primary prevention) and to provide early detection and retard the progression of disease in those with established ASCVD (secondary prevention). It becomes necessary in a patient with a family history of premature death due to myocardial infarction in first degree relatives (<55 years in male and <65 years in female), history of familial hypercholesterolemia or patient with clinical stigmata of familial hypercholesterolemia (xanthoma/xanthomata or eyelid xanthelasma), patients with comorbidities that are likely to be benefitted from statin use, i.e., patients with diabetes, chronic kidney disease, HIV, etc. and risk stratification for patients without the disease, who might benefit from hypolipidemic drug treatment.[5]

Collection of Samples

1 mL of venous blood is collected from the antecubital vein and in a gel-barrier transport, green-top (heparin) or lavender-top ethylenediaminetetraacetic acid (EDTA) vial. Sample should be preserved at ambient room temperature and serum (gel barrier tubes) or plasma (heparin/EDTA vial) obtained after centrifugation is allowed to separate within 45 minutes. The universal recommendation is that patient should remain fasting for more than 8 hours (preferably 12 hours of complete dietary restriction with exception to water and medicines) but a non-fasting sample would suffice for total cholesterol and HDL-c estimation, diagnosis of metabolic syndrome and CV risk estimation in patients being considered for primary prevention. The reason being the utility of LDL-c estimation is limited apart from the management of familial hyperlipidemia. Most of the risk estimators meant for cardiovascular diseases, e.g., Reynolds risk score, Framingham risk score, ASCVD pooled cohort risk estimator, etc., take into account only total cholesterol and HDL-c among all risk factors and these components have negligible variation whether the patient is fasting or in fed-state. Fasting sample becomes mandatory in cases of suspected genetic causes of hyperlipoproteinemia, premature ASCVD, hypertriglyceridemia and in those at risk of pancreatitis. The rationale behind fasting is to avoid a post-meal surge of triglycerides, and reference values that we measure were worked out on fasting blood samples. Moreover, non-HDL cholesterol (total cholesterol − HDL cholesterol), a secondary target of lipid-lowering therapy can also be estimated in the non-fasting sample.[6]

Pre-Analytical Errors

In addition to food intake, certain other factors also affect the LP estimation that needs consideration prior to interpretation of the test results. Applying a tourniquet too long (2–5 mins) prior to sample collection can falsely elevate cholesterol levels by 5-15%. It is observed that there is the effect of seasonal variation on lipid profile, e.g., in winter the cholesterol is found to be little increased and the same happens for triglycerides in summer. When one assumes supine from an upright posture there is a pooling of blood and the resultant dilutional effect cab bring down triglycerides by 15% and cholesterol by 10%. Nephrotic syndrome can cause hypercholesterolemia including total, LDL and VLDL fractions and the same happens in hypothyroidism also. In any acute infection or inflammatory states, there is an alteration of the usual lipid profile with the triglycerides going high and cholesterol coming down. The changes usually become apparent within the first 24-48 hours after MI, become most obvious in 4-7 days, and then come to the baseline after some months when we can re-estimate the lipid profile.[7]

Upper Limits of Normal for Components of Lipid Profile (Tables 1-3). (Targets for Low-Density Lipoprotein and non-High-Density Lipoprotein may not Apply to Those with Diabetes or for Secondary Prevention)

Table 1. Testing during non-fasting conditions[8]

Components	Upper limit of normal (mg/dL)	Upper limit of normal (mmol/L)
Triglycerides	175	2
Total cholesterol	190	5
LDL cholesterol	115	3
Remnant cholesterol (IDL+VLDL)	35	0.9
Non-HDL cholesterol	150	3.9
HDL cholesterol	40	1

Table 2. Testing during fasting conditions [when triglyceride in non-fasting state is more than 5mmol/L (440 mg/dL)][8]

Components	Upper limit of normal (mmol/L)	Upper limit of normal (mg/dL)
Triglycerides	1.7	150
Remnant cholesterol (IDL+VLDL)	0.8	30
Non-HDL cholesterol	3.8	145
Total cholesterol	Same as fasting	Same as fasting
LDL cholesterol		
HDL cholesterol		

