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Research Paper



Use of friedewald equation for dyslipidemia in metabolic syndrome

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Abstract

Objective: The Friedewald equation is frequently used to estimate low-density lipoprotein cholesterol (LDL-C) in routine patient care; however, recently many limitations have emerged regarding its use.
Aim: Analyse the use of Friedewald equation for dyslipidemia in metabolic syndrome.
Methods: Subjects were selected with metabolic syndrome that fulfilled consensus statement for Asians Indians and excluded those with triglyceride (TG) ≥400mg/dl, and chronic liver and/or kidney disease. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TGs, and LDL-C were measured with direct assays. LDL-C was further estimated using the equation and compared with LDL-C by direct assay.
Results: The mean and standard deviation of TC, TGs, HDL-C, and LDL-C were 194.77±24.38mg/dl, 174.84±60.27mg/dl ($p<0.0001$), 40.68±5.40mg/dl ($p<0.05$), and 122.30±19.30mg/dl among subjects with metabolic syndrome. On the other hand, Friedewald estimated LDL-C and VLDL-C were 121.29±18.84mg/dl and 35.08±12.65mg/dl ($p<0.0001$). Furthermore, a statistically significant higher TGs/HDL-C ($p<0.0001$) and LDL-C/HDL-C ratios was observed in subjects with metabolic syndrome. However, no significant difference was recorded between the two methods of estimating LDL-C.
Conclusion: TGs/HDL-C was found significantly higher among subjects with metabolic syndrome; however, no significant difference between both Friedewald equation and direct measurement method for LDL-C estimation was observed. Hence, the accuracy of LDL-C estimation formulas and direct methods for measurement in patients with the metabolic syndrome requires further exploration.

Keywords: *Dyslipidemia, Friedewald Equation, Metabolic Syndrome.*

1. Introduction

The metabolic syndrome is a major escalating public-health and clinical challenge worldwide in the wake of urbanization, surplus energy intake, increasing obesity, and sedentary life habits. It represents as a constellation of interconnected physiological, biochemical, clinical, and metabolic risk factors including hypertension, atherogenic dyslipidemia, central obesity, glucose intolerance, pro-inflammatory and pro-thrombotic state which reflects underlying insulin resistance. This atherogenic dyslipidemia is characterized by a spectrum of qualitative lipid abnormalities reflecting perturbations in the structure, metabolism, and biological activities of both atherogenic lipoproteins and antiatherogenic high density lipoprotein cholesterol (HDL-C) which includes an elevation of lipoproteins containing apolipoprotein B (apoB), elevated triglycerides (TGs), increased levels of small particles of low density lipoprotein cholesterol (LDL-C), and low levels of HDL-C (Kaur 2014).
High level of LDL-C is a major risk factor for ischemic heart diseases, and its relation to premature coronary artery disease (CAD) has been demonstrated (Grundy *et al.* 2004), where each 1% reduction in LDL can reduce the risk of CAD by 1% (Robinson *et al.* 2005). The use of statins in lowering LDL-C is clearly efficacious in the treatment and prevention of CAD. However, despite current therapeutic use of statins as monotherapy even in optimal doses and achieving target LDL-C reduction, a significant number of patients with mixed atherogenic dyslipidemia are at high risk for coronary events (Grundy *et al.* 2004).
The Friedewald equation which is frequently used to estimate LDL-C in routine patient care; is a central focus of clinical practice guidelines throughout the world, including in the United States (Smith *et al.* 2011), Europe (Reiner *et al.* 2011), and Canada (Genest *et al.* 2009). The Friedewald equation indirectly estimates LDL-C as total cholesterol minus high-density lipoprotein cholesterol minus triglycerides/5 in milligrams per deciliter. The equation introduced into clinical practice in 1972 because the automatic methods are time-consuming and/or require costly equipments and trained personnel (Friedewald *et al.* 1972). However, the essential limitations are known for the equation which includes hypertriglyceridaemia, type III hyperlipidaemia, low LDL-C concentration and secondary hyperlipidaemias observed in patients with diabetes mellitus, renal disease, hepatic failure, and on hormone replacement therapy (Friedewald *et al.* 1972, Bairaktari *et al.* 2004, Jun *et al.* 2008).
LDL-C should be measured accurately due to its involvement in CAD risk assessment. Otherwise, clinicians will frequently misclassify patients based on National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) LDL-C categories when LDL-C is low and triglycerides are elevated using the Friedewald equation to estimate LDL-C. The magnitude of underestimation of LDL-C is often sufficient to lead to under-treatment based on ATP III categorization. However, current guidelines address this issue by recommending non-HDL-C as a secondary

