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Determination of LDL-cholesterol: direct measurement by homogeneous assay versus Friedewald calculation among Makerere University undergraduate fasting students

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ABSTRACT

The treatment of patients for coronary heart disease risk requires knowledge of the plasma lipid levels. Low density lipoprotein cholesterol (LDL) levels make a strong basis for therapeutic decisions. Although there are incongruities among values of LDL from different methods of determining LDL, the clinician is not routinely informed of the method used. The purpose of this study was to compare LDL levels determined by the Friedewald equation with those assayed by the Kyowa Madox method. The lipid results previously measured by Kyowa Madox method among Makerere University fasting students and reported earlier were retrieved. The measured values of total cholesterol (TC), High Density Lipoprotein cholesterol (HDL) and triacylglycerols (TG) were used to calculate LDL using Friedewald equation in which LDL= TC-HDL-TG/2.2 mmol/L. The values obtained were compared non parametrically with the assayed values previously reported. Our results showed a high value of correlation between measured and calculated LDL so that in general, the two methods can be used interchangeably in this population. However, in cases of dyslipidaemia, the calculated values tend to be lower than the assayed values. It is therefore recommended that clinical laboratories should report the LDL values along with the determination method used, the alert values, the reference ranges, the desirable ranges and the therapeutic targets.

Keywords: Homogeneous assay, LDL cholesterol, direct measurement, Friedewald equation, comparison.

INTRODUCTION

The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program (NCEP) recommends using low density lipoprotein (LDL) cholesterol levels as the basis for the decision to treat patients for coronary heart disease (CHD) risk (NCP III). Assessing CHD risks requires knowledge of the patients` LDL cholesterol levels matched against recommended cut points shown in Table 1.

Although LDL values make a strong basis for therapeutic decisions, the clinician is not routinely informed of how the LDL values

Table 1: LDL cholesterol goals based on risk categories (NCEP III, 2002)

LDL mmol/L	Classification
<2.59	Optimal
<3.34	Near optimal/above optimal
<4.11	Borderline high
<4.89	High
>4.91	Very High

were obtained. The ultracentrifugal measurement of LDL is time-consuming and too expensive for routine work. In clinical laboratories LDL is most often obtained by using Friedewald formula which links total cholesterol (TC), triacyl glycerols (TG), high density lipoprotein cholesterol (HDL), very low density lipoprotein cholesterol (VLDL) with low density lipoprotein cholesterol as: LDL = TC – HDL –VLDL(Friedewald et al.,1972).

The VLDL also requires expensive ultracentrifugation and is routinely approximated as TG/2.2 mmol/L. So, practically, the Friedewald algorithm is: LDL= TC-HDL – TG/2.2 mmol/L (Nauck et al., 2002). This formula is flouted with analytical errors of TC, TG and HDL assays. Besides, the empirical relationship of VLDL = TG/2.2 as derived by Friedewald et al. (1972), assumes that virtually all the plasma TG is carried in VLDL and that the TG: cholesterol ratio of VLDL is virtually constant at 2.2. Neither assumption is universally true and even when it is, a proviso of fasting for a minimum of 11 hours is imposed. Such is inconvenient for routine clinical work.

In search of better accuracy and universal application, the American National Reference system for Cholesterol licensed five homogenous methods for directly measuring LDL (Nauck et al., 2002). These methods include International Reagents Corporation, Denka Seiken, Wako, Daiichi and Kyowa Medox(ibid), of which the last one is applied in automated instruments commonly used in Uganda referral hospitals. It was indeed the method used to study lipids in 183 Makerere University fasting students reported previously (Bimenya et al., 2006), thereby beckoning this work.

Many studies elsewhere have reported incongruities between the values of LDL obtained by Friedewald equation and by any of the licensed homogeneous methods (Rubies-Prat, 1993). The incongruity is neither uniform nor linear because of multiplicity of causes including the diseases, ethnicity, and diet. None the less, it has been of concern in many clinical situations. Consequently, some laboratories, especially in Britain, gave a numerical report of LDL, followed by its method of determination and its possible interpretation (Kroll, 2000), a practice not done routinely in Uganda.

Principle of Kyowa Madox LDL assay

In this homogeneous assay, plasma or serum is made to react with various reagents to produce a measurable signal without centrifugation as follows:

1. HDL, VLDL and chylomicrons (CM) are blocked by surfactants and sugar compounds.
2. LDL is solubilized by enzymes and surfactants and made to react to give cholestenone and H_2O_2
3. The H_2O_2 reacts with a leuco-dye to produce measurable intensity of blue colour whose absorption is proportional to the

peroxide, and therefore to the LDL substance concentration.