Table 3. Categorization of increased cholesterol values in dyslipidemia adults and children according to the National Cholesterol Education Program (NCEP) guidelines[9]

Categories	LDL cholesterol values (mg/dL)	
	Adults	Children and adolescent
Acceptable	<129	<110
Borderline high	130-159	110-129
High	≥160	≥130

Laboratory Methods

Total cholesterol is measured by the cholesterol oxidase phenol 4-amino antipyridine method (CHOD-PAP) where cholesterol esters are hydrolyzed and then oxidized to produce a red hue. After incubation of the resultant at 37° C for 10 minutes, absorbance from the quinoneimine dye is measured at 505 nm. Other estimation methods are the Lieberman Burchat method and the Sulkowski method. Triglycerides are measured with glycerol-3-phosphate oxidase-phenol-4-amino pyrophosphate (GPO-PAP) method. It is hydrolyzed and oxidized to produce a red colour; the absorbance of which is measured similarly as

before. LDL, VLDL and chylomicrons are precipitated by the addition of polyethylene glycol (PEG) 6000. After centrifugation HDL remains in the supernatant and is measured with the CHOD-PAP method as described earlier. The LDL cholesterol estimation can be done in two ways; indirect and direct. The indirect approach is based on the Friedewald cholesterol estimation formula.[5] The formula is as follows-

LDL-c (mg/dL) = TC (mg/dL) – HDL-c (mg/dL) – TG (mg/dL)/5

The direct approach utilizes homogeneous enzymatic direct assays. In the initial chemical reaction, LDL-c is separated by incubation with different sets of reagents (containing ascorbic acid, oxidase, peroxidase, buffer, detergent, etc. to dissolve non-LDL lipid fractions). Then hydrogen peroxide is formed by the action of cholesterol esterase and oxidase in the reagent which then reacts with 4-aminoantipyrin to emit a blue complex. The absorbance of this complex gave the LDL-c concentration in the sample. In few situations, such as when triglyceride exceeds 400 mg/dL, the direct methods are employed to validate the results obtained from Friedewald equation. These methods are more reliable as they comply with the standard requirements recommended by the NCEP for LDL-c assays. Its precision and accuracy (CV and bias <4%) hold for an even fed state.[10]

The apoprotein components B and A-1 represents the pro and anti-atherogenic lipids in the body. Therefore, the ratio of apoB: ApoA1 is a strong indicator and predictor of atherosclerotic cardio vascular disease particularly in patients with type 2 diabetes. In addition, it is a stronger predictor than any cholesterol ratio for that matter viz total cholesterol: HDL-c ratio, LDL-c: HDL-c or non-HDL-c: HDL-c. The cut-off value for apoB: apoA1 for prediction of coronary artery disease 0.72-0.8.[11]

Conditions Where Friedewald Equation is Less Reliable

Friedewald's formula is precluded for LDL-C derivation when the individual is not fasting. A fasting sample is needed for Friedewald's formula in its essence abides by the law that the triglyceride to cholesterol ratio in VLDL is fixed. In non-fasting samples (containing chylomicrons and chylomicron remnants), this ratio is deranged. Hence, VLDL-C would be overestimated, and LDL-C will be falsely low. Chylomicrons are particles enriched in triglycerides and can interfere with the VLDL calculation from triglyceride levels without considering the significant contribution of chylomicrons towards a total pool of triglycerides. Chylomicrons could also be found in the genetic syndromes of hyperlipidemia, e.g., type I (familial hyperchylomicronemia), type III (familial dysbetalipoproteinaemia), and type V familial hyperlipidemia (combined hypertriglyceridemia. In these conditions, there would be different isoforms of VLDL which could not be easily calculated from TG levels and put in the formula. It is better to go for elementary procedures, viz., ultra-centrifugal isolation and electrophoretic separation of components.

Friedewald *et al.* observed when patients with serum TG is beyond 400 mg/dL were not excluded, the correlation between direct and indirectly estimated cholesterol measurement weakened.[12-14]

Conclusion

Lipid profile is an important laboratory test with significant ramifications. The clinician should be adept in optimum utilization of this test when indicated keeping in mind the associated pros and cons to help in the maximal reduction of ASCVD risk.

Declaration of conflicting interests

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