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ORIGINAL ARTICLE

Interpretation of Traditional Lipid Profile: A Reference Interval of a Nigerian Population

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ABSTRACT

Background: The interpretative goal in dyslipidemia is based on a well-established laboratory reference interval in a low economic resource country. However, such establishment by the standard direct method is quite expensive and cumbersome, which makes the dependence on the Western reference values with a different reference individual of a different lifestyle and socio-economic setting. This has led to over or under-diagnosis of dyslipidemia and its burden. The aim of this study was to establish local multivariate references and compare them with the currently used western values.

Methods: A hospital-based indirect method was used to establish population-based reference interval values from 1,125 observations of a Nigerian population at a tertiary health centre between January and December 2022.

Results: The overall mean age of the participants was 52.3±13.7 years, while the males' and females' ages were 53.3±14.7 and 51.7±13.7 years, respectively. The sex ratio was 1:1.4. The established reference intervals were triglyceride (0.6-2.0mmol/L), total cholesterol (3.1-5.9mmol/L, low-density lipoprotein cholesterol (1.7-4.1mmol/L) and high-density lipoprotein cholesterol of 0.7-1.5mmol/L respectively. The values for triglyceride and HDL-C were not affected by age and sex; the total cholesterol and the LDL-C levels increased with age until the 6th decade, with a subsequent decrease after.

Conclusion: Interpreting the lipid profile result with caution, given the observed upper reference limits in age and sex stratification, is important to identify CHD risk and avoid over diagnosis of dyslipidemia. Age and sex stratification should be considered in the interpretation.

Keywords: Dyslipidemia; Interpretation; Traditional lipid; Reference interval, Nigeria.

INTRODUCTION

Dyslipidemia is a common manifestation and complication of many common diseases in Nigeria. The laboratory's request for a traditional lipid profile in the clinical assessment of cardiovascular diseases and many other diseases cannot be over-emphasized. However, the drawback of appropriate interpretation of the report of such requests has continued to pose a challenge of misdiagnosis because of the continued use of the Caucasian reference interval in a practice area of different socio-economic and dietary patterns. Reference limits are some of the most widely used tools in the medical decision-making process.[1] However, despite the recommendations of the International Organization for Standardization (ISO) 15189,[2] that biological reference intervals must be reviewed every decade, the processes involved are very cumbersome and highly technical.

There are two significant methods involved in the determination of a reference interval; population-based direct and hospital-based indirect data methods. The population-based direct method is the recommended standard and more reliable method because of the well-defined criteria for selecting sufficient reference individuals. However, it is not an easy process for most laboratories in developing countries because of the long duration, logistics difficulties in selection, and cost.[1] In addition, there is a need for common standardization and traceability as well as an external control system specifically designed for the process.The procedure also requires the use of matrix-correct control materials with concentration values traceable to the same reference methods and the validation of results in accordance with analytical quality specifications created for the use of common reference intervals.[3]

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This has, to a greater extent, promoted the practice of duplicating reference values from nearby or regional laboratories instead of considering a simpler, more widely used, and less costly approach.
In contrast, the indirect hospital-based method uses the data generated within the laboratories based on the observation that the majority of analysis results produced in the laboratory are "normal" but may sometimes not be reliable. Moreover, it is readily available, numerous, cheaper, and faster, and does not involve patient inconvenience, discomfort, or the risks associated with generating new patient health information. Indirect methods also use the same pre-analytical and analytical techniques used for patient management and can provide very large numbers for assessment. Limitations to the indirect methods include the possible effects of diseased subpopulations on the derived interval. However, the limitations of the indirect methods include the possible effects of diseased subpopulations on the derived interval[4] because of the obsolete use of the word "normal" for healthy individuals and the characteristics of the individuals.

Following the International Federation of Clinical Chemist Committee on Reference Interval and Decision Limits (IFCC C-RIDL) protocol[4], which aims to encourage the use of indirect methods to establish and verify reference intervals, to promote the publication of such intervals with a clear explanation of the process used, and to support the development of improved statistical information used to derive a representative population-based appropriate reference interval. The aim of this study was to establish local multivariate references and compare them with the currently used western values.