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treatment target when TGs exceed 200mg/dl (2.26mmol/l) (Grundy *et al.* 2004). Yet, the equation has remained in routine use due to its cost effective nature for the general population with remarkably little scrutiny.

Furthermore, hypertriglyceridemia is considered as an independent predictor of CAD, where the ratio of TG/HDL-C has been proposed as an easily obtainable atherogenic marker (Bittner *et al.* 2009). The ratio further correlates inversely with the plasma level of small, dense LDL-C particles; where small dense LDL-C particles are more atherogenic than larger buoyant ones. The HDL-C particles correlate inversely with serum TGs and small dense LDL-C; where the larger but less dense HDL-C particles are more protective than the atherogenic small dense HDL-C particles (Da luz *et al.* 2005).

Hence, the current design is an effort to study lipid panel and ratios in patients with metabolic syndrome so that it can be used as an assessment tool for CAD risk profile in primary care settings and resource poor situations.

2. Methodology

A cross-sectional study was designed to analyze the use of Freidewald equation for dyslipidemia in metabolic syndrome. The samples were recruited from ex-servicemen who had been retired from the defense services; their family members comprising spouse, parents and children; both genders aged above 20 years; and those had attended the polyclinic from Aug, 2013 to Sept, 2013. Under the Ex-Servicemen Contributory Health Scheme (ECHS), all registered members are entitled to free at-the-point-of-access medical attention and services from the ECHS polyclinic located at Sultanpur Lodhi, Kapurthala, Punjab (India). Institutional ethical committee approval was obtained prior to the study start, and informed written consent was taken from all the recruited subjects who attended polyclinic during the study period.

2.1. Definition of metabolic syndrome

It was defined on the basis of consensus statement for Asians Indians with three out of five variables abnormal for the diagnosis: Obesity >25.00kg/m^2, fasting blood glucose >100mg/dl (>5.6mmol/l), hypertension ≥130/≥85mmHg, triglycerides(TGs) >150mg/dl (>1.7mmol/l) and/or High Density Lipoprotein Cholesterol(HDL-C) <40mg/dl (<1.03mmol/l) in men or <50mg/dl (<1.29mmol/l) in women. It further includes those previously diagnosed with hypertension, high TGs, low HDL-C, high LDL-C (>130mg/dl; 3.36mmol/l), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diabetes mellitus and being on treatment for these disorders (Misra *et al.* 2009). All subjects were categorized into two groups on the basis of presence or absence of the diagnosis of the metabolic syndrome; however, those with TGs ≥400mg/dl (≥4.52mmol/l), and chronic liver and/or kidney disease were excluded.

2.2. Freidewald equation

LDL cholesterol (mg/dl) = Total cholesterol (mg/dl) – HDL cholesterol (mg/dl) – Triglyceride/5 (mg/dl).

VLDL cholesterol = Triglycerides/5 (mg/dl).

2.3. Clinical measurements

Participants were weighed to the nearest 0.1kg wearing minimal clothes and without shoes; and height was measured to the nearest 0.1cm with a wall mounted non-extendable measuring tape. BMI was calculated as weight per square meter (kg/m^2). A standard mercury sphygmomanometer with suitable calibrated cuff was used to take blood pressure after subject seated and rested for five minutes.

