Comparison between Fasting and Non-Fasting Sample for the Determination of Serum Lipid Profile

Shivangi Ghildiyal[1], Ashish Prakash Anjankar[2], Prakash Keshaorao Kute[3]

[1]Department of Biochemistry, Jawaharlal Nehru Medical College, Sawangi, Meghe, Wardha, Maharashtra, India.
[2]Department of Biochemistry, Jawaharlal Nehru Medical College, Sawangi, Meghe, Wardha, Maharashtra, India.
[3]Department of Biochemistry, Jawaharlal Nehru Medical College, Sawangi, Meghe, Wardha, Maharashtra, India.

ABSTRACT

BACKGROUND

For determination of serum lipid profile, traditionally we use fasting blood sample. Though it has been the most reliable method for testing lipid profile, it has some drawbacks. Fasting is not easy for some people specially children, diabetics and also it is a barrier for population screening. So, intent of our study is to check the authenticity of results obtained using non-fasting samples by correlating it with the results obtained using fasting samples. We wanted to estimate & compare fasting and non-fasting lipid profile values in severe diabetic patients and in apparently healthy controls.

METHODS

This analytical cross sectional study included 40 apparently healthy controls and 40 diabetic patients as participants confirmed by history and biochemical tests. Blood sample was collected from each patient two times; once after 10-12 hours fasting and other as a random sample. Lipid profile parameters were estimated using standard tests. Statistical analysis was done by using Pearson's correlation coefficient. Data analysis was carried by Statistical Package SPSS and Microsoft Excel and $p<0.05$ was considered as level of significance.

RESULTS

In apparently healthy controls differences between fasting and non-fasting concentrations were small and clinically insignificant for lipid profile parameters like total cholesterol ($p=0.861$), LDL-cholesterol ($p=0.203$) and HDL-cholesterol ($p=0.916$). The difference was statistically significant ($p=0.001$) for triglycerides.

CONCLUSIONS

Fasting samples are preferable for serum lipid profile measurement in all individuals with serum triglyceride levels greater than 350 mg/dL. But, non-fasting samples for lipid profile can be used for cardiovascular risk determination in the general people as it reduces patient's inconvenience and promotes patient acquiescence towards lipid profile checking.

KEY WORDS

Fasting, Non-Fasting, Lipid Profile, Cardiovascular Disease

Corresponding Author:
Dr. Ashish Prakash Anjankar,
Department of Biochemistry,
Jawaharlal Nehru Medical College,
Sawangi, Meghe, Wardha-442001,
Maharashtra, India.
E-mail: ashish.anjankar@gmail.com

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BACKGROUND

We routinely perform serum lipid profile test for cardiovascular disease (CVD) risk prediction.[1] Lipid profile test is routinely done in fasting blood specimen. It includes four basic parameters: total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Fasting means overnight 12-14 hours dietary constraint with the exclusion of water and medicines. Fasting sample is favoured for lipid profile due to two important reasoning: (a) post prandial (after a meal) triglycerides remain inflated for several hours,[2] (b) most reference values for serum lipids are fixed on fasting blood specimen. National Cholesterol Education Program (NCEP)[3] and European guidelines[4] also endorse measuring lipid profile in fasting blood specimen for appraisal of CVD risk. Interestingly, while assessing lipid profile for CVD risk appraisal, superiority of fasting specimen over non-fasting specimen has not proven by ancillary evidence. An online report in Archives of Internal Medicine (November 12) found that there is only marginal difference in lipid levels when measured in a fasting or non-fasting state.[5] NCEP guidelines also granted total cholesterol (TC) and HDL-C in non-fasting specimen as these lipid parameters are nearly similar in fasting and non-fasting state.[6,7] Adult treatment panel III recommended non- HDL-C (TC—HDL-C) may also be measured in the non-fasting state. Eberly et al. (2003),[8] Bansal et al. (2007),[9] and Nordestgaard et al. (2007)[10] have advised that levels of non-fasting triglycerides may better or similarly anticipate CVD events than levels of fasting triglycerides.

Nevertheless, in routine clinical practice the lipid profile is generally measured after 12-14 hours of fasting. Though it has been the most reliable method for testing lipid profile, it has some drawbacks. Fasting is not easy for some people especially children and diabetics. Lipid profile in fasting state acts as a barrier for population screening. Also for patients, physicians and testing laboratories, it would be more convenient and efficient to measure lipid levels in a non-fasting setting.[11-15] So, unless there's a really good reason for fasting samples to be tested, non-fasting sampling is much more convenient for all, from a practical perspective. So, intent of our study is to check the authenticity of results obtained using non-fasting samples by correlating it with the results obtained using fasting samples.

