INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND
MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

CONTENTS

RESEARCH

SMALL DENSE LOW DENSITY LIPOPROTEIN WITH ANGIOGRAPHICALLY ATHEROSCLEROSIS IN CORONARY HEART DISEASE

(Small Dense Low Density Lipoprotein dengan Aterosklerosis Secara Angiografi di Penyakit Jantung Koroner)

Yuliani Zalukhu[1], Siti Muchayat Purnamaningsih[1], Nahar Taufik[2], Suwarso[1]

ABSTRAK

Penyakit Jantung Koroner (PJK) merupakan penyebab kematian utama di berbagai negara maju maupun yang berkembang dan biasanya terjadi bagi mereka yang memiliki kadar kolesterol tinggi, serta berusia lanjut. Namun baru-baru ini, penyakit jantung koroner lebih sering terjadi pada usia muda dan kadar kolesterol yang normal. Beberapa telitian menunjukkan bahwa LDL terdiri atas tujuh subtype yaitu small dense Low Density Lipoprotein (sd-LDL) merupakan subtipe LDL yang jauh lebih aterogenik, sehingga sangat mudah menyebabkan aterosklerosis. Tujuan penelitian ini adalah untuk mengetahui apakah terdapat hubungan antara sd-LDL dan persentase aterosklerosis secara angiografi di penyakit jantung koroner. Rancangan penelitian adalah potong lintang, subjek penelitian 54 pasien PJK dipilih secara berurutan, yang menjalani pemeriksaan angiografi di RSUP. Dr. Sardjito, Yogyakarta. Penyakit jantung koroner ditetapkan berdasarkan gejala klinis dan pemeriksaan EKG, aterosklerosis ditetapkan dengan pemeriksaan angiografi, sedangkan sd-LDL merupakan angka banding LDL-C/Apo-B yang <1,2 diukur dengan metode enzimatik homogeneous dan Immunoturbidimetry. Kenasaban antara sd-LDL dengan aterosklerosis dianalisis dengan uji Spearman. Pada penelitian ini terteliti terdiri atas 37 laki-laki dan 17 perempuan dengan gejala terbanyak nyeri dada 47(87%), diagnosis didominasi oleh angina pektoris stabil 49(90,8%) dan pengobatan paling banyak adalah golongan statin. Ciri angka banding LDL-C/Apo-B serta persentase aterosklerosis subjek penelitian ditunjukkan di Tabel 4, terlihat bahwa sd-LDL mempunyai rerata 1,06 dengan nilai minimal 0,81 dan maksimal 1,16, serta large buoyant LDL memiliki rerata 1,34 dengan nilai minimum 1,20 dan nilai maksimum 1,48, sedangkan persentase aterosklerosis bernilai rerata 46,68% dengan nilai minimal 0% dan maksimal 100%. Ditemukan kenasaban negatif, sedangkan yang bermakna antara small dense Low Density Lipoprotein (sd-LDL) dengan persentase aterosklerosis secara angiografi (r=-0,451; p=0,014).

Kata kunci: *sd-LDL, aterosklerosis, penyakit jantung koroner, angiografi, potong lintang*

ABSTRACT

Coronary Heart Disease (CHD) is one of the leading cause of death in developed as well as the developing countries and usually occurs in a population with high cholesterol level, as well as elderly. However, recently CHD occur more often in young age who has normal cholesterol levels. Some studies showed there was an indication that LDL consisted of seven different subfractions (subtypes) based on its scale, density and cholesterol composition. Small dense low density lipoprotein (sd-LDL) is much more atherogenic LDL, so it is very easy to cause atherosclerosis. This study was conducted to find out the correlation between sd-LDL and the percentage of atherosclerosis in coronary heart disease by assessing them. This study used a cross-sectional design, with 54 subjects consecutively enrolled in this study at Dr. Sardjito Hospital, Yogyakarta, who underwent angiography examination. Angiography was performed to diagnose atherosclerosis. CHD was determined based on the clinical picture and ECG, meanwhile sd-LDL was measured by immunoturbidimetry assay method. To find the correlation between sd-LDL and atherosclerosis, Pearson correlation was used if the data were not skewed or Spearman correlation if the data were skewed. The data distribution normality was tested with Kolmogorov Smirnov, p<0.05. This study consisted of 37 males and 17 females with the most common symptom was chest pain (87%). The diagnosis of stable angina pectoris was found in 49 subjects (90.8%) and most of them underwent a therapy known as statin. Characteristics of the ratio between LDL-C/ApoB and the atherosclerosis percentage in the study subjects were shown the Table 4, in which the mean of sd-LDL was 1.06 with the minimum value of 0.81 and the maximum value of 1.16 and the mean of large buoyant of LDL was 1.34 with the minimum value of 1.20 and the maximum value of 1.48, while the mean of the atherosclerosis percentage

---

[1] Department of Clinical Pathology, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito Hospital, Yogyakarta, Indonesia.
   E-mail: pkugm@yohoo.com
[2] Department of Heart Disesase, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito Hospital, Yogyakarta, Indonesia.

was 46.68% with the minimum value of 0% and the maximum value of 100%. The study found a significant negative correlation between small dense low-density lipoprotein (sd-LDL) and angiographically atherosclerosis percentage (r=-0.451, p=0.014).

