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Minimizing pre analytical variables for estimation of lipids profile level: An update

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Abstract
Requisition for estimation of lipid profile comes as a routine biochemical parameter. The required specimen for analysis is a fasting blood sample. The fasting condition of an individual for clinical analysis of sample is defined as time atleast after 8 hour after last food intake. Many attempts have been carried to simplify the lipid analysis by conducting it on non fasting sample. Apart from fasting, certain precautions to be taken care with respect to posture of the patient, diet, smoking, alcohol and drug intake. This update addresses the issue with these pre analytical variables and the way to reduce the affect of these confounders.

Keywords: lipids, biochemical, precautions, apart

Introduction
The biochemical analysis either for diagnosis or screening has become very important nowadays due to increased dependency and reliability of the physicians. So the overall performance of the analysis that include pre analytical, analytical and post analytical phase should be errorproof. The technical, analytical and post analytical part of the analysis is becoming more sound so various pre analytical variables that can affect the analysis should be taken care of. Many of the pre analytical variables are well known and standardized guidelines are available to overcome the undesired effects, but some variables/confounders are still present and no standard guidelines have been mentioned to overcome or avoid their influence on analysis.

Requisitions for estimation of Lipid profile in the clinical laboratory comes as a routine parameter. Lipid analysis is mainly done for screening of cardiovascular risk in patients as low density lipoprotein cholesterol (LDL-C) has a positive correlation with atherosclerotic cardiovascular disease (ASCVD) and High density lipoprotein cholesterol (HDL-C) has a negative correlation with ASCVD [1]. The lipid profile analysis includes measurement of Triacylglycerol (TAG), Total Cholesterol (TC), High density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c). There are many pre analytical factors that affect the serum or plasma level of lipid parameters. Among Preanalytical part, preparation of the patient is very important, therefore apart from laboratory personnels clinicians should also be aware of the proper instructions to be given to the patients. Preparation of the patients include the instruction about fasting, diet, smoking and alcohol ingestion, drug intake, sleep, stress and exercise etc therefore these factors sould be considered in clinical practice [2]. Here the possible detailed discussion of the variables are provided.

Fasting
The fasting condition of an individual for clinical analysis of sample is defined at as time after 8 hour after last food intake [3] and thus for larger part of the day most individuals are supposed to be in nonfasting (less than 8 hour of fast) condition apart from few hours at the start of the day. Most of the Guidelines as NCEP [4], AACE, 2017 [5] and European guidelines [6] all recommend a fasting lipid profile and the reference value mentioned by them is for a 12 hour fasting sample. Fasting is defined as no dietary intake for that specified time apart from water and medication. In normal daily life situation this schedule is not followed usually. Therefore the serum lipid levels obtained after 12 hour fasting will not be able to reflect the routine basic lipid levels and thus the risk of atherosclerosis associated [7]. Among Routine lipid profile parameters TAG is the most influenced by the fasting or non fasting condition. This fasting affects the TAG level as it remains high for many hours after food intake [8].

Benefits of fasting: the most acceptable logic behind the established standards for fasting lipid analysis is the elevated TAG level observed after a fat tolerance test [9] but this level of discrepancy had not been seen after habitual diet in majority [10]. though fast foods contain high level of fats and their intake may simulate the discrepancy of fat tolerance test so it is advisable to avoid the this kind of diet on the particular day of analysis.

LDL-C is calculated by the Friedewald equation (LDL-C= TC-HDL-[TAG/5]) and this calculation is valid for fasting TAG values. Therefore, if non fasting TAG value is used for calculation of LDL-c then it will be underestimated. And the treatment goal of the most of the guidelines are focused on LDL-c level. LDL-c can be estimated directly in these cases but again there are few lilmitations. As direct estimation of LDL-c in non fasting blood samples also leads to false low values sometimes [11, 12]. Further it has been reported that there is lack of association of direct LDL-c measurement in non fasting sample with cardiovascular disease in women [11]. Additionally, these direct estimations /assays add to health care cost. False low value of LDL-c will lead to underestimation of risk assessment for cardiovascular diseases according to NCEP-ATPIII guidelines and patients and individuals may miss the particular intervention needed.

Non fasting benefits: But it has been recommended by AACC that HDL-C, non HDL-C and TC can be analyzed in non fasting blood samples [5]. As the difference in the

fasting levels and nonfasting levels of these parameters is not much. The benefits of non fasting lipid analysis over fasting lipids level is quite evident. The whole process of blood collection becomes very simple for everyone starting from patients to laboratories. The clinicians are also likely to be benefitted from increased patient compliance as patients are spared from one extra visit at specific time.

