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RESEARCH

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**COMPARISON OF sLDL-C ANALYSIS USING SRISAWASDI METHOD AND HOMOGENEOUS ENZYMATIC ASSAY METHOD ON HYPERTRIGLYCERIDEMIA CONDITION**

*(Perbandingan Analisa sLDL-C metode Srisawasdi dan Homogeneous Enzymatic Assay di Kondisi Hipertrigliseridemia)*

**Gilang Nugraha<sup>1</sup>, Soebagijo Poegoeh Edijanto<sup>2</sup>, Edhi Rianto<sup>3</sup>**

**ABSTRAK**

Small Dense Low Density Lipoprotein (sdLDL) merupakan fraksi terkecil dari partikel Low Density Lipoprotein (LDL) yang memiliki diameter  $\leq 25,5$  nm. Partikel sdLDL merupakan lipoprotein sangat aterogenik bahkan telah dilaporkan meningkatkan bahaya Penyakit Jantung Koroner (PJK) hingga tiga kali lipat. Pengukuran sdLDL dilakukan dengan alat dan teknik yang rumit sehingga kurang cocok diterapkan dalam praktek klinis sehari-hari. Tahun 2011, Srisawasdi dkk mengembangkan teknik pengukuran perkiraan sdLDL-cholesterol (sdLDL-C) menggunakan persamaan dengan menghitung profil lipid rutin. Dilaporkan bahwa peningkatan kepekatan trigliserida (TG) menurunkan keasaban perkiraan sdLDL-C Srisawasdi. Penurunan nilai keasaban dapat mempengaruhi ketepatan yang mengakibatkan penurunan mutu pemeriksaan laboratorium. Diambil 88 sampel yang dilakukan pengukuran Total Cholesterol (TC), TG, high density lipoprotein-cholesterol (HDL-C) dan direk low density lipoprotein-cholesterol (dLDL-C) di RSUD Dr. Soetomo, sdLDL-C metode homogeneous enzymatic assay dilakukan di Laboratorium Parahita Dharmawangsa. Hasil analisis menunjukkan, tidak ada perbedaan hasil pemeriksaan sdLDL-C formula Srisawasdi dkk dengan metode homogeneous enzymatic assay ( $P=0,000$ ). Penurunan nilai keasaban ditemukan di kelompok kepekatan TG  $<100$  mg/dL sampai dengan kelompok kepekatan TG 200-299 mg/dL. Perbedaan nilai keasaban di setiap kelompok TG tidak mempengaruhi ketepatan pemeriksaan sdLDL-C formula Srisawasdi ( $P=0,720$ ) hingga kepekatan TG  $<400$  mg/dL, dengan nilai bias pada seluruh sampel yaitu 34,15%. Keterbatasan sdLDL-C formula Srisawasdi dkk hanya dapat digunakan di kepekatan TG kurang dari 200 mg/dL dengan pemantapan mutu intralaboratorium yang terkendali baik. Saran penelitian, perlu diteliti lebih lanjut untuk menentukan nilai normal sdLDL-C formula Srisawasdi.

**Kata kunci:** sdLDL-C, formula Srisawasdi, keasaban, ketepatan

**ABSTRACT**

Small dense low-density lipoprotein (sdLDL) is the fraction of the smallest particle of low density lipoprotein (LDL) which has a diameter of  $\leq 25.5$  nm. SdLDL particles are highly atherogenic lipoprotein and have even been reported to increase the risk of Coronary Heart Disease (CHD) up to three times. Unfortunately, sdLDL measurements are usually made by complex instruments and intricate techniques, thus making them less suitable to be applied in everyday clinical practice. In 2011, Srisawasdi *et al* developed a measurement technique to estimate sdLDL-cholesterol (sdLDL-C) using an equation by counting routine lipid profile. It was reported that the increase in the concentration of triglycerides (TG) can lower the estimated correlation of sdLDL-C proposed by Srisawasdi. A decline in correlations can affect the accuracy, resulting in decreased quality of laboratory tests. Therefore, this research aimed to evaluate the examination of sdLDL-C using Srisawasdi's formula on hypertriglyceridemia condition. Eighty-eight samples were taken to measure Total Cholesterol (TC), TG, High Density Lipoprotein-Cholesterol (HDL-C) and direct low density lipoprotein-cholesterol (dLDL-C) at Dr. Soetomo Hospital. Meanwhile, measurement of sdLDL-C using homogeneous enzymatic assay method was performed at Parahita Laboratory in Dharmawangsa. Results of the analysis show that there was no difference between sdLDL-C using Srisawasdi's formula and sdLDL-C using homogeneous enzymatic assay method ( $P=0.000$ ). A decline in correlation found in from the TG concentration group of  $<100$  mg/dL to the TG concentration group of 200-299 mg/dL. The difference correlation