Key words: sd-LDL, atherosclerosis, coronary heart disease, angiography, a cross-sectional

INTRODUCTION

Coronary Heart Disease (CHD) is a health problem in both the developing and developed countries such as Indonesia. Coronary heart disease is the leading cause of death worldwide. According to data from the World Health Organization (WHO), death due to CHD worldwide in 1999 reached 7.1 million and in 2001 it reached 7.2 million, in which between 40–75% died before reaching the hospital and in 2020 it is estimated to increase to 11.1 million.[1]

The data from Household Health Survey showed that death due to CHD continued to increase from year to year. In 1957, it was at the eleventh place (1–3%), in 1980 it was at the fourth place (5.2%), in 1986 it was at the second place (9.77%), in 1992 it was at the first place (16.4%) and in 1995 it was still at the first place (26%). Developing as well as developed countries, such as Indonesia, CHD is the leading cause of death.[2]

Atherosclerosis or the formation of plaque on the walls of blood vessels is a process that occurs slowly for years, which ultimately leads to CHD. The process of atherosclerosis begins with the non-functioning of the endothelial due to CHD risk factors mainly sd-LDL. This condition causes monocytes and sd-LDL easily enter the endothelium. Small dense low-density lipoprotein that has been trapped is then oxidized so that monocytes that have turned into macrophages will seize or phagocyte sd-LDL. Small dense low-density lipoprotein accumulates in macrophages to form foam cells that are increasingly large and form plaque or atherosclerosis. Small dense low-density lipoprotein is a small and dense LDL particle, making it easy to get into the blood vessel. It is atherogenic because it is very easily oxidized and has a high affinity for LDL receptors in the liver and has a high affinity for proteoglycans on the walls of arteries mediated by Apo-B.[3]

Each lipoprotein (chylomicrons, VLDL, IDL, LDL) contains only one apo-B molecule and more than 90% of Apo-B contained in LDL. The level of total Apo-B total reflects the total number of Apo-B lipoprotein and the total number of atherogenic particles. There still is no simple and inexpensive examination of sd-LDL, but sd-LDL can be estimated based on the ratio of LDL cholesterol (LDL-C)/apolipoprotein B (Apo-B) which is less than 1,2.[4]

The presence of atherosclerosis in the coronary artery can be ascertained through angiography using a special program, either automatically or manually, to determine the atherosclerosis percentage.[5]

The aim of this study was to the correlation between small dense low-density lipoprotein (sd-LDL) and the angiographically atherosclerosis percentage in coronary heart disease by determined.

METHODS

This study was an analytical observational study with cross sectional design conducted at the Cardiology Department, Clinical Laboratory Unit and Radiology Department of Dr. Sardjito Hospital. The research was conducted from September to November 2010. The cross-sectional design was intended to see the correlation between sd-LDL and the angiographically atherosclerosis percentage related to CHD.

Inclusion criteria were patients with CHD clinically who underwent coronary heart angiographiarteri with or without atherosclerosis, both males and females. The exclusion criterion was pregnant woman. The minimum sample size in this study was 54.

Atherosclerosis was measured by two (2) trained cardiographers using Philips Integris 5000, XTV 16 angiography. If the results of the reading were different and the difference was more than 10%, the reading would be performed by a cardiologist who had also been trained to use the same equipment. The measuring result was the summation of the mean of angiography measurement by atherosclerosis in percentage (%). If atherosclerosis was found in more than one coronary artery, the atherosclerosis percentage would be summed up and then divided by the number of arteries with atherosclerosis.

Sd-LDL examination was performed indirectly in which if the ratio of LDL cholesterol or LDL-Cholesterol (LDL-C)/Apolipoprotein B 100 or apolipoprotein B (apo-B) was <1.2, known as sd-LDL. The examination used Hitachi 902 in which sd-LDL was examined using homogeneous enzymatic method and apo-B test with Immunoturbidimetry.

A statistical analysis was carried out with SPSS version 2012. Mann Whitney test was used for continuous comparison. Spearman correlation test was carried out to correlate sdLDL with the angiographically atherosclerosis percentage.

