



Exploring the Novelty in Lipid Profiling of Patients: A Non-fasting Approach from Eastern India

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Abstract

Objective To date, no reference interval is available for lipid profile, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), or low-density lipoprotein (LDL)-cholesterol, etc., in a non-fasting state. Hence, the study was taken up with the objective of exploring the possibility of establishing a reference interval for non-fasting lipid profile consisting of serum TC, TG, LDL, HDL, and very low-density lipoprotein (VLDL) cholesterol.

Materials and Methods A total of 1,350 apparently healthy subjects, including 636 healthy men and 714 healthy women of 18 years and beyond of age, were enrolled in the study. Reference individuals were recruited using cluster sampling method from various villages and semi-urban regions irrespective of their sex, religion, socioeconomic status, or any other demographic profile, and samples were analyzed in Beckman Coulter AU480 analyzer.

Results The mean age of 1,350 participants was 38.23 ± 15.94 years. We found that all the test parameters require a different reference interval than the established fasting reference range, except for HDL cholesterol in females. The data were subdivided into subjects below 40 years, between 40 and 60 years, and older than 60 years of age. All five parameters in the lipid profile were individually analyzed and were compared age group-wise and gender-wise with the total study population. Significant differences in the various dataset were found.

Conclusion A shift toward non-fasting lipid interval measurement is, thus, a piece of evidence-driven mechanism. Even from a patient's perspective, it sets in ease and convenience in lipid-profile testing, subsequently leading to a more compliant cardiovascular management and monitoring.

Keywords

- ▶ cholesterol
- ▶ HDL
- ▶ LDL
- ▶ lipid profile
- ▶ non-fasting
- ▶ reference interval
- ▶ triglyceride

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Introduction

An average human spends most of his life in the non-fasting state, and yet in every clinical guideline, a fasting lipid profile has been standardized. The rationale implied was that the fasting lipid profile reduced the variability and accounted for the low-density lipoprotein (LDL) cholesterol values by the Friedewald formula.¹ Nonetheless, in clinical practice, the conventional 8-hour fasting period lipid profile may not reflect the daily mean of plasma lipid and lipoprotein levels.² The European Atherosclerosis Society/European Federation of Clinical Chemistry and Laboratory Medicine (EAS/EFLM) joint consensus statement recommends fasting lipid profile as not essential for assessment unless there are certain conditions as serum triglyceride (TG) greater than 440 mg/dL or if there is a high-risk or life-threatening conditions which require an immediate specialist referral.³ Estimation of serum lipid profile is an integral part of overall cardiovascular risk assessment. A standard lipid profile consists of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and TG. All the parameters can be directly assessed, and LDL-cholesterol can be directly measured or estimated using the Friedewald formula. The adequacy of studying these levels in a non-fasting state for cardiovascular disease risk has been studied and verified by large prospective studies in the past decade. In studies involving both fasting and non-fasting lipid profiles, it has been seen that the results have been similar, or at times non-fasting lipid profiles have had a better correlation with the risk.⁴ Among certain other studies comparing the two modalities of lipid profile, minor and non-significant changes in serum LDL-cholesterol and TG levels were observed. In the case of HDL-cholesterol, there was no change. It has also been stated that a non-fasting state is the primary physiological condition of a person. Therefore, a non-fasting lipid profile may enhance the strength of association with the various consequences like cardiovascular diseases.⁵

A non-fasting lipid profile is a much easier and less cumbersome procedure from a patient's perspective. As it does not interfere with the daily routine and allows for a sample to be drawn at any instant, it is more practical for the patient as well as from the laboratory perspective. It helps reduce the number of visits by the patient as well as makes it a convenient process on economic and safety grounds for a certain group of patients, like the elderly or diabetic.¹ Especially in the case of diabetics, where hypoglycemia may be a concern because of fasting along with hypoglycemic drugs, non-fasting profiling is a safer option.⁶