The Friedewald equation for calculating LDL (Friedewald et al., 1972)

This equation was established empirically by Friedewald et al. in 1972 to estimate the concentration of LDL cholesterol in plasma without use of the expensive and time consuming preparative ultracentrifuge. The method involves measurement of fasting plasma cholesterol, triglyceride, and high-density lipoprotein cholesterol concentrations, none of which requires the use of preparative ultracentrifuge. Comparison of this algorithm with the more direct procedure in which the ultracentrifuge largely reserved for research was used, yielded correlation coefficient ranges of 0.94-0.99, depending on patient population (Nauck et al., 2002).

The purpose of this study was to derive LDL values by Friedewald equation from those of TC, TG and HDL already reported among Makerere University undergraduate fasting students and to compare the derived values with those already reported having been assayed by Kyowa Madox direct homogeneous method and to make recommendation on the reporting essentials.

MATERIALS AND METHODS

The lipid results measured and reported among Makerere University undergraduate fasting students (Bimenya et al., 2006) were retrieved. In the study, duly approved by the Faculty Research and Ethics Committee, 183 undergraduate over–night fasting students with informed consent had each donated 5 ml of antecubital venous blood for plasma lipid assays. In the current study, the measured values of TC, HDL and TG in each individual were retrieved and used to derive calculated LDL using Friedewald equation in which: LDL= TC-HDL-TG/2.2 mmol/L (Friedewald et al., 1972). The values obtained were compared with the values assayed by Kyowa Madox method for each individual as previously published. Non parametric statistics were used.

RESULTS

The empirical data of measured LDL cholesterol and Friedewald-calculated cholesterol are presented in separate histograms presented hereunder.

Measured LDL cholesterol

The distribution of measured LDL cholesterol (mLDL) is displayed in Figure 1.

The distribution is polymodal, leptokurtic and positively skewed albeit with Gaussian approximation. With mean (SD) of 3.80 (1.223), the distribution ranges from 2 to 8.17 mmol/L, making 84.6% of the subjects worthy of medical scrutiny of LDL above optimal levels of <2.59 mmol/L.

The calculated LDL cholesterol

The distribution of calculated LDL cholesterol (c-LDL) is displayed in Figure 2.

The distribution is polymodal, leptokurtic and positively skewed albeit with Gaussian approximation. With mean (SD) of 3.80(1.223), the distribution ranges from 1.99 to 8.17 mmol/L, making 84.2% of the subjects worthy medical scrutiny of LDL above optimal levels of <2.59 mmol/L (Table 1).

Correlaton of m-LDL with c-LDL cholesterol

The cholesterol m-LDL values regressed against c-LDL among Makerere University undergraduate fasting students are shown in Figure 3.

Apart from the two trans-line values, the plotted points are collinear, signifying a high degree of correlation between the measured and the calculated LDL cholesterol in this population sample. However, the regression angle is less than 45 degrees,

