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ANALYSIS OF LDL-C MEASUREMENT USING DIRECT AND FRIEDEWALD FORMULA IN TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT

LDL-C is important to evaluate the risk of cardiovascular disease. LDL-C can be measured directly or by using the Friedewald equation. Type 2 Diabetes Mellitus (DM) patients have tighter LDL-C target compared with normal population. This research is aimed to analyze the difference of LDL-C level measured by direct test and Friedewald equation in DM and non-DM. This research was a cross-sectional study using LDL-C data of 208 patients who were tested in Laboratory of Clinical Pathology, Hasanuddin University Hospital from a period of August 2015 to January 2016. LDL-C and other lipid were measured using ABX Pentra 400 meanwhile Friedewald LDL-C was calculated with equation LDLC= Total Cholesterol-HDL-C-(1/5 Triglycerides). Type 2 DM patients were diagnosed by ADA 2015 criteria or who had previous DM history. Friedewald LDL-C estimates lower than direct method (139.07+50.60 mg/dL vs 155.33+51.74 mg/dL, p=0.000). Delta of direct LDL-C and Friedewald equation measurement is higher in DM than non-DM patients (11.97+11.52% vs 8.49+11.27%, p=0.030) Fridewald LDL-C estimates LDL-C lower than direct method and the difference is wider in DM than non-DM. It is suggested to measure LDL-C directly in DM type 2 to reach the actual LDL-C target.

Key words: Direct LDL-C, Friedewald, diabetes mellitus

INTRODUCTION

Hypercholesterolemia is a common condition related to atherosclerosis. Low-density lipoprotein cholesterol (LDL-C) can be used as a marker of the major risk factor of cardiovascular events in the future of hyperlipidemia patients and as therapy goal in those patients. Accuracy and precision of LDL-C measurement are very important for coronary heart disease patients.[1]

Reference method of LDL-C measurement in serum is b-Quantitation procedure which needs an ultracentrifugation technique. This procedure is time consuming, expensive, and needs more volume of serum, therefore it is not suitable for routine testing. LDL-C quantification is often measured by two methods, direct measurement and quantification by using Friedewald Formula (FF) which is commonly accepted.[2]

Friedewald *et al.* in 1972 described a formula for estimating LDL-C value by using total cholesterol, High-Density Lipoprotein Cholesterol (HDL-C) and triglycerides measurements. The limitation of this formula is that the patients must be in a fasting condition and the level of triglycerides may not exceed 400 mg/dL.[3]

Type 2 Diabetews Mellitus (DM) is characterized by dyslipidemia. Dyslipidemia in diabetes shows low level of HDL-C, abnormal Very Low-Density Lipoprotein (VLDL), high triglycerides with normal or slightly increased of LDL-C, and total cholesterol. This condition is related to coronary heart disease.[4] Type 2 DM patients often have dyslipidemia, so routine LDL-C measurement is performed to stratify the risk factor of cardiovascular events in the future.[5] Low density lipoprotein cholesterol analysis is usually performed by using direct test or by calculating it using Friedewald Formula, therefore the accuracy of this formula in estimating exact LDL-C level in diabetes patients is needed to stratify cardiovascular risk and as therapy target.

In this study, measurement and comparison of LDL-C in type 2 diabetes mellitus patients using direct test and Friedewald Formula was conducted. The aim of this study was to evaluate the accuracy of this formula compared with the direct method.

METHODS

A cross-sectional study performed by taking data of diabetic and non-diabetic patients who were for tested LDL-C in the Hasanuddin University Hospital, Makassar from the period of August 2015 to January 2016. A direct test of LDL-C, total cholesterol, HDL-C, and triglycerides were performed by using ABX Pentra 400. Quantification with Friedewald Formula was performed by using an equation: LDL-C = Total cholesterol – HDL-C – trigycerides/5.[5] Type 2 diabetes mellitus patients were diagnosed by the American Diabetic Association (ADA) 2015 criteria or those who had a previous diabetic history. Non -diabetic subjects as a control were also recruited

for the comparison. LDL-C test was performed after at least 8 hours period of fasting. Samples with triglycerides exceeding 400 mg/dL were excluded. Normality of data were analyzed with the Kolmogorov-Smirnov test. Normally distributed data were percentage of rLDL while age, total cholesterol, HDL-C, LDL-C triglycerides, and LDL-C calculated with the Friedewald Formula (LDL-FF) were not normally distributed. rLDL was described as direct LDL minus LDL-FF while % rLDL was equated as ((LDL direct -LDL FF)/ LDL direct) x 100 % The difference of normally distributed data was measured with T-test while the Mann-Whitney test was used for abnormal distributed data.

RESULTS AND DISCUSSION

Total samples of this study were 208 subjects (Table 1) consisting of 90 type 2 diabetes mellitus patients and 118 non-diabetic patients. Male subjects were 85 patients (40.9%) while female were 123 patients (59.1%). Briefly, in both groups, the mean difference of direct LDL-C and LDL -FF (rLDL) was 16.26 ± 18.62 mg/dL and the mean percentage of difference was 9.99±11.48%. From all samples, 170 (81.73%) samples had a higher direct LDL-C level than LDL-FF, 37 (17.79%) had a higher LDL-FF than direct LDL-C meanwhile 1 (0.48%) sample had the same value of direct LDL-C and LDL-FF.