METHODOLOGY

This was a descriptive cross-sectional study conducted between January and December 2022 at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The laboratory request form documented 1,125 observed values from individuals aged 15 years and above who came for routine medical evaluation, in line with the posterior selection strategy of reference individuals. Data on age, gender, and the following parameters were collected: serum Triglyceride (Trg), Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), and High-Density Lipoprotein Cholesterol (HDL-C).

Multivariate reference intervals were established by the in-direct, nonparametric method, which relies on a statistical method that has become a gold standard based on the assumption that observed laboratory values are "normal." It relies on some distributional assumptions that the data has a Gaussian distribution and that the peak of the distribution is composed of mainly normal values. For the determination of reference intervals, this method is intended to separate the healthy and diseased distributions of the measured analyte and estimate 2.5 and 97.5 centiles from the dataset, the simplest ones being based on a cut-off value below or above which the observed data are discarded. In this study, the participants were stratified based on age and sex.

Conversely, for participants diagnosed with obesity, drugs, and disease conditions which are usually complicated with dyslipidemia such as diabetes; cardiovascular disease; neoplasm (cancer/malignancies, liver disease, kidney disease, medication, transfusion, or recent surgery; heavy smoking and alcohol consumption and significant recent illness were excluded. Serum triglycerides and total cholesterol were determined enzymatically using the LiquiCHEK (Agappe) kit, while both LDL-C and HDL-C were determined by the phosphotungstic precipitation method using the El-Shalom biomedical kit.

All statistical analyses were carried out using SPSS Version 20 (SPSS version 20.0, SPSS Inc. Chicago, IL, USA). The determination of reference intervals was based on CLSI C28-A3 guidelines. Age and sex-stratification data were assessed for Gaussian distribution using the Kolmogorov–Smirnov test and Shapiro–Wilks test. Dixon method was used to identify outliers. The 2.5nd and 97.5th percentile, mean, median, and range were determined. Depending on data distribution, the observed differences between males and females were evaluated using the student *t*-test or the Mann–Whitney U test where applicable. A two-sided p-value of < 0.05 was considered significant. Reference limits were calculated as mean±1.96 standard deviation (SD).

RESULTS

The overall mean age of the participants was 52.3±13.7 years, while the mean age for the males and females was 53.3±14.7 and 51.7±13.7 years, respectively. The sex ratio was 1:1.4 in favour of females. The serum triglyceride level increased as the age progressed (from 1.0 to 1.4 mmol/L) while both serum TC and LDL-C increased to age 60 years (from 3.9 to 5.1mmolL and 2.6 to 3.4mmolL respectively) and subsequently decreased thereafter (to 4.2mmolL and 2.6mmolL respectively). The HDL-C remained unchanged with ageing (1.1mmolL). The population reference interval values by age for the various analytes of the lipid profile are as displaced in Table 1.

The sex-stratified reference interval values were: Triglyceride (Male and female:0.5 to 2.1mmol/L

Table 1: Stratification of the reference intervals according to age

Age (years)	Triglyceride (Trg)	Total Cholesterol (TC)	Low-Density Lipoprotein Cholesterol (LDL-C)	High-Density Lipoprotein Cholesterol (HDL-C)
10 – 19	1.0	4.4	3.0	1.0
20 – 29	1.0	3.9	2.6	0.9
30 – 39	1.2	4.3	2.7	1.1
40 – 49	1.2	4.3	2.8	1.1
50 – 59	1.4	4.9	3.2	1.1
60 – 69	1.3	5.1	3.4	1.1
70 – 79	1.3	4.5	3.0	1.1
Above 80	1.4	4.2	2.6	1.1