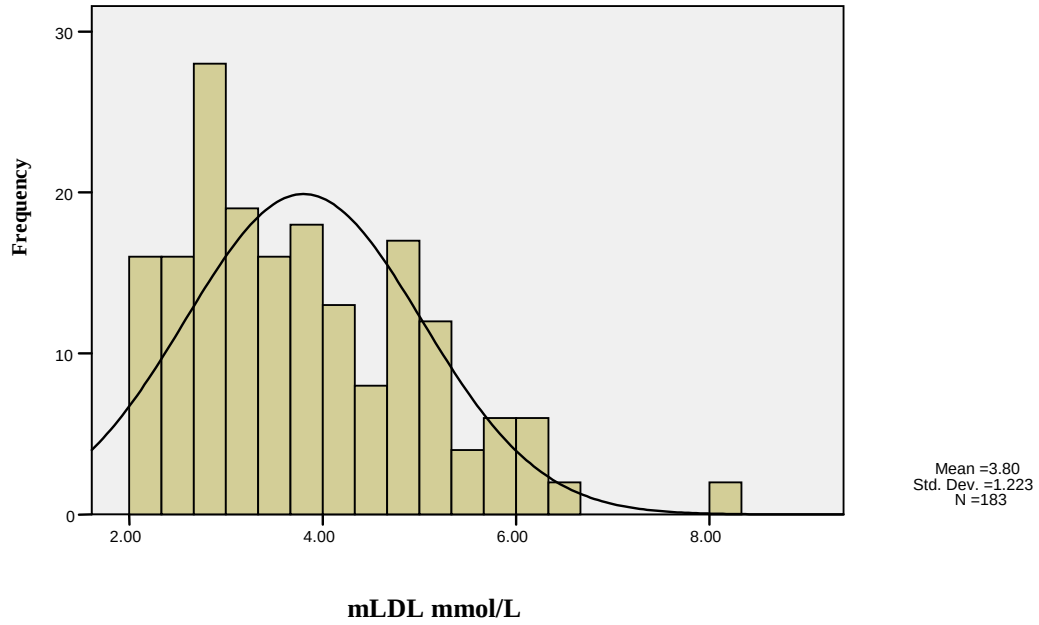

Figure 1: Frequency distribution curve for measured LDL cholesterol values among Makerere University undergraduate fasting students.

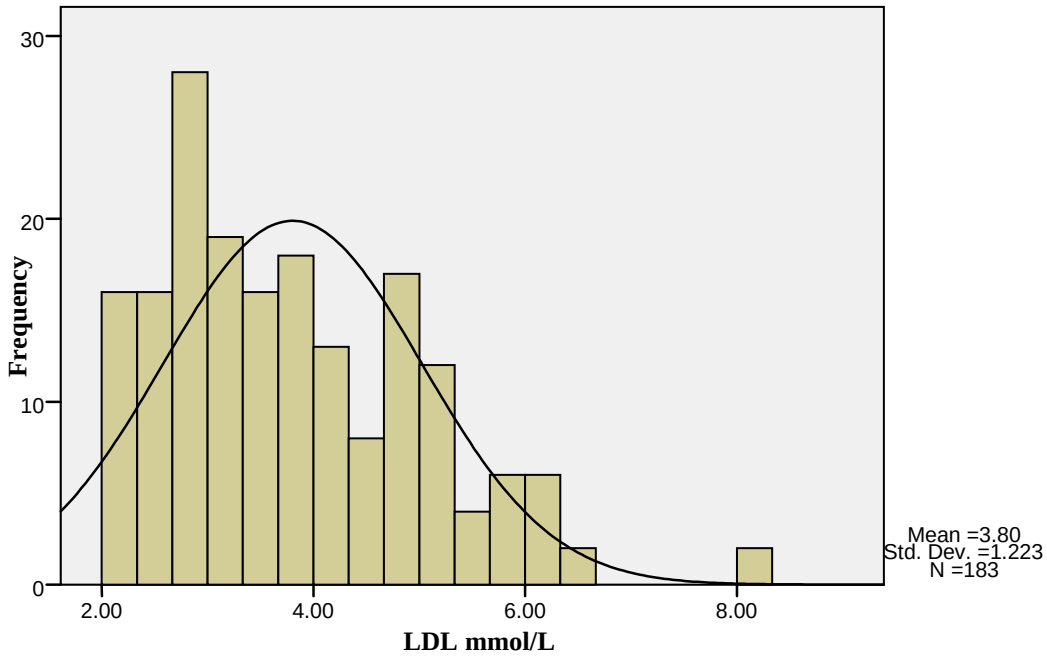

Figure 2: Frequency distribution curve for calculated LDL cholesterol levels among Makerere University undergraduate fasting students.

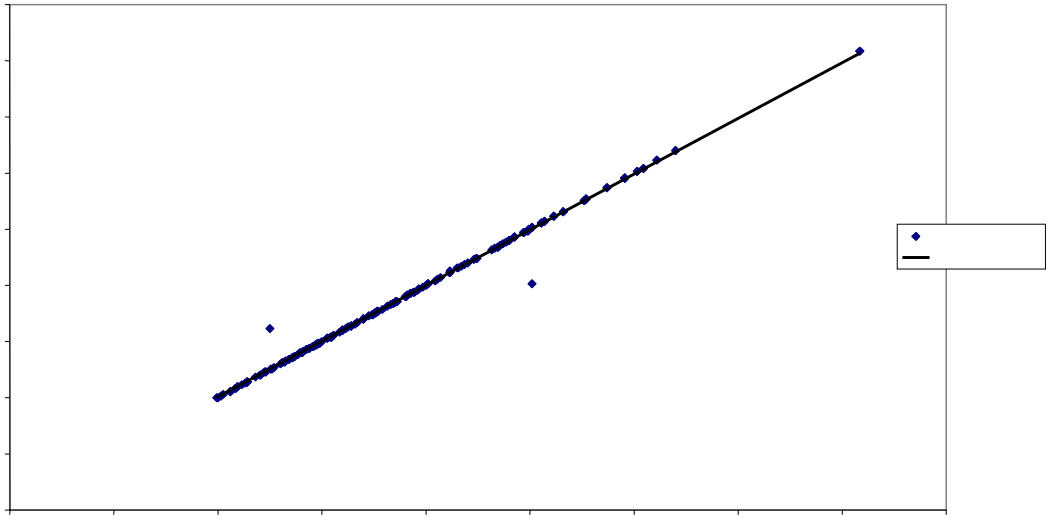

Figure 3: Values of m-LDL cholesterol regressed on those of c-LDL among Makerere University undergraduate fasting students.

signifying that the two methods do not give identical results.