However, reporting the non-fasting lipid profile may be challenging in some instances. Clinicians often raise the concern regarding wrong stratification of individuals into a lower risk category when assessing LDL cholesterol in non-fasting state due to variability in TGs affecting LDL-C calculation via the Friedewald formula. The European consensus has recommended that the laboratories should offer re-measurement of fasting TGs if non-fasting TG levels are 350 mg/dL or higher, because TG concentrations are more stable in the fasting state.⁵ But, risk algorithms use TC and

HDL cholesterol and not TGs or LDL cholesterol, hence there is almost nil impact of non-fasting state on risk estimates using these methods. An alternative for concerned clinicians is to use the Martin-Hopkins equation which is a modified version of Friedewald formula.⁷ Sometimes, additional fasting lipid testing may be required under certain clinical conditions. The fasting sample may be required if samples are also to be analyzed for fasting glucose or therapeutic drug monitoring. Known cases of hypertriglyceridemia on follow-up or subjects taking medications that can cause hypertriglyceridemia should be assessed with fasting lipids only.⁸

Analyzing the current evidence, it can be argued that the use of non-fasting lipid profile is evidence-driven. In contrast, fasting is more likely belief-driven with a "we have always done it that way" notion of having accepted it as a part of an age-old practice.⁹ Although reference intervals for fasting lipid profile are widely mentioned in various textbooks of clinical chemistry and kit inserts from reagent manufacturers, to date, no reference interval is available for lipid profile or any of the individual components, e.g., TC, TG, or LDL-cholesterol in a non-fasting state. Hence, the study was taken up with the objective of exploring the possibility of establishing a reference interval for lipid profile consisting of serum TC, TG, LDL, HDL, and VLDL cholesterol in a non-fasting state.

Materials and Methods

Study Design and Setting

The present study was undertaken in the Clinical Biochemistry Laboratory of the Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha, India. This cross-sectional study was performed within the period from April 2018 to March 2020.

Patient Selection

The study population was drawn from a previously conducted study on anemia from our institute with 1,732 participants.¹⁰ Reference individuals had been recruited using cluster sampling method from various villages and semi-urban regions across Khordha district of Odisha province, India, irrespective of their sex, religion, socioeconomic status, or any other demographic profile. All the participants were screened for history of smoking and alcohol intake, and had been duly excluded from the study. History of diabetes, obesity, recent surgery, cardiac illness, and regular strenuous exercise was taken, and excluded if found positive. Subjects in the study population were also asked to inform about any endocrine abnormalities, hormone therapy, or any other chronic illness for which medications are being taken. They were also questioned about medication of any diseases causing lipid alterations or drugs which affect lipid concentration. All such subjects had been duly excluded from this study. In addition to these pregnant women, women actively using any form of oral contraceptives were also excluded from this study. After these, only the subjects with a blood hemoglobin level above 13 g/dL for men and 12 g/dL for women were selected. Only individuals with normal

Table 1 %CV of the internal quality control data of the test parameters during the study period^a

Parameter	%CV of level 1	%CV of level 2	%CV from reagent manufacturer
Cholesterol	0.79	0.69	1.06
Triglycerides	1.35	1.17	1.46
Low-density lipoprotein (LDL) cholesterol	1.42	1.24	2.34
High-density lipoprotein (HDL) cholesterol	1.90	2.14	1.92

^aNumber of runs = 27.

hemoglobin concentrations were included in the study, as there is evidence that low hemoglobin concentration tends to influence serum lipid concentrations.¹¹ Finally the total participants for the study of non-fasting reference interval were 1,350 non-anemic apparently healthy population that included 636 males and 714 females who accorded to all the conditions specified. The reference individuals were aged from 18 years and over with body mass index between 18 and 24.99 kg/m². No participant was advised for overnight fasting, and blood samples were obtained in the forenoon irrespective of time and type of last meal. Verbal confirmation about non-fasting state was taken before taking blood sample from each participant.