Table 2: Stratification of the reference intervals according to gender

Analytes	Mean (mmol/L)	-1.96SD (mmol/L)	+1.96SD (mmol/L)	Reference Interval (mmol/L)
Triglyceride	M = 1.3	0.5	2.1	0.5 – 2.1
	F = 1.3	0.6	2.0	0.6 – 2.0
Total Cholesterol	M = 4.3	3.0	5.6	3.0 – 5.6
	F = 4.7	3.3	6.1	3.3 – 6.1
Low-Density-Lipoprotein Cholesterol (LDL-C)	M = 2.8	1.6	4.0	1.6 – 4.0
	F = 3.1	1.9	4.3	1.9 – 4.3
High-Density-Lipoprotein Cholesterol (HDL-C)	M = 1.0	0.6	1.4	0.6 – 1.4
	F = 1.1	0.7	1.4	0.7 -1.4

M = Male; F = Female; SD = Standard deviation

Table 3. Determined serum mean and the established reference interval values for traditional lipid profile

Analytes	Mean (mmol/L)	SD (mmol/L)	-1.96SD (mmol/L)	+1.96SD (mmol/L)	Reference-Interval (mmol/L)
Triglyceride	1.3	0.7	0.6	2.0	0.6 – 2.0
Total Cholesterol	4.5	1.4	3.1	5.9	3.1 – 5.9
Low-Density-Lipoprotein Cholesterol (LDL-C)	2.9	1.2	1.7	4.1	1.7 – 4.1
High-Density-Lipoprotein Cholesterol (HDL-C)	1.1	0.4	0.7	1.5	0.7 – 1.5

SD – Standard deviation

and 0.5 to 2.0mmol/L respectively), TC (male and female:3.0to 5.6mmolL and 3.3 to 6.1 mmol/L respectively), LDL-C (Male and female: 1.6 to 4.0mmol/L and 1.9 to 4.3 mmol/L respectively), HDL-C (Male and female: 0.6 to 1.4mmol/Land 0.7 to 1.4mmol/L respectively). Others as presented in Table 2. The p-values for all the analytes were insignificant among both genders. The calculated reference interval from the mean values and the SD is as stated in Table 3.

DISCUSSION

Health is a relative concept that implies that an individual is in a state of well-being.[5] However, the concept of being well must be compared to an established population reference interval using reference individuals and data. The reference interval is a clinically significant tool in the interpretation of laboratory results which in turn determines the clinical decision taken on

a case management. Therefore, the goal of every medical diagnostic laboratory is to determine its own local population-based reference interval from the lower and upper limits of the reference values from apparently healthy individuals.[6]

However, in the lowest resource area of practice, the cost and logistics of this apriori methodology are often insurmountable, and this has led to many diagnostic laboratories using either Caucasian, nearby state, regional, or national hospital values as their reference without regard for diet and sociocultural differences of the areas which in turn can affect an analyte of interest. This, more often than not, may lead to under or over-diagnosis of a disease condition, leading to huge medical errors and the subsequent events of complication. The significance of locally established reference values is to avoid this common above scenario as well as lay credence to the fact that a laboratory result is not clinically useful unless and until it has been subjected to the established reference data.[5] Therefore, the only option left in such low-resource areas is the indirect posterior method, which is less expensive but has disadvantages regarding some assumptions on population distribution and clinical diagnosis.

The mean age of the participants in this study showed that it was an adult population similar to previous reference interval studies in some Nigerian and Caucasian reports. [7,8,9,10,11.] This was to lay credence to the aim of the study even though it is preferable to interpret the lipid result along the stratification of age and gender because the observed mean values are usually affected by age, sex, food habits, lifestyle changes, socio-economic status, and race. Stratification has also been shown to enhance the sensitivity of reference intervals rather than a mean value.[6] Therefore, the established mean values are useful in calculating the interval using the 95% confidence limit rather than being used in the interpretation of the lipid profile.