According to Wilcoxon signed rank test, the two distributions are positively skewed with Gaussian approximation at P value of 0.7114 with median, standard error of skewness, and standard error of kurtosis not significantly different. These qualities would support the interchangeable use of c-LDL with m-LDLin this population. However, the modes, the standard deviations, the variances, the skews, the kurtoses, the ranges and the sums are significantly different, inducating that precautions should be taken about the permutability of the two methods in this population.

DISCUSSION

As LDL is a modifiable risk for coronary heart disease, its measurement is recommended in the evaluation and management of hypercholesterolemia (NCP III). However, the concerned professional organizations that give guidelines on clinically critical cut off ranges of LDL do not specify how the LDL should be obtained (Bimenya et al., 2006). Many workers recommend the "time honoured" Friedewald algorithm (Faas, 2003) while others recommend new directly homogeneous methods that circumvent the compounded errors inherent in the three measurements of TC, TG and HDL that are a pre-requisite for the Friedewald derivation (Nauck et al., 2002).

This work compared the Friedewald LDL with that assayed directly with a licensed homogeneous method of Kyowa Madox currently used on automated chemistry equipment in referral hospitals in Uganda. This was done on apparently healthy Makerere University under graduate fasting students who acted as surrogates of Uganda ethnicity, with varied dietary and life style back grounds. The sample used did not present hypercholesterolemia, dyslipidemia, liver disease, kidney disease, hypothyroidism or, indeed, self-reporting heart disease.

Therefore, these results must be interpreted in the context of seemingly normalcy.

Otherwise, the caveats for Friedewald calculation from the beginning were that the algorithm is inaccurate in non fasting samples, in specimens of TG>4.52 mmol/L and in patients with hyperlipoproteinemia or chylomicronemia (Friedewald et al., 1972). In Uganda, these caveats are particularly important with hyperlipidaemia of any origin including Diabetes mellitus, and antiretroviral drugs. In such patients m-LDL which, moreover, does not require fasting (Nauck et al., 2002) is highly recommended.

However, this recommendation comes with a cost. A kit of Kyowa Madox direct LDL assay costs Uganda shillings 285 000 for 50 tests (Segirinya, 2009), translating to an extra Shs 57 000 per sample

Given the currently recommended optimal target LDL level of less than 1.8 mmol/L (Nichols et al., 2006), the mean c-LDL and mean m-LDL of 3.80 mmol/L obtained in this work, were above the optimal (Table 1). This implies that some of the Makerere undergraduate students needed medical attention with respect to hyperlipidemia. In general terms, c-LDL and m-LDL could be used interchangeably in this population.

The plot of measured LDL against calculated LDL indicates in fig 3 that the measured value of LDL will usually be higher than the calculated value of LDL. This means that using c-LDL instead of m-LDL some times would deny medical attention to the deserving client in this population. Such has been reported elsewhere that the calculated method placed the average below 2.586 mmol/L, appearing within the desired range whereas the value obtained by the measured method placed the average above the LDL goal of 2.586 mmol/L outside of the desired range originally recommended in ATP III (Table1).

As a result of the great significance attached to clinically critical cut off ranges of LDL levels which may be misunderstood if the method used to determine them is not revealed, clinical laboratories should therefore report the LDL determination method used; the reference values, alert values, desirable ranges and the therapeutic targets for the clinical condition also. This is obviated in Uganda due to the high prevalence of carbohydrate diets, antiretroviral treatment and metabolic diseases all of which are on the rise and need checks and controls through LDL management.

Conclusion and recommendation

The results show that generally, the Friedewald algorithm and the Kyowa Madox methods can be used interchangeably in this population given that they have identical mean, median, the skew, the kurtosis, and the high correlation coefficient. However, in cases of dyslipidaemia, the calculated values tend to be lower than the assayed values. It is therefore recommended that clinical laboratories should report the LDL values along with the determination method used, the alert values, the reference ranges, the desirable ranges and the therapeutic targets.

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