Further AACE, 2017 has recommended direct LDL-c measurement in case of certain high risk such as fasting TAG >250mg/dl, diabetic patients and cases with vascular diseases. Further they also recommended that Lipids, including TAG, can be measured in the non-fasting state if fasting determinations are impractical [6]. Several studies have reported that the most interesting part is that non-fasting TAG levels are a better risk predictor for ASCVD than the fasting TAG levels [13, 14]. The Canadian Cardiovascular Society's dyslipidemia guidelines 2016, recommends non-fasting lipid and lipoprotein testing which can be performed in adults in whom screening is indicated as part of a comprehensive risk assessment to reduce CVD events. Further they suggest that for individuals with a history of triglyceride levels >4.5 mmol/L, lipid and lipoprotein levels to be measured in fasting sample.

To conclude fasting lipid profile estimation is more reliable and recommendable. Though the recommendation for non fasting lipid analysis would simplify the blood collection and increase compliance of patient for screening or follow up but there are many limitations.

The term non-fasting is not very clear as it can be anytime from a previous meal and TAG increase in blood will be different at different time interval so non fasting TAG level in blood will depend on time after meal. TAG level in blood increases in a specific pattern where maximum blood level will be attained at around 4-5 hours after meal [13]. No definitive guidelines for cutoff value for a non fasting lipid level has yet been defined. Further, non fasting sample with lipemia will interfere with measurement of other parameters apart from lipid profile. It is important to compare serum lipid profile in fasting and at different time interval after a representative meal in terms of prediction of cardiovascular risk.

Diet

NCEP recommends a normal diet / unrestricted diet for at least two weeks prior to investigations. Change of a normal diet with high protein diet leads to change in levels of urea, cholesterol, phosphate, ammonia and uric acid within four days of change. Large protein meal at lunch or in evening also increases serum TC and growth hormone. A high fat diet increases the serum concentration of TAG. TAG level also decreases with decreased intake of sucrose. High starch diet – Reduces cholesterol levels [15]. Divided small meals rather than a single heavy meal has a beneficial role in reduction of TC.

Intake of monounsaturated fat instead of saturated fat leads to decrease in TC and LDL-c. Vegetarians have lower levels of LDL-c, VLDL-c and HDL-C by 37%, 12% and 12% than non- vegetarians. So in strict vegetarians change in diet or even intake of egg will lead to change in parameters.

Smoking and Alcohol

The effect of smoking on different parameters is related to the number of cigarettes smoked and duration of smoking. Apart from its chronic effects, smoking also has acute effects on various analytes that starts immediately. For example glucose concentration may be increased by 10mg/dl within 10 minutes of smoking a cigarette and plasma insulin secretion is delayed in response to glucose and rise in insulin level has been noted one hour after a cigarette is smoked. Plasma cortisol concentration increases by as much as 40% within 5 minutes of the start of smoking. These hormones can lead to alteration in lipid profile also. Smoking causes an increase in level of TC, TAG, and LDL-c (3%, 9.1% and 1.7%) and decrease in HDL. smoking of 1-5 cigarette leads to change in the level of fatty acid, free glycerol, aldosterone, cortisol and epinephrine [16]. No standard guideline has been mentioned regarding smoking before lipid analysis, but to be on safe side acute effects of smoking can be minimized by avoiding sample collection within one hour of smoking.

Laboratory analysis of many parameters may give false results even after a single moderate amount of alcohol intake. When moderate amount of alcohol are ingested for one week, there is an increase in TAG concentration by 20mg/ dl or more. Alcohol intake has chronic effects on the HDL level by affecting the cholesterol ester transfer protein (CETP) level of plasma. Prolonged moderate intake of ethanol leads to reduction in CETP and thus increase in HDL-c.

Stress

Any surgery, trauma, acute illness leads to acute phase response where due to release of various inflammatory proteins there is alteration in lipid levels. Blood lipid levels alter following attack of myocardial infarction and these aterations in blood levels may be for weeks so it advisable to the screening for lipid profile in these cases within 24 hour of myocardial infarction [17, 18]. In a study by Nawaz *et al*. This has been reported that in patients admitted with acute illness all lipid parameters vary significantly with the lipid parameter after discharge from the hospital but in these cases ratio of TC:HDL remains relatively stable [19].