This research related to human use has complied with all the relevant national regulations, institutional policies, and is in accordance with the tenets of the Helsinki Declaration. Approval of the Institutional Ethics Committee was taken with AIIMS reference no. T/IM-NF/Biochem/17/38 before collecting samples. Informed consent was obtained from all the participants. Blood sample that had been collected from the previous study and stored at -20°C after serum separation, was used.¹⁰

Measurement of Lipid Profile

Tests for serum TC, TG, LDL, and HDL cholesterol were performed by a photometric method with the AU480 analyzer (Beckman Coulter, Brea, California, United States). All the parameters were assayed under standard assay protocol using reagents, and reference intervals from Beckman Coulter, United States, and serum concentration of very low-density lipoprotein (VLDL) cholesterol was calculated. Serum cholesterol was assayed by the cholesterol oxidase-peroxidase method, and serum TG was assayed by the glycerol phosphate oxidase-peroxidase method. HDL cholesterol was quantified with an enzyme chromogen system where anti human- β -lipoprotein antibody in first reagent binds to lipoproteins other than HDL (LDL, VLDL, and chylomicrons). The antigen-antibody complexes formed, block enzyme reactions when second reagent was added. Estimation of LDL cholesterol was performed with the help of a protecting agent which protects LDL from enzymatic reactions. All non-LDL lipoproteins (HDL, VLDL, and chylomicrons) were broken down by reaction with cholesterol esterase and cholesterol oxidase (CHO). Hydrogen peroxide produced by this reaction is decomposed by catalase of first reagent. When second reagent was added, the protecting reagent was released from LDL and catalase inactivated by sodium azide. LDL was then quantified by the CHO/PAP system. Quality control was

maintained using two levels of internal quality control samples daily from Bio-Rad Laboratories (Hercules, California, United States) and external quality control by monthly samples from the Association of Clinical Biochemists of India prepared by Christian Medical College (CMC), Vellore, India. The coefficient of variation (%CV) of the parameters TC, TG, LDL, and HDL cholesterol was satisfactory during the study period. The %CV were compared with the values provided by the reagent manufacturer as mentioned in **Table 1**. Hb% was measured in samples collected in EDTA tubes using the XP100 analyzer (Sysmex, Kobe, Japan). There was no change in the equipment, reagents, calibration standards, and quality control lot during the study period.

Statistical Analysis

We calculated the lipid profile's reference range based on the Committee on Reference Intervals and Decision Limits (C-RIDL), International Federation for Clinical Chemistry and Laboratory Medicine.¹² In the non-parametric method, data are arrayed in increasing order depending on the reference ranks.¹³ The most important considerations in developing reliable reference intervals were the proper selection of reference subjects, testing an adequate number of subjects, and avoiding preanalytical sources of error.¹⁴ The data were subjected to the Shapiro-Wilk test to determine whether it was normally distributed or not. All statistical analysis of the data was done using RefVal,¹⁵ which follows the procedures established by the various guidelines and adds bootstrapping. The data were thus analyzed using both parametric and non-parametric methods, after removal of outliers. The outliers were removed by Horn's Algorithm that has been pre-initiated in the RefVal software. All the data points are internally converted into a Box-Whisker plot, and a Tukey's Fence Factor (in our case 1.5) determines the outliers. So, any data points further from 1.5 times of the IQR from the first and third quartile are considered outliers. This has previously been demonstrated by Solberg and Lahiti as an efficient way to remove outliers for reference interval calculations.¹⁶ A bootstrapping process was suggested by Solberg in which a random draw to create a subset was used. The bootstrapping was done such that the random resampling number was high (in this case 500). This was done with a 95% confidence interval, and reference limits were set at 2.5 and 97.5 for the lower and upper limits, respectively. The decision to partition the data and the partitioning criteria followed the recommendation of Lahti et al.¹⁷ All other statistical analyses and visualization of data were done using IBM SPSS

v23.0 and JASP v0.12.2. The significance level was expressed as a *p*-value, and *p* of less than 0.05 was considered significant.