The established reference interval for triglyceride (0.7-1.9mmol/L) in this study showed a higher lower and upper reference limit when compared with the currently used values (0.3-1.7 mmol/L). This difference was, however, not statistically significant (p-value = 0.001) when compared. It was also observed that the serum levels increased with age but were also not significant (p-value = 0.001). However, the serum mean values are the same between males and females. Ageing is associated with progressive increases in serum triglyceride levels. The import of this observation is that diagnostic laboratories should adjust their reference interval even though the current values have not influenced the under/over-diagnosis of triglyceridaemia.

The serum TC concentration increases progressively after the age of 20 and reaches a plateau between the ages of 50 and 60 in males, whereas, in women, it reaches a peak between 60 and 70.[12] Our established mean serum TC increased with age up to the sixth cascade of life and subsequently began to decrease in both genders. As earlier documented, the Total cholesterol and triglyceride levels tend to increase up until 50 years of age, and then a gradual decline starts to occur, and this is what is responsible for the positive correlation that exists between total cholesterol and/or triglyceride levels with the incidence of cardiovascular disease up to the age of 50 years and thereafter, the ability of total cholesterol to predict coronary heart disease in very old individuals remains controversial.[12]

However, the gender stratification showed higher serum values in the females. This could be due to the effect of reproductive hormones as menopause has been reported to be associated with a progressive increase in total cholesterol, with, in particular, an increase in low-density lipoprotein (LDL-C) and triglycerides with a decrease in high-density lipoprotein (HDL-C)[13,14,15] raising the risk factor for CVD significantly. Serum TC levels peak in women at 55–65 years of age and about 10 years later than the peak in men. This is similar to observations in previous apriori studies as noted earlier. This study established serum TC upper reference limit of 5.9mmol/L is, however, in the diagnostic value of mild hypercholesterolemia in the currently used limit. This is a significant finding related to the discussion of over diagnosis of dyslipidemia when compared to previous studies. The need to adjust to this new observed upper limit is supported by the reason that the previous studies were over ten years, and lifestyle changes have occurred during this period. Also, the number of participants in those studies is small. It is, therefore, important to exercise caution in the interpretation of the current upper limit reference of TC as mild hypercholesterolemia without consideration for age/gender and the serum value of LDL-C.

The serum LDL-C values were observed to be higher at the two extreme decades of life and this could be attributable to the decreased muscle mass/adiposity ratio in these ages. This observation also showed that the gender ratio is in favor of females, and this is due to the higher serum levels of TC observed earlier in the same gender group. This would suggest the need to be more careful in the interpretation of a lipid profile in the pediatric and elderly age groups. It was recommended that the elderly should maintain an LDL-C level of 1.91-2.47 mmol/L and a TG level of not less than 1.66 mmol/L.[16]

Age and gender stratification do not influence serum HDL-C levels. This is similar to reports from other studies, and the established reference interval (0.7-1.5mmol/L) agrees with other studies.[13,14,15]

High-density lipoprotein cholesterol (HDL-C) is considered a protective factor for CHD. HDL-C levels have been associated with good health status, while reduced HDL-C values are recognized as risk factors for CHD in both middle-aged and older persons.[17] Furthermore, it has been shown that reduced HDL-C also predicts non-CHD/stroke mortality in older persons. Thus, low HDL-C may also be considered a valid biomarker for chronic disease and poor health status in old age.

Limitations of the study

i. The Indirect Method used may introduce potential bias as the dataset may include individuals with undiagnosed or subclinical diseases that could affect the lipid profile values.
ii. Generalizability: The study was conducted in a single tertiary health centre and may not fully represent the diverse Nigerian population with varying socio-economic and dietary patterns.
iii. Potential Lifestyle and Environmental Factors: The study did not account for specific lifestyle factors such as diet, physical activity, or environmental influences, which are known to affect lipid levels.

Conclusion: Chemical Pathologists must exercise great caution in interpreting the traditional lipid profile result, given the observed upper reference limits of some parameters about age and gender stratification. This is to identify CHD risk and avoid over diagnosis of dyslipidemia and unnecessary treatment costs incurred by clients. Interpretation of the lipid profile should be done with age and gender-stratified reference intervals.

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