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value in each group, however, did not affect the accuracy of sdLDL examination using Srisawasdi's formula ( $P=0.720$ ) in up to the TG concentration group of  $<400$  mg/dL, with a bias in the whole sample of 34.15%. There are also some limitations of sdLDL-C using Srisawasdi's formula. For instance, it can only be used on a TG concentration of less than 200 mg/dL with well-controlled intralaboratorium quality assurance. Thus, further researches need to be conducted to determine the normal value of sdLDL-C using Srisawasdi's formula.

**Key words:** sdLDL-C, Srisawasdi's formula, correlation, accuracy

## INTRODUCTION

Cardiovascular disease (CVD) has been the first disease causing death in the world. In 2012, the number of people who died from CVD reached 17.5 million people (31%), consisted of 42.3% of CHD cases and 38.3% of stroke cases.<sup>1</sup> In Indonesia, the prevalence of stroke in 2013 was estimated to reach 7.0% to 12.1 % while the prevalence of CHD was approximately 0.5% to 1.5%.<sup>2</sup>

Cardiovascular disease usually occurs due to the formation of atherosclerotic plaque caused by LDL.<sup>3</sup> But, other researchers found many patients with CVD had normal levels of LDL-C.<sup>4-6</sup> The population of LDL reported is heterogeneous with a diameter of  $\leq 25.5$  nm related closely with CVD because they are highly atherogenic to increase CHD risk up to three times, known as *small dense* LDL (sdLDL).<sup>6-8</sup>

SdLDL measurements can be performed with a variety of techniques, such as using ultracentrifuge, electrophoresis, nuclear magnetic resonance, ion mobility analysis, dynamic light scattering, and photometer.<sup>9-10</sup> Many clinical laboratories implement examinations with photometer technique using *homogeneous enzymatic assay* method, consisted of two stages. At the first stage, chylomicron elimination occurs, in which VLDL, IDL, lLDL and HDL change into water and oxygen by cholesterol esterase, cholesterol oxidase and catalase enzymes. At the second stage, sdLDL-C reacts into colored compounds by cholesterol esterase, cholesterol oxidase and peroxidase enzyme and then measured at a wavelength of 600nm.<sup>7,11,12</sup> The method has a complicated construction technique, especially without the use autoanalyzer, the need of laboratory instrument and expensive fees that can add to the cost of patients' examinations.

In 2011, Srisawasdi *et al* developed an examination technique by estimating sdLDL-C levels using the results of the lipid profile in the population of Thailand at Ramathibodi Hospital. The use of sdLDL-C using Srisawasdi's formula can provide an advantage because it does not require laboratory instrumentation as well as uses special reagents and routine lipid profile test results that can be easily applied, especially in clinical

laboratories as simple as basic clinical laboratories and health centers that have limited abilities of laboratory tests. Another advantage of this method is that it can reduce the cost of laboratory tests that can help patients who are poor.