Results

Distribution of the Data

The data was non-parametric for all the parameters as the Shapiro-Wilk *p*-value was less than 0.001. Also, on log₁₀ transformation, the Shapiro-Wilk *p*-value was still less than 0.001 except for LDL cholesterol (*p*=0.200). Therefore, all data were primarily expressed as a mean, median, and interquartile range. All statistical analysis was done based on the data being not normally distributed. The median age of 1,350 participants was 36 years with an IQR of 26 to 50 years. 52.9% of the study participants were male. ► **Table 2** presents the descriptive data of the study population and shows that the outliers for each parameter were excluded from the reference interval analysis.

Reference Interval

The reference values were calculated by bootstrapping 500 iterations. Non-parametric reference ranges were used as the parametric transformations didn't have the required Goodness-of-Fit, and hence it was not recommended to use those values. The partitioning was done based on gender. Accordingly, it was portioned into male and female gender, and there was a total of 714 female and 636 male participants in our study as shown in ► **Table 3**. As per the recommendation of Lahti et al, if the % difference between the reference limits exceeds 4.1 or is less than 0.9, the partitions require a different reference range than the one for the population.¹⁷ From ► **Table 3**, it is found that all the test parameters require a different reference interval than in their fasting state except for HDL cholesterol in females.

After the overall data analysis, we have subdivided the data into three groups of subjects below 40 years of age (*n* = 733), subjects between 40 and 60 years of age (*n* = 520), and subjects older than 60 years of age (*n* = 97). When analyzed for differences in the required reference range it was noted that according to our criteria all the group needed a separate reference range for serum cholesterol, serum TG, and VLDL. However, in case of HDL the age group of less than 40 years of age did not need a separate reference interval. Similarly, for LDL only the age group between 40 and 60 years needed a separate reference interval. This has been summarized in ► **Fig. 1**.

Discussion

The primary objective of this study was to explore the possibility to determine the non-fasting lipid profile reference range. No such reference currently exists as fasting lipid estimation in serum has been the norm. However, the ease non-fasting lipid profile would bring in for all the stakeholders has been effectively known for a long. Still, a study in this regard is lacking. Again, stratification according to age and sex is essential as the physiological state differs within

Table 2 Descriptive data of the study population with details of lower and upper determined reference range and detail of number of outliers removed

Parameter (mg/dL)	Outliers deleted	Values remain	Median (IQR)	Lower reference limit (2.5 percentile)	95% CI for lower limit	Upper reference limit (97.5 percentile)	95% CI for upper limit
Total cholesterol	19	1,331	117.3 (94.5–144.6)	62.7	58.3–64.4	206.9	196.4–214.2
Triglyceride	21	1,329	77.8 (58.4–110.9)	35.6	33.70–36.76	230.55	214.70–248.55
HDL cholesterol	34	1,316	80.3 (64.8–100.2)	21.4	19.90–22.40	64.7	63.50–66.00
LDL cholesterol	13	1,337	40.6 (34.2–47.8)	42.7	40.4–44.1	143.9	140.1–149.9
VLDL cholesterol	2	1,348	14.0 (6–23.5)	0.8	0.5–1.1	56.3	50.3–71.6

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Table 3 Gender-specific reference intervals for lipids in a non-fasting state