RESULTS AND DISCUSSION

Examination of LDL-C and Apo-B was carried out with pooling system. Within one month of study, 54 samples that met the inclusion criteria were obtained. Samples were collected every day and processed immediately into serum and then stored in a freezer for approximately one month at -80° C. The second phase was observation in which prior to the examination of sd-LDL (LDL-C and Apo-B), calibration, precision and accuracy tests were carried out.

The results of examining comparative material Apo-B were respectively 78mg/dL, 75 mg/dL, 82 mg/dL, 83 mg/dL, 81 mg/dL, so that the bias value was 3.7%. Accuracy test showed that the examination of LDL-C and Apo-B were within the recommended bias <10%.[6] Precision test carried out daily resulted in KV LDL-C at 1.96% and 4.72% and Apo-B at 2.99% and 3.39%. These results were still within the allowed

Table 1. Characteristics of subject

Characteristics		n	%
Sex and age			
male:	-<55 yr	16	43.2
	-≥55 yr	21	56.8
Female:	-<65 yr	13	76.5
	-≥65 yr	4	23.5
Education			
Elementary school		1	1.9
Junior high school		5	9.3
Senior high school		19	35.2
College		29	53.7
Occupation			
Civil servant		20	37.0
Private sector		12	22.2
Retirement		16	29.6
Farmer		1	1.9
Housewife		5	9.3
Risk factor			
DM		26(54)	48.1
Hypertension		29(54)	53.7
Smoking		19(54)	35.2
Overweight		13(54)	24.1
Dyslipidemia		12(54)	22.2
Symptom			
Chest pain		47	87
Out of breath		6	11.1
Burning sensation in the chest		1	1.9
Diagnosis			
Stable angina pectoris		49	90.8
Unstable angina pectoris		3	5.6
AMI		2	3.7
Treatment			
Statin		46(54)	85.2
Fibrate		0(54)	0
Others		35(54)	64.8
ECG			
Normal		29	53.7
ST depression		21	38.9
ST elevation		4	7.4

range values for accuracy test in which the LDL-C was <7.8% and Apo-B was <6.4%.[7] Thus, the accuracy test showed good results.

The research data showed that most subjects were male (37) while the number of female subjects was 17.

These results were consistent with the theory stating that the number of male patients with CHD was greater than female patients because the latter were protected by estrogen.[8] In terms of education, most of the subjects (29) went to college (53.7%) and most of the subjects (20) were civil servants (37%).

Based on the risk factors, out of 54 subjects of the study, 29 subjects (53.7%) had hypertension, followed by DM (48.1%) as many as 26 subjects, 19 subjects (35.2%) smoked, 13 subjects (24.1%) were overweight and 12 subjects (22.2%) had dyslipidemia. The most common symptom was chest pain, experienced by 47 subjects (87%). The most common diagnosis was stable angina pectoris experienced by 49 subjects (90.8%) and and 46 subjects (85.2%) received statins.

Electrocardiography (ECG) revealed that 29 subjects (53.7%) showed a normal ECG, 21 subjects (38.9%) showed ST depression and only 4 subjects (7.4%) showed ST elevation. It was in accordance with the article written by Pope in 2005[9] i.e. APS patients commonly showed a normal ECG. The details are shown in Table 1.

The results of the analysis of the difference in the means of the atherosclerosis percentage based on the characteristics of the subjects that could also serve as a risk factor and treatment are shown in Table 2.

There was no significant difference in the mean of atherosclerosis percentage between subjects with risk factors and subjects without risk factors except dyslipidemia. The values of p were as follows: for sex and age, the value of $p=0.226$, for DM, the value of $p=0.185$, for hypertension, the value of $p=0.619$, for smoking, the value of $p=0.120$ and for overweight, the value of $p=0.271$, whereas dyslipidemia showed a significant difference with $p=0.030$ ($p<0.05$).

Similarly, in the two previous studies conducted by Koba et al[10] the prevalence of high sd-LDL was found in type 2 DM. The studies concluded that DM was an independent predictor of sd-LDL.[9–11]

The research conducted by Mohan et al[11] stated that the subfraction of LDL was small (pattern B or sd-LDL), but was significantly higher in subjects with diabetes with $p<0.001$.[9-11] Regarding other risk factors, there was no significant difference for hypertension with the value of $p=0.453$, smoking ($p=0.075$), overweight ($p=0.988$) and dyslipidemia ($p=0.920$).