2.4. Biochemical analysis

A venous blood sample was obtained from all the pre-informed individuals after 8–10 hours of fasting. All instruments were checked for calibration before using them to measure blood glucose using Erba glucose kit (GOD-POD method, end point), TC by Erba cholesterol kit (CHOD-PAP method, end point), TGs with Erba triglyceride Des kit (GPO-Trinder method, end point), HDL-C by cholesterol kit (Phosphotungstic acid method, end point), and LDL-C by Erba kit (modified polyvinyl sulfonic acid and polyethylene-glycol methyl ether coupled classic precipitation method).

2.5. Statistical analysis

All evaluated variables are presented as means and standard deviation (means±SD) using Microsoft Excel 2007. Comparisons between two groups were done by paired t test with statistically significance at $p<0.05$.

3. Results

The present study analyzed total 110 samples; 43.64% of these were males and 67.36% were females. The mean age of the study subjects was 58.02±12.57years. The demographic characteristics of the study subjects were further elaborated in Table 1.

Table 1: Demographic Characteristics of All the Study Subjects (N=110)

Category	Number of subjects	Frequency (%)
Males	48	43.64
Females	62	67.36
Age	58.02±12.57	58.02±12.57
Hypertension	58	52.73
Hyperglycemia	48	43.64
Obesity (>25kg/m^2)	60	54.55
Hypercholesterolemia	33	30.00
Low High Density Lipoprotein-Cholesterol	37	33.64
High Low Density Lipoprotein-Cholesterol	32	29.09
Hypertriglyceridemia	49	44.55

The study further divided subjects into two groups on the basis of metabolic syndrome: with (N=55; 50.00%) and without (N=55; 50.00%) the syndrome (Table 2). The mean and standard deviation of TC, TGs and HDL-C were 194.77±24.38mg/dl, 174.84±60.27mg/dl, and 40.68±5.40mg/dl among subjects with metabolic syndrome; and 192.30/dl±26.64, 130.02±26.97mg/dl, and 43.15±7.06mg/dl were the values for the same variables among subjects without metabolic syndrome, respectively. It shows statistically significant higher ranges of TGs ($p<0.0001$), HDL-C ($p<0.05$), and TC in subjects with the diagnosis of metabolic syndrome than without it.

Table 2: Comparison of Lipid Panel in Subjects With and Without Metabolic Syndrome

Category	Metabolic Syndrome		P value
	Yes (55)	No (55)	
Total Cholesterol	194.77±24.38	192.30±26.64	0.614
Triglycerides	174.84±60.27	130.02±26.97	**<0.0001**
High Density Lipoprotein-Cholesterol	40.68±5.40	43.15±7.06	**<0.05**
Low Density Lipoprotein-Cholesterol (calculated)	121.29±18.84	122.60±22.34	0.740
Low Density Lipoprotein-Cholesterol (measured)	122.30±19.30	123.70±23.34	0.7324
Very Low Density Lipoprotein- Cholesterol	35.08±12.65	26.00±5.40	**<0.0001**

Further, the Friedewald calculated LDL-C were 121.29±18.84mg/dl and direct measured LDL-C were 122.30±19.30mg/dl among subjects with metabolic syndrome. This shows no significant differences between two methods of direct measurement and Friedewald estimation for LDL-C with mean difference of 1.01mg/dl, correlation coefficient of 0.0354,

and p value of 0.714. Contrarily, the Friedewald calculated VLDL-C were 35.08±12.65mg/dl and 26.00±5.40mg/dl in both groups; which shows statistically significant (p<0.0001) higher ranges in subjects with metabolic syndrome (35.08±12.65mg/dl) as compared to individuals with no syndrome (26.00±5.40mg/dl). Figure 1 represents the graphical correlation of Friedewald estimated LDL-C levels in subjects with and without metabolic syndrome.