We wanted to estimate & compare fasting and non-fasting lipid profile values in severe diabetic patients and in apparently healthy controls.

METHODS

It is an analytical cross-sectional study carried out in Department of Biochemistry, of a tertiary care hospital, among 80 patients of diabetes mellitus (de-novo, follow cases of diabetes mellitus, patients with complications of diabetes mellitus). The sample size was determined using Krejcie and Morgan methodology of calculation of sample size-

$$N = \frac{X^{2*}N^{*}P^{*}(1-\mathrm{P})}{(\mathrm{M}E^{2*}(N-1)) + (X^{2*}P^{*}(1-P))}$$

Where:
N= sample size
X^2= Chi-square for the specified confidence level at 1 degree of freedom
N = Population Size
P = Population Proportion (.50 in this table)
ME = Desired Margin of Error (expressed as a proportion)

It included 40 apparently healthy controls and 40 diabetic patients (cases) who were confirmed by history and biochemical tests. All diabetic subjects had chronic HbA1c greater than 8.0, a clinical diagnosis of mild or moderate NPDR, and no other retinal disease. The control subjects did not have diabetes or other retinal disease. Written consent was gathered from each subject. Institutional Ethical Committee (IEC) has affirmed this study protocol.

Inclusion Criteria
Diagnosed cases of type 2 diabetes mellitus of all age groups and of either sex.

Exclusion Criteria
- Hypo-functioning of thyroid gland.
- Chronic renal failure, Nephrotic syndrome.
- Patients on corticosteroids or oral contraceptives.
- Patients on lipid lowering drugs.

Blood sample was collected from each patient two times: once after 12- l4 hours fasting and other as a random sample. All the blood samples were collected into plain bulbs. Lipid profile includes measurements of TC, TG, LDL-C and HDL-C. Analysis of lipid profile was done on Randox Biochemistry Fully automated autoanalyser.

No.	Parameter	Method
1.	Total Cholesterol	Enzymatic method - Cholesterol esterase, cholesterol oxidase and peroxidase
2.	HDL-C	Direct Enzymatic method
3.	Triglycerides	Enzymatic method- Liquid stable Glycerol phosphate oxidase and peroxidase; End point
4.	VLDL-C and LDL-C	Indirect method- Friedwald Equation [16] VLDL-C = TG/5 LDL-C = TC - HDL-C – (TG/5).

Table I. Lipid Profile Parameters with Their Method of Estimation

Statistical Analysis
Data was entered in Microsoft Excel. Statistical analysis was done by using Pearson's correlation coefficient. Data analysis was carried by Statistical Package SPSS and Microsoft Excel and $p<0.05$ is considered as level of significance.

RESULTS

Parameter	Fasting	Non-Fasting	p Value
TC	174.33 (±20.86)	172.26 (±20.95)	0.861
TG	147.83 (±15.04)	178.20 (±16.84)	0.001 S
LDL-C	131.77 (±9.83)	129.65 (±8.63)	0.203
HDL-C	41.96 (±5.87)	42.17 (±5.24)	0.916

Table 2. Serum Lipid Profile in Fasting and Non-Fasting Sample in Apparently Healthy Controls

S-Significance, $p<0.05$

From table 2, in apparently healthy controls differences between fasting and non-fasting concentrations were small and clinically insignificant for lipid profile parameters like total cholesterol (p=0.861), LDL-cholesterol (p=0.203) and HDL-cholesterol (p=0.916). The difference was statistically significant (p=0.001) for triglycerides.

Parameter	Fasting	Non-Fasting	p value
TC	269.24 (±22.33)	266.54 (±24.75)	0.804
TG	230.53 (±18.99)	281.92 (±24.88)	0.001 S
LDL-C	188.44 (±10.32)	185.39 (±9.93)	0.189
HDL-C	38.01 (±5.11)	36.38 (±4.68)	0.901

Table 3. Serum Lipid Profile in Fasting and Non-Fasting Sample in Severe Diabetic Patients

S-Significance, p<0.05

From table 3, in severe diabetic patients differences between fasting and non-fasting concentrations were small and clinically insignificant for lipid profile parameters like total cholesterol (p=0.804), LDL-cholesterol (p=0.189) and HDL-cholesterol (p=0.901). The difference was statistically significant (p=0.001) for triglycerides.