Equation set by Srisawasdi *et al* is as a follow:

$$\text{sdLDL-C (mg/dL)} = 0,580 (\text{non-HDL-C}) + 0,407 (\text{dLDL-C}) - 0,719 (\text{cLDL-C}) - 12,05$$

CLDL-C value is determined using the Friedewald formula,  $\text{LDL-C (mg/dL)} = \text{TC} - \text{HDL-C} - \text{TG}/5$ .<sup>13</sup>

The measurement of sdLDL-C using Srisawasdi's formula needs to be evaluated before it is used widely outside of Thailand since the determination of the calculation parameters has some risks of precision and accuracy examination, such as the number of parameters used in the formula, which means the more number is used, the higher error can occur. In addition, Srisawasdi uses cLDL-C proposed by Friedewald that has limitations on hypertriglyceridemia condition due to using the constant of five dividing TG that can provide imprecise results of VLDL calculation, thus leading to the wrong result.<sup>14,15</sup> Therefore, Srisawasdi reported a decline in the correlation of lipid profile parameters, namely TC, TG, HDL-C and dLDL-C against the estimated value of sdLDL-C at the triglyceride concentration of 200–400 mg/dL and a decline in the correlation value in triglycerides over 400 mg/dL.<sup>13</sup>

A decline in correlation value on a method actually will affect the accuracy of the examination and cause reduction in quality of laboratory tests. As a result it cannot help in establishing the diagnosis. If this problem cannot be solved then the low quality of laboratory tests may not help to reduce the number morbidity and mortality caused by sdLDL-C.

Therefore, this research aimed to compare the results of sdLDL-C measurements using Srisawasdi's formula and the results of sdLDL-C measurements using *homogeneous enzymatic assay* method. There is no difference in accuracy of sdLDL-C using Srisawasdi's formula as a bias of the examination on hypertriglyceridemia condition.

## METHODS

Subjects of this research were patients undergoing routine lipid profile examination at Clinical Pathology of Integrated Diagnostic Center at Dr. Soetomo Hospital (Instansi Patologi Klinik Gedung Pusat Diagnostik Terpadu RSUD Dr. Soetomo Surabaya). Samples of serum then were taken from patients who fasted 10 to 12 hours with TG less than 400 mg/dL. Eighty-eight samples were taken in sequence and divided into four groups based on the concentration of TG. Each group consisted of 22 samples.

Next, all the serum samples were measured for levels of TC, TG, HDL-C, and dLDL-C by *autoanalyzer Siemens Dimension® RxL Max® clinical chemistry system* using *Flex® reagent cartridge* with enzymatic method from Monday to Friday in Dr. Soetomo Hospital. The results of the serum examination then were divided into four research groups consisted of 22 samples for each, namely the TG group of <100 mg/dL (group I), the TG group of 100-199 mg/dL (group II), the TG group of 200-299 mg/dL (group III) and the TG group of 300-399 mg/dL (group IV). There were three *autoanalyzers* used in this study.

Afterward, SdLDL-C level measurement was performed in the Parahita *Laboratory Diagnostic Centre*, Surabaya using TMS 24i Premium with Randox reagents enzymatically on Monday and Thursday. The serum samples not immediately examined were stored in a freezer with a temperature of  $-70^{\circ}\text{C}$ .

Examination of non-HDL-C and cLDL-C then was conducted by calculation. The concentration of non-HDL-C obtained from non-HDL-C (mg/dL)=TC-HDL-C, whereas the concentration of LDL-C (mg/dL) was found by using the formula of Friedewald: cLDL-C (mg/dL)=TC-HDL-C-(TG/5).

Finally, a statistical analysis, paired t-test, was performed to determine whether there was a difference between sdLDL-C using Srisawasdi's formula and sdLDL-C using *homogeneous enzymatic assay* method as referent methods. Next, accuracy test was also conducted to determine the effects of increased TG levels of the accuracy of sdLDL-C using Srisawasdi's formula. ANOVA test then was carried out to establish determine whether there were an increase and decrease in the accuracy values of sdLDL-C in each group using Srisawasdi's formula affected by the concentration of TG. The last, correlation and linear regression tests were performed to determine the correlation between the estimated sdLDL-C using Srisawasdi's formula as well as their variables and the measurement of sdLDL-C using *homogeneous enzymatic assay* method.