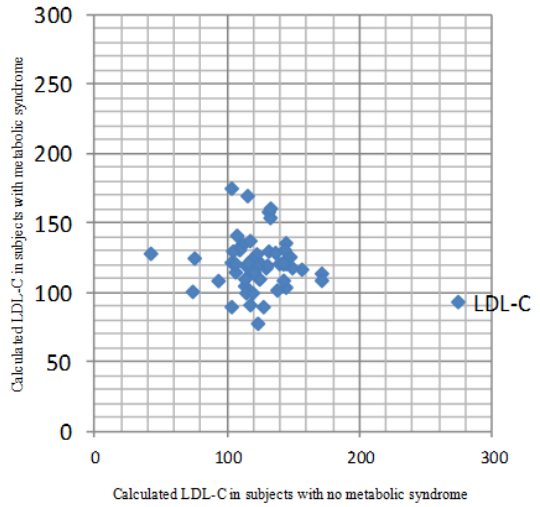


Fig.1. Graphical correlation of calculated LDL in subjects with and without metabolic syndrome

Table 3 reveals the lipid ratios in all the study subjects. The calculated mean and standard deviation of TG/HDL-C, TC/HDL-C, and LDL-C/HDL-C ratios were 4.35±1.53, 4.86±0.75, and 3.03±0.61 for subjects with metabolic syndrome; and 3.08±0.76, 5.21±5.11, and 2.91±0.68 were calculated for the same ratios among subjects with no metabolic syndrome. It further reveals a statistically significant higher TG/HDL-C (p<0.0001) and LDL-C/HDL-C ratios in subjects diagnosed with metabolic syndrome, with no significant difference between TC/HDL-C ratios in both groups.

Table 3: Comparison of Lipid Ratios in Subjects With and Without Metabolic Syndrome

Category	Metabolic Syndrome		P value
	Yes (55)	No (55)	
TGs/HDL-C	4.35±1.53	3.08±0.76	**<0.0001**
TC/HDL-C	4.86±0.75	5.21±5.11	0.6163
LDL-C/HDL-C	3.03±0.61	2.91±0.68	0.3321

TGs: Triglycerides, HDL-C: High Density Lipoprotein- Cholesterol, TC: Total Cholesterol, LDL-C: Low Density Lipoprotein- Cholesterol.

4. Discussion

The current study (Table 2) noticed no significant differences among the LDL-C values measured by Friedewald equation (121.29±18.84mg/dl) and direct method (122.30±19.30mg/dl). Similarly, Knopfholz *et al.* (2014) found that Friedewald formula is a good method for estimating LDL-C in patients with metabolic syndrome. Furthermore, Sahu *et al.* (2005) reported that the use of Friedewald formula is a cost saving and reliable estimate of LDL-C when HDL-C and TC were determined.

However, Martin et al. (2013) observed that Friedewald equation tends to underestimate LDL-C in the setting of high TG levels, especially at low LDL-C levels, which could result in under treatment of high-risk patients, and further suggested additional evaluation, especially if TGs are ≥150mg/dl (>1.7mmol/l). Similarly, Charuruks & Milintagas (2005) indicated the direct measurement of LDL-C when TG ≥200mg/dl (2.26mmol/l) because they found that the direct method was more precise and accurate than Friedewald equation, even for TG levels between 200 and 399 mg/dl (2.26-4.49mmol/l). On the other hand, Piva & Fernandes (2008) observed that Friedewald formula tends to significantly overestimates LDL-C values when compared to direct measurement methods.

Moreover, some studies have shown that Friedewald equation can also display discrepancies in low TG values (Sahu *et al.* 2005, Piva & Fernandes 2008). When TG was <70mg/dl (0.8mmol/l), the estimated LDL-C using the Friedewald equation showed slightly lower values than that using the direct method (Piva & Fernandes 2008). Contradictory results have been demonstrated by Sahu *et al.* (2005), in which serum LDL-C using the Friedewald formula was higher than the homogeneous assay for TG < 100 or 200mg/dl (1.12 or 2.26mmol/l).