Parameter (in mg/dL)	Gender	Outliers deleted	Values remain	Lower reference limit (2.5 percentile)	95% CI for lower limit	% Diff from overall for lower limit	Upper reference limit (97.5 percentile)	95% CI for upper limit	% Diff from overall for upper limit
Total cholesterol	Male	4	632	59.4	55.2-64.7	5.26	208.4	196.4-220.6	0.72
	Female	17	697	62.9	58.8-67.6	0.32	204.4	190.1-214.8	1.21
Triglyceride	Male	7	629	32.65	31.1-34.8	8.28	248.85	214.8-276.3	7.93
	Female	13	701	37	35.7-39.3	3.93	218.3	198.1-235.3	5.31
HDL cholesterol	Male	25	611	20.8	19.5-23.3	2.8	61.5	58.7-63.7	4.94
	Female	15	699	21.7	19.0-22.7	1.40	66.1	65.0-66.9	2.16
LDL cholesterol	Male	5	631	39.9	38.1-42.8	6.55	142.8	139.4-151.7	0.76
	Female	11	703	45	43.6-46.7	5.39	145.3	137.9-152.0	0.97
VLDL cholesterol	Male	6	630	0.5	0.1-0.9	37.5	47.1	43.5-51.3	16.34
	Female	2	712	1	0.8-1.3	25.00	55.7	47.4-74.4	1.07

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

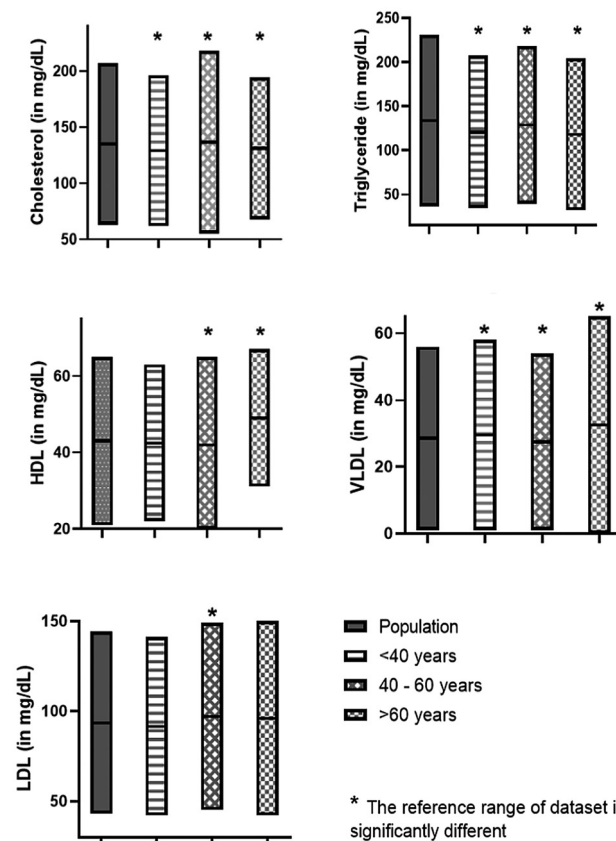


Fig. 1 Agewise distribution of reference intervals for lipids in non-fasting state.

these groups. Non-fasting lipid profile testing does have a significant difference from the currently proposed references as proposed in Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th edition.¹⁸

The established reference values provided for the various lipid profile parameters are from a range of 116 to 274 mg/dL for TC (with an age limit of 20–79 years); 35 to 301 mg/dL for TGs (6–79 years); 31 to 89 mg/dL for HDL-cholesterol (15–79 years), and 62 to 189 mg/dL for LDL-cholesterol (25–79 years). This has been adapted from the Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th edition.¹⁸ The reference values for the various parameters of a lipid profile that we estimated had a difference of 85 and 32% for lower and upper limit of TC, 1.7 and 23.4% for lower and upper limit of TGs, 31 and 27.3% for lower and upper limit of HDL-cholesterol, and 31.1 and 23.4% for lower and upper limit of LDL-cholesterol as compared with that of the already established fasting range of data.

It has been previously seen that the state of fasting has a minimal change in the lipid content of the serum that is estimated. However, the concentrations of TG slightly increase 1 to 7 hours post meals.¹⁹ The partitions that were done were based on various implications of lipid study on a population predominantly of the cardiovascular diseases risk.²⁰ Upon analysis, it was clear that when adjusted for sex, there is a need to define a reference range different from the general population data. A similar scenario was seen in

the case of partitioning based on age as well. Also, this should be considered that as the population in this study belongs to the Eastern Indian population setting, the dietary and life-style habits are mostly homogenous throughout the sample size. Keeping that in mind, the confounding factors of both dietary factors as well as broad sociodemographic factors may well be negated. This aligns with the major purpose of the study, i.e., to find out a less cumbersome and more generalized lipid profile reference system akin to the general populace without any preparations or constraints.