## RESULTS AND DISCUSSION

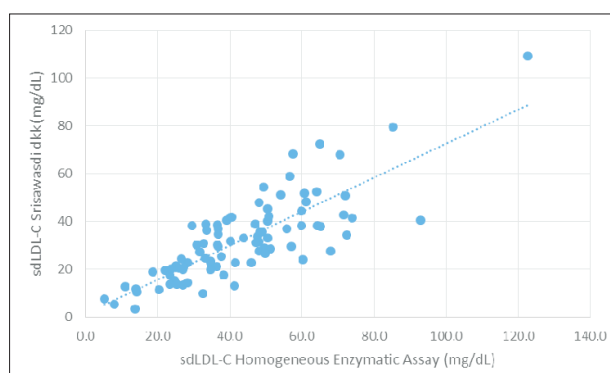
A total of 88 serum samples was taken for the research. The samples consisted of 47 female and 41 male patients at the average age of 53 years with a range of 16-76 years. Characteristics of lipid profile in this research are summarized in Table 1.

**Table 1.** Characteristics of lipid profile of the research samples

PARAMETERS	MEAN (mg/dL)	SD	RANGE (mg/dL)
Total Cholesterol	199	57.85	80-471
Triglycerides	201	108.70	20-398
HDL-C	41	13.50	5-82
dLDL-C	119	43.98	42-335
cLDL-C	118	50.29	32-342
Non-HDL-C	158	57.55	45-421
<i>homogeneous</i> sdLDL-C	32.3	17.25	3.2-109.1
sdLDL-C using Srisawasdi	43.4	19.61	5.4-122.7

Based on the results of T-test, there was no difference in the concentration values of sdLDL-C determined using Srisawasdi's formula and using homogeneous enzymatic assay method as a referent method ( $P=0.000$ ). Correlation analysis of sdLDL-C using Srisawasdi's formula was conducted to determine how well Srisawasdi's formula in determining the level of actual sdLDL-C in the blood by determining how strong the correlation of sdLDL-C using Srisawasdi's formula and sdLDL-C directly measured by homogeneous enzymatic assay as a referent method.

Figure 1 shows that sdLDL-C using Srisawasdi's formula and sdLDL using *homogeneous enzymatic assay* method had a positive correlation. But, the correlation became weaker as the increasing concentrations of sdLDL-C. The best correlation in sdLDL-C was <40.0 mg/dL as shown in the following scatter diagram.



**Figure 1.** The scatter diagram of sdLDL-C using *homogeneous enzymatic assay* method and Srisawasdi's formula.



**Table 2.** Correlation of sdLDL-C using Srisawasdi's formula and sdLDL-C using *homogeneous enzymatic assay* method on each group

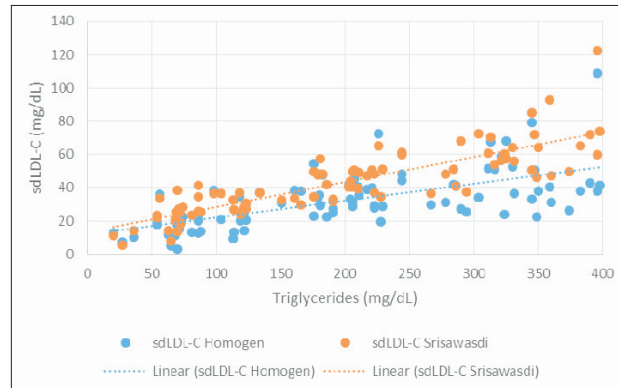
	Group I (<100 mg/dL)	Group II (100-199 mg/dL)	Group III (200-299 mg/dL)	Group IV (300-399 mg/dL)
Coefficient Correlation	0.670	0.568	0.513	0.709
Sig	0.001	0.006	0.015	0.000

Correlation of sdLDL-C using Srisawasdi's formula in each research group is shown in Table 2. Impairment correlation occurred in from the TG concentration group of <100 mg/dL to the TG concentration group of 200–299 mg/dL. The table also shows that the correlation value in the TG concentration group of 300–399 mg/dL was higher than other groups.

The results of this research, moreover, show that increased TG levels could significantly affect the correlation of an examination. Nevertheless, there was a discrepancy in the correlation value in this research compared to the findings of the research conducted by Srisawasdi *et al.* There was an increase in the coefficient value of the TG concentration group of 300–399 mg/dL (0.513), which should be lower than the TG concentration group of 200–299 mg/dL (0.709).