This shows the controversy exist between studies in comparing direct method with Friedewald formula. Various reasons for these differences might be in regard to other serum lipid levels, especially TGs; the influence of fasting and non-fasting samples on lipid measurement; and biochemical tests quality control in each laboratory. Moreover, it has been suggested that Friedewald formula is required to define the lipid phenotype (Yu *et al.* 2000).

Furthermore, Puavilai *et al.* (2009) found more accuracy in the new modified Friedewald equation, which is LDL =TC-HDL-1/6TG, to calculate serum LDL-C level from non-fasting TG levels, regardless of the time of last meal if serum TGs <300mg/dl (3.39mmol/l). Diabetes is the epitome of metabolic syndrome; however, new studies are demonstrating the limited efficacy of Friedewald formulae in diabetic patients. In diabetic patients, with or without insulin use, the Friedewald equation on average underestimates 8% serum LDL-C, but it can underestimate more than 10% in patients with TG levels between 200 and 400mg/dl (2.26-4.5mmol/l) (Hirany *et al.* 1997). Conversely, Whiting *et al.* (1997) observed that the Friedewald formula provide an accurate estimation of LDL-C among diabetic patients with plasma TGs <400mg/dl (<4.5mmol/1); and further suggested that the direct immunoseparation method significantly overestimated LDL-C at TGs between 178 and 400mg/dl (2-4.5mmol/1).

The study further reveals a statistically significant higher TG/HDL-C (4.35±1.53; p<0.0001) and LDL-C/HDL-C (3.03±0.61) ratios in subjects diagnosed with metabolic syndrome (Table 3). TG/HDL-C ratio especially more than four (Da luz *et al.* 2005) is a powerful predictor of total mortality independent of important prognostic variables including age, race, smoking, hypertension, diabetes, and severity of coronary artery disease; even stronger than TC/HDL-C and LDL-C/HDL-C (Bittner *et al.* 2009). The Copenhagen Male Study showed triglycerides on their own to be another strong risk factor, but found that stratifying TGs levels by HDL-C levels led to more accurate detection of increased coronary disease risk (Jespersen *et al.* 1998). The atherogenic link between high TGs and low HDL-C is due to the higher plasma concentration of TG-rich, very low-density lipoprotein that generates small, dense LDL during lipid exchange and lipolysis. These LDL particles accumulate in the circulation and form small, dense HDL particles, which undergo accelerated catabolism, thus closing the atherogenic circle (Da Luz et al. 2008). Thus, this ratio is an easy, non-invasive means of predicting the presence and extent of coronary atherosclerosis.

Limitation: The present study did not take into account the current use of medication or the inflammatory state of the patients. Since the commonly used statin, angiotensin converting enzyme inhibitors and angiotensin II receptor blockers may alter the inflammatory state, and may weaken the relationship between TC and LDL-C due to their action more on LDL-C and less on HDL-C and TGs. However, more studies using larger samples taken from different ethnic and geographic populations and preferably compared with reference method of ultracentrifugation and precipitation would further accomplish this work. Additionally, considering the different ranges of TGs will certainly give more information about the degree of inaccuracy of Friedewald equation.

5. Conclusion

TGs/HDL-C was found significantly higher among subjects with metabolic syndrome; however, no significant difference between both Friedewald equation and direct measurement method for LDL-C estimation was observed. Subsequent research including β-quantification by ultracentrifugation validation is required in order to explore the accuracy of LDL-C estimation formulas and direct methods in patients with the metabolic syndrome who exhibit abnormal abundance of small dense LDL-C particles. Hence, LDL-C estimation warrants consideration in contemporary patient care, as clinicians care for patients, as experts formulate clinical practice guidelines, and as investigators design future research studies.

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