The principle proposed by Lahti et al was followed to determine the separation of reference range from the general population. If the percentage difference between the reference limits exceeds 4.1 or is less than 0.9, then the partitions require a different reference range than the one for population.¹⁷

Our study is one of the few studies on determining reference intervals for non-fasting lipid profiles in adults. A major strength of this study is that it is a community-based study, thus, recruiting a large study population from their place of residence, and no recruitment was done in a hospital set up. As our study is monocentric, we cannot state that our study population is representative of the whole Indian subcontinent in general as dietary and geographical diversities are there. A further multicentric study needs to be performed to present the reference intervals for the general population in India.

The belief-driven practice of advising fasting lipid profile to avoid substantial variability in results due to food intake has been extant for a long and hence seemed to be the only right option. If we look at the present global scenario, the utility of non-fasting testing in clinical decision making was first recognized in 2009 by the Danish Society for Clinical Biochemistry when they recommended its use over fasting profiles as the tests of choice for their national laboratories. Since then, several societies have approved the non-fasting lipid testing for routine screening and clinical decision making.^{5,21-23} In 2018 the American College of Cardiology/American Heart Association (ACC/AHA) cholesterol guidelines modified previous 2013 recommendations for fasting, and allowed non-fasting testing for routine screening which was reiterated again in the 2019 ACC/AHA prevention guidelines.^{24,25} There was a concern that population level risk associations would not capture individual variability based on fasting status which was used against adopting widespread non-fasting lipid testing. But as per the data published on 8,270 participants from the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm trial with prospective follow-up, they provided robust evidence addressing this concern.²⁶ The results of this study suggested that such a strategy would be highly effective and offer many advantages for cardiovascular risk screening and treatment decisions, including for initiating statin or antihypertensive therapy. This study conclusively provided robust evidence supportive of broader adoption of non-fasting lipid level measurement in clinical practice.

Hence, given the increasing adoption of a non-fasting lipid profile, it will evenly aid diagnosis and early treatment. A shift toward non-fasting lipid interval calculation is thus a

step toward precise evidence-driven mechanism procedure. Even from the perspective of a patient, it sets in ease and augment convenience for the laboratory services.^{9,27} This may lead to more increased compliance from patients and could usher in newer future research to modify and better the norms improving the practice, thus making health care availability more convenient by rendering it more accessible and affordable.

Conclusion

Our study aimed at exploring a novel approach for lipid profile testing, i.e., by a non-fasting modality. Based on the data derived from the population and the subsequent study it can be well suggested that the non-fasting approach to lipid profile testing does require a different set of reference ranges as compared with currently used ones. In addition to that, separate references should be used for patients based on sex and age. All this has been suggested as per the guidelines drawn from previous literature as well as IFCC recommendations. Such a novel approach might go a long way into easing the case for patients and providing availability of lipid profile testing to the masses at their convenience.

Authors' Contributions

G.K.S. conceptualized and designed the study, and drafted the manuscript. S.N. participated in analysis and interpretation of data. P.B.M. has participated in recruitment of reference individuals and sample collection. M.M. participated in interpretation of data and critically revised the manuscript. All authors have contributed in final preparation of the manuscript, accepted responsibility for the entire content of this manuscript, and approved its submission.

Ethical Approval

The study followed guidelines enshrined in the Declaration of Helsinki and Tokyo and was duly granted ethical clearance by the Institute Ethical Committee of All India Institute of Medical Sciences (AIIMS) Bhubaneswar (Reg No: ECR/534/Int/OD/2014/RR-17) vide their approval number T/IM-NF/Biochem/17/38 dated March 28, 2018.

Conflict of Interest

None declared.

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