The discrepancy found in the correlation value can be caused by variation in lipid profiles caused by the use of different auto-analyzers in lipid profile analysis and the high various coefficient values of the lipid profile examinations on the parameters of HDL-C, still above 5%. The various coefficient values can also be caused by the use of cLDL-C using Friedewald causing various results in the TG concentration group of >200 mg/dL.<sup>16</sup>

In addition, accuracy is defined as the bias value of sdLDL-C using Srisawasdi's formula as the referent method. The bias value of sdLDL-C using Srisawasdi's formula on the entire research samples was 34.15%, higher than the other referent method. Figure 2 shows the effect of TG on the accuracy of sdLDL-C using Srisawasdi's formula. The table show that the accuracy of sdLDL-C using Srisawasdi's formula decreased (increased bias value) as the increasing concentrations of TG. The decline in the accuracy can be seen in the linear line of sdLDL-C using Srisawasdi's formula, far from the linear line of sdLDL-C using *homogeneous*



**Figure 2.** The scatter diagram of sdLDL-C using *homogeneous enzymatic assay* method and Srisawasdi's formula against triglycerides.

*enzymatic assay* as the increasing concentration of TG.

The accuracy of sdLDL-C using Srisawasdi's formula in each research group is shown in Table 3. This table shows that a decline in bias values from the TG concentration group of <100 mg/dL to the TG concentration group of 100-199 mg/dL. It means increased accuracy triggered an increase in bias values from the TG concentration group of 100–199 mg/dL to the TG concentration group of 200–299 mg/dL. In other words, a decline in accuracy caused a decline in bias values up to the TG concentration group of 300–399 mg/dL.

Based on the observation of the correlation values and the accuracy values, it is known that there was no effect of the correlation values on the accuracy value in increased levels of TG because of different results. In groups II and IV, the correlation values were in the opposite direction to the accuracy of inspection, while group III had a direct correlation value with the accuracy.

**Table 3.** The bias values of sdLDL-C using Srisawasdi's formula against sdLDL-C using *homogeneous enzymatic assay* method

	Group I (<100 mg/dL)	Group II (100-199 mg/dL)	Group III (200-299 mg/dL)	Group IV (300-399 mg/dL)
Bias Value (%)	+39.3	+26.1	+30.8	+40.4

Next, ANOVA test was performed to determine differences in accuracy of each research group. The results show that there was no significant difference in accuracy values of each TG concentration group (P=0.720). The results of the ANOVA test prove that the increased TG concentration up to 400 mg/dL does not affect the accuracy of sdLDL-C using Srisawasdi's formula. The differences in the values occur as a variation in the calculation.

Differences between the accuracy values and correlation values can be caused by the use of the referent method using Srisawasdi *et al* in determining the estimation equation of sdLDL-C. Srisawasdi *et al* uses *homogeneous enzymatic assay* method which is basically not the gold standard method. *Homogenous enzymatic assay* method can only measure sdLDL-C on a density of >1.044 g/mL, while sdLDL-C based on the gold standard method of *density gradient ultracentrifugation* which has a density of  $\geq 1.034$  g/mL. It means that sdLDL-C cannot be measured in a *homogenous enzymatic assay* method if the density is from 1.034 to 1.044 g/mL. There also has been no report about sdLDL density range that can be measured with Srisawasdi formula. But the density difference can affect the accuracy, resulting in giving the quite high bias values and the various correlation values.

Afterward, multiple linear regression analysis was conducted on sdLDL-C using *homogeneous enzymatic assay* method as the dependent variable and non-HDL-C, dLDL-C, as well as cLDL-C as independent variables. The results show the correlation coefficient was 0.852 and the determination coefficient was 0.725 with P=0.000.

Moreover, Table 4 shows that the concentration of sdLDL-C using Srisawasdi's formula in this research was significantly affected by non-HDL-C and cLDL-C, whereas the concentration of dLDL-C did not affect significantly the concentration of sdLDL-C using Srisawasdi's formula.

Furthermore, the results of multiple linear regression analysis using non-HDL-C, dLDL-C and

cLDL-C variables against sdLDL-C using *homogeneous enzymatic assay* method show that dLDL-C variable did not significantly affect sdLDL-C using *homogeneous enzymatic assay* method. Hence, it can be expected that these factors can contribute to errors in determining the estimated values of sdLDL-C using Srisawasdi's formula.