In the DM group (Table 2), direct LDL-C was higher than LDL-FF (156.63±58.58 vs. 138.07±60.94 mg/dL) with a mean difference 18.56±19.69 mg/dL or 11.97±11.52% meanwhile in the non-diabetic group the same was found, direct LDL-C was also higher than LDL-FF (154.34±46.08 vs. 139.83±41.28 mg/dL) with a mean difference of 14.51±17.63 mg/dL or 8.49±11.27%. The mean difference of direct LDL-C and LDL-FF was significantly higher in the diabetic group than non-diabetic one (p=0.030) with a percentage of difference of 3.48±1.59%.

Controlled LDL-C is one of the therapeutic targets in DM patients. LDL-C target in DM patients was tight, and considered the same as patients with coronary heart disease.[5] Measurements of LDL-C are commonly performed by direct method or by calculated Friedewald formula using total cholesterol, HDL-C and triglycerides for estimating LDL-C level. Fridewald formula uses Triglycerides/5 equation (in mg/dL) for estimating Vey Low-Density Lipoprotein Cholesterol (VLDL-C) because VLDL-C carries most of circulating triglycerides. This formula is often used by most clinical laboratories because it is simple, easy to perform and can reduce cost. Unfortunately, this formula has several limitations such as chylomicron and triglycerides level of >400 mg/dL may caused tendency of false low calculated LDL-C than the exact level and must be performed in a fasting condition.[1]

In type 2 DM patients, dyslipidemia commonly occurs, marked by low HDL-C, abnormal VLDL-C and high triglycerides.[4] The high level of triglycerides reported has a correlation with false low calculated LDL using Friedewald formula. Even though direct LDL and LDL-FF has a strong correlation but the difference of both is wider as the triglycerides level increases, and the correlation is weakened as the triglycerides level exceeds 400 mg/dL.[6-8] In the children population with normal and dyslipidemia lipid profile, the same finding was also reported. Majority of calculated Friedewald Formula gave a false lower LDL-C calculation than the direct method in children.[9]

Table 1. Characteristics of samples

Variable	Mean±SD
Age (years)	57.06±9.36
Cholesterol total (mg/dL)	214.04±54.69
HDL-C (mg/dL)	50.07±14.28
Triglycerides (mg/dL)	124.50±64.37
LDL-C (mg/dL)	155.33±51.74
LDL-FF (mg/dL)	139.07±50.60
ΔLDL (mg/dL)	16.26±18.62
%ΔLDL (%)	9.99±11.48

In this study, a significant difference between direct method and Friedewald Formula was found. There were 81.73% samples which had lower LDL-FF than direct LDL-C. This finding was consistent with Garoufi et al. who reported that 75.6% of children with normal cholesterol and 77.3% of children with dyslipidemia had LDL-FF lower than direct LDL-C.[9]

Table 2. The difference of age, lipid profile, LDL-C, LDL-FF, and ΔLDL in DM, and

Variable	Non-DM	DM	p
	Mean±SD	Mean±SD	
Age (years)	56.94±10.12	57.21±8.31	0.825[a]
Total Cholesterol (mg/dL)	212.93±43.15	215.48±67.02	0.543[a]
HDL-C (mg/dL)	49.86±14.18	50.36±13.90	0.754[a]
Triglycerides (mg/dL)	116.24±59.33	135.32±69.29	0.078[a]
LDL-C (mg/dL)	154.34±46.08	156.63±58.58	0.75[a]
LDL-FF (mg/dL)	139.83±41.28	138.07±60.94	0.204[a]
ΔLDL (mg/dL)	14.51±17.63	18.56±19.69	0.106[a]
%ΔLDL (%)	8.49±11.27	11.97±11.52	0.030[b]

[a] Mann-Whitney test
[b] T test

The difference of direct and calculated Friedewald LDL-C mean was increased as the increase of triglycerides and fasting glucose level in type 2 DM patients.[10-12] Viera *et al.* reported that the difference of LDL-C was higher in patients with HbA1c >8% than those with HbA1c <8%.[12] Contrary, Kopfholz *et al* reported that there was no significant difference of direct and calculated Friedewald LDL-C in metabolic syndrome patients at triglycerides levels <150 mg/dL and >150 mg/dL.[13]

One of the long-term diabetes mellitus complication is coronary heart disease. Risk of coronary heart disease can be measured with LDL-C so the accuracy of LDL-C measurement is important for evaluating cardiovascular risk and therapy. In this study, was found that calculated LDL-C using Friedewald Formula mostly gave false lower LDL-C value, therefore, this formula was not reccomended to be used for evaluation of cardiovascuar risk in type 2 DM patients. The researchers suggested a direct method for LDL-C measurement.

Evaluation of several formulas for estimating LDL-C is suggested and compare them with a direct method as alternating of Friedewald Formula which seems not ideal to be used in type 2 DM population.[14-17]

CONCLUSION AND SUGGESTION

The majority of calculated LDL-C levels using Friedewald Formula showed false low LDL value than the direct method and the mean difference was higher in type 2 DM patients than in non-diabetic ones. The researcher suggest the use of direct method to measure LDL-C for evaluation of cardiovascular risk in type 2 DM patients.

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