Medications or Drugs

Many medications that are used for other indications can lead to abnormality in lipid levels due to altered lipoprotein metabolism [20]. Medications have both positive and negative effect on plasma or serum lipid levels either directly on indirectly through effect on weight or glucose metabolism. These factors should be considered when evaluating a person with high levels of TAG, TC, LDL-c and reduced level of HDL-c [21]. Other scenario is what are the possible ways that can help to avoid the effect of drugs on lipid analysis in circumstances where drug is unavoidable. Antihypertensive, anticonvulsants, hormonals, antipsychotics, immunosuppressive agents etc are the common medications that are unavoidable and known for altered effect on lipid profile parameters.

Effect of these drugs can be either due to class effect or some drugs belonging to the same class but have opposite effect on lipid levels e.g. beta blockers [22]. The effect of beta blockers depend on specific drug and dose. Carvedilol and labetolol have no effect on lipid level wheras atenolol, metoprolol, betaxolol, nadolol, propanol and timlol have pronounced effect on lipid level and they cause increased TAG (upto 40%) and decreased HDL-c (5-20%) [23]. This can be a matter of consideration for discontinuation or dose reduction or replacement of the drug prior to screening for

lipid profile.

Further the knowledge of half life of the drug can be very helpful. Drugs can be administered after withdrawal of the sample so that its effect will be minimal. For example loop diuretics have been reported to increase the levels of TAG and LDL-c but this effect is acute and expected only for the duration of action of furosemide that is for 6-8 hours [24].

The dose of the drug may be an adjustable factor, for example thiazide diuretics in a dose of 12.5 to 25 mg/day has not been found to affect the lipid level [25], whereas a dose of more than 50mg/day negatively affect the lipoprotein level and causing an increase in LDL-c by 5-10% and TAG by 5-15% [26]. Antidiabetic drugs- patients with monotherapy with metformin has been shown insignificant but favorable effect on lipid profile [27] and combination therapy of metformin and glibeclamide produced a significant effect on total cholesterol and LDL-c [28] but a non-significant favorable effect on serum triglyceride [27]. Therefore patients on metformin monotherapy can be screened without any precaution.

Apart from this, ingestion of recreational drug also causes alteration in lipid profile but the results reported are varied and conflicting. Some studies has reported in a study on patients who were candidates for coronary artery bypass grafting, the levels of LDL and TAG were significantly higher in opium addicts than non-addicts [29]. In another study, it has been shown that the serum levels of TAG, TC, and LDL in opium addicted individuals were significantly higher [30] or heroin addicts had lower TC and TAG, and higher VLDL than the non-addicts [31]. But reports contradicting these findings are also present. In a study by Masoomi *et al, no* significant relations have been observed between opium addiction and TAG, TC, LDL, and HDL levels [32, 33].

Many medicines may affect the results of this test. A brief review of drugs and its effect on lipid profile have been presented in table1. Be sure to take the history about the drug intake of any kind, including nonprescription medicine, herbs and natural substance.

Table 1

Drugs	LDL-c	HDL-c	TAG
Loop diuretics	↑5-10%	---	↑5-10%
Thiazide diuretics (high dose)	↑5-10%		↑5-15%
Beta blockers*	----	↓5-20%	↑10-40%
Cyclosporine and Tacrolimus	↑0-50%	↑0-90%	↑0-70%

*Some beta blockers not all

Posture

A change from an upright to a supine position due to dilutional effect can reduce the cholesterol levels by 10% and triglycerides by 12% [16].

Prolonged tourniquet application

Applying tourniquet for long (2–5 min) can increase cholesterol from 5 to 15% [18].

Seasonal effect

Cholesterol is slightly higher in winter than in summer and the opposite is true for triglycerides [34].

Diseased condition

The disease conditions like nephrotic syndrome increase total cholesterol, LDL cholesterol and VLDL cholesterol [35] and hypothyroidism increases LDL cholesterol and total cholesterol. Infection and inflammation may decrease total cholesterol and HDL cholesterol and increase triglycerides [36].

Conclusion

It is therefore, important that all these factors should be kept in mind while interpreting the lipid profile. Interpretation of the results has a high impact on the decision making in selection of the therapeutic approach to the patients. So some possible suggestion on these unnoticed variables will minimize the influence on these variables and the clinical laboratories will be able to contribute the responsible and reliable part in the healthcare system.

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