Similarly, the results of a research conducted by Cho Y *et al*<sup>17</sup> on the estimation of sdLDL-C using Srisawasdi's formula in healthy patients and metabolic syndrome in Korea show that dLDL-C variable did not significantly affect the coefficient value of determination (0.625).<sup>17</sup> Meanwhile, the coefficient value of determination in this research population was 0.725 better than in the Korean population, but still below the coefficient of determination specified by Srisawasdi *et al* (0.879).

In addition, multiple linear regression analysis shows differences in the coefficient values of determination in researches conducted in Thailand, Korea, and Indonesia. One factor causing incompatibility in the correlation of the estimation accuracy of sdLDL-C using Srisawasdi's formula may be related to the ethnic factor. However, a research showing the effects of ethnicity on sdLDL-C using Srisawasdi's formula still has not been reported. But, the research on differences in sdLDL particle in Asian ethnicity has been reported by Annurad E *et al*<sup>18</sup> showing that there are differences in sdLDL caused by high carbohydrate diet.<sup>18</sup> It means that indirect estimation of sdLDL-C can be affected by ethnicity through routine lipid profile due to the different concentration of the routine lipid profile in each ethnic.<sup>19</sup>

Another factor influencing the discrepancy in the declined correlation values of sdLDL-C estimation using Srisawasdi's formula is a screening method. Srisawasdi *et al* conducted a measurement of TC, TG, and HDL-C according to the standards derived from *Centers for Disease Control and Prevention-National Heart, Lung and Blood Institute Lipid Standardization Program*, but not for dLDL-C.<sup>11</sup> The use of the methods that are not standard can cause bias so that the values of dLDL-C in this research and in the research conducted by Cho Y *et al*<sup>17</sup> become insignificant. Therefore, to minimize the factors, quality assurance on the parameters dLDL-C is necessary with very low percentage of CV.

In addition, Table 5 shows that there was no significant correlation between sdLDL-C using Srisawasdi's formula and the concentration groups TG of <100 mg/dL and 100–199 mg/dL (P<0.05) with the correlation coefficients of 0.659 and 0.600. Similarly, there was also no significant correlation between sdLDL-C using Srisawasdi's formula and

**Table 4.** Correlation and Effects of Lipid Parameters Measured Against sdLDL-C using *Homogeneous Enzymatic Assay* method

VARIABLES	REGRESSION		
	Variable coefficients	Std. error	Sig
Constant	-8.302	3.097	0.009
Non-HDL-C	0.450	0.051	0.000
dLDL-C	-0.009	0.080	0.907
cLDL-C	-0.249	0.068	0.000



**Table 5.** Correlation of TG and sdLDL-C using Srisawasdi's formula in each research group

Group of Triglycerides	Correlation Coefficients	Sig
<100 mg/dL	0.659	0.001
100–199 mg/dL	0.600	0.003
200–299 mg/dL	0.137	0.545
300–399 mg/dL	0.327	0.137

the concentration groups TG of 200-299 mg/dL and 300–399 mg/dL ( $P>0.05$ ) with the correlation coefficients of 0.137 and 0.327.

Based on the correlation analysis, it is known that the concentration of TG used in setting sdLDL-C using Srisawasdi's formula in this research was <200 mg/dL. Finally, the results of ANOVA analysis show that there was no difference in the accuracy between the TG concentration group of <100 mg/dL and the TG concentration group of 100–199 mg/dL.

## CONCLUSION AND SUGESSTION

Based on the results of this research, it can concluded that changes in correlation values during hyper-triglyceride condition in determining estimation of sdLDL-C using Srisawasdi's formula do not affect the accuracy of the examination since there are some limitations in sdLDL-C using Srisawasdi's formula. First, it can only be used at a concentration of triglycerides less than 200 mg/dL. Second, determination of intra-laboratorial quality of lipid profile should be well controlled. Thus, further researches are needed to determine the normal value of sdLDL-C using Srisawasdi's formula since the bias value of sdLDL-C using Srisawasdi's formula is 34.15% higher than the value using *homogeneous enzymatic assay* method.

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