DISCUSSION

TC and LDL-C levels were slightly reduced in non-fasting as compared to fasting specimen. The possible cause for small reduction in TC & LDL-C levels in non-fasting specimen is most likely haemodilution following fluid intake in association to the meal.[17] Triglycerides increased and HDL-C decreased in non-fasting as compared to fasting specimen. These changes are mostly possible due to food intake rather than fluid intake. Although triglyceride increase are owed directly to fat intake, the parallel reduction in HDL cholesterol is possibly due to bidirectional lipid exchange between triglyceride-rich lipoproteins and HDL particles.[18]

Our study results corresponded well with the studies carried out by Langsted et al. (2008)[11], Mora et al. (2008)[12], Steiner et al. (2011)[19], Langsted and Nordestgaard (2011)[20] Sidhu and Naugler (2012).[21] These large-scale, population-based studies have now proven that there is only a slight change in lipids and lipoproteins levels in response to habitual food intake. Among all other studies comparing non-fasting with fasting lipid profiles, minor increase in plasma triglycerides and minor decreases in TC and LDL-C concentrations were noticed, with nominal change in HDL-C concentrations. These minor changes in lipid profile parameters appear to be clinically unimportant.

Since 2009, International guidelines are recommending non-fasting lipid profiles. Several societies' guidelines in the United Kingdom, Europe, Denmark, United States, Canada and Brazil also advocate non-fasting lipid profiles. In 2009, the Danish Society for Clinical Biochemistry endorsed that all laboratories in Denmark should use random non-fasting specimen for lipid profile measurements rather than fasting specimen while offering physicians the option of remeasurement of triglyceride concentrations in the fasting state if non-fasting values aremore than 350 mg/dL.[20,22] Since 2009, non-fasting lipid testing has become a clinical benchmark in Denmark and practically after 2015 all laboratories in Denmark have adopted non-fasting lipid profiles.

Sidhu and Naugler (2012)[21] expressed that fasting for routine lipid level measurement is broadly needless and is improbable to affect patient clinical prospect stratification, while non-fasting lipid measurement might boost patient conformity and safety. Furthermore, UK NICE guidelines have advocated non-fasting lipid testing in the primary prevention setting since 2014.[23] Requirement of fasting makes sample collection for lipid profile avoidably difficult for all the patients. The fasting state is that used conventionally; however, it would be easier for all the patients if a lipid profile sample could be drawn at any time of the day, irrespective of the timing and the content of the last meal.

Drawbacks of Fasting Samples

a. Fasting is difficult for children.
b. Fasting is a barrier for population screening.
c. Some people might not be true regarding their food intake/ fasting state.
d. Fasting restricts the use of point-of-care testing.
e. Fasting can be a risky affair for the patient of diabetes as they might end up in hypoglycaemic shock.
f. Patients are often annoyed by return on a separate visit for a fasting lipid profile and may fail on essential testing.
g. Fasting requirements can add to the overall costs of lipid testing.
h. Laboratories are burdened by a large number of patients for fasting lipid profile samples in the morning.

Advantages of Non-Fasting Lipid Profile

a. The most obvious advantage of non-fasting rather than fasting lipid profile measurements is that it simplifies blood sampling for patients, physicians and testing laboratories.[11-15]
b. Patients who have not fasted do not have to make another appointment to have their blood drawn.
c. Non-fasting specimen collection is more convenient for patients; it promotes patient acquiescence towards lipid profile checking.[11-15]

Among all studies comparing non-fasting with fasting lipid profiles, only minimal changes in lipid profile concentrations were noted which were clinically insignificant. Also, studies have persistently reported that non-fasting lipid profile suffice for screening of cardiovascular disease risk.[8-12] However, limitations of non-fasting lipid levels include (i) non-fasting levels may be less standardized, (ii) abnormal/normal lipid profile cut-points are not established.

Limitations

a. Sample size was small.
b. Second sample was not taken at fixed intervals after the last meal (random sample).
c. No diet record of participant before blood sampling. Practically also such information is not available before blood sampling.
d. As superiority of fasting lipid testing was not proven over non-fasting lipid profile, it is justifiable to consider non-fasting lipid testing in persons who present for a routine clinic visit.

CONCLUSIONS

Fasting samples are preferable for serum lipid profile measurement in all individuals with serum triglyceride levels greater than 350 mg/dL (in whom the Friedwald equation for calculating LDL should not be used). But, non-fasting samples for lipid profile can be used for cardiovascular risk determination in the general people as it reduces patient's inconvenience and promotes patient acquiescence towards lipid profile checking.

Recommendations

a. Fasting is not normally required for determining the plasma lipid profile in the general population.
b. When non-fasting serum triglyceride concentration is very high (>350 mg/dL), lipid profile may be repeated in fasting state.

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