These results were consistent with studies conducted by Tokuno et al[12] in which sd-LDL decreased significantly after being treated with statin compared to before the treatment with $p<0.001$.[9–11]

Table 2. Comparison of the atherosclerosis percentage based on risk factors

Risk factor		n	Atherosclerosis Mean±SD (%)	p
Sex and age				
male:	<55 years	16	50.19±33.62	0.226
	≥ 55 years	21	54.43±31.64	
female:	<65 years	13	31.63±33.54	
	≥65 years	4	37.01±37.13	
DM				
	Yes	26	58.49±28.64	0.185
	No	28	35.72±34.38	
Hypertension				
	Yes	29	68.49±22.55	0.619
	No	25	21.37±25.30	
Smoking				
	Yes	19	48.73±28.66	0.120
	No	35	45.57±36.18	
Overweight				
	Yes	13	39.66±30.10	0.271
	No	41	48.90±34.53	
Dyslipidemia				
	Yes	12	51.75±23.86	0.030*
	No	42	45.23±35.87	

Remarks: Kruskal Wallis test for group comparison >2 and Mann Whitney test. For comparison two goups, significant if p(*)<0.05

Table 3. Feature of ratio of LDL-C/Apo-B and the atherosclerosis percentage in the research subjects

Criteria	N	Mean	Minimum	Maximum
sd-LDL	29	1.06	0.81	1.16
Large LDL	25	1.34	1.20	1.48
Atherosclerosis (%)	54	46.68	0	100

Figure 1. Correlation between the ratio of LDL-C/Apo-B and the atherosclerosis percentage
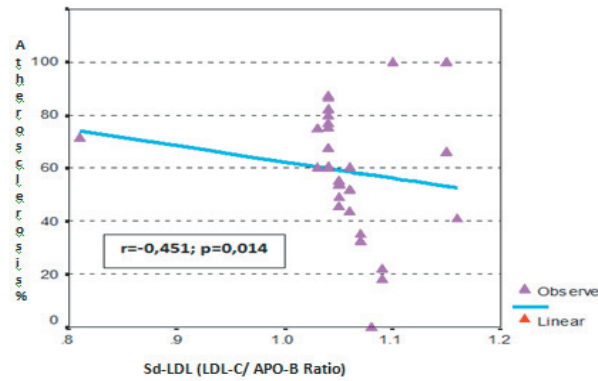
Figure 2. Correlation between sd-LDL and the angiographically atherosclerosis percentage

The feature of ratio of LDL-C/Apo-B and the atherosclerosis percentage in research subjects are shown in Table 3. It shows that the mean of sd-LDL was 1.06 with a minimum value of 0.81 and a maximum value of 1.16. The mean of large LDL was 1.34 with a minimum value of 1.20 and a maximum value of 1.48. The mean of the atherosclerosis percentage was 46.68% with a minimum percentage of 0% and maximum percentage of 100%.

The correlation between the ratio of LDL-C/Apo-B and an increase in the atherosclerosis percentage was presented in figure 1. The results of the study showed that there was a significant moderate negative correlation between the ratio of LDL-C/Apo-B and the atherosclerosis percentage, (r=-0.502, p=0.000), indicating that the smaller LDL (sd-LDL and large LDL), the greater the atherosclerosis percentage.

Correlation between sd-LDL and the angiographically atherosclerosis percentage in coronary heart disease was shown in Figure 2.
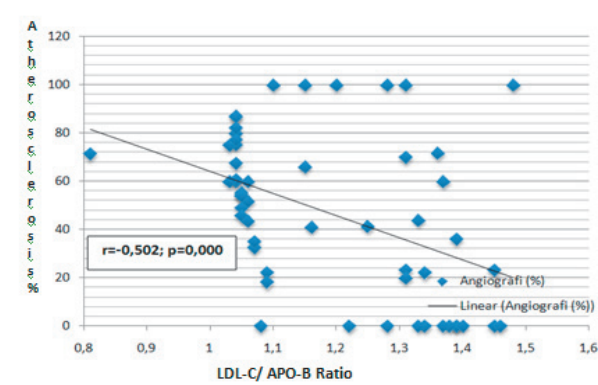
The results showed that there was a significant moderate negative correlation between sd-LDL and the atherosclerosis percentage (r=-0.451, p=0.014) indicating that the smaller the sd-LDL, the higher the atherosclerosis percentage. The strength of the correlation in this study was similar to the one in the study conducted by Kwon et al[13], which examined the correlation between sd-LDL and atherosclerosis in Coronary Artery Disease (CAD). The results showed that there was a significant moderate negative correlation between sd-LDL and atherosclerosis (r=-0.4; p=<0.001). The difference between the current study and the study conducted by Kwon et al[13] was the sample size and the location. In the research conducted by Kwon et al[12] in Korea, the sample size was 156 subjects. The research conducted by Gohari et al in 2009[14] found higher sd-LDL level and ratio of sd-LDL/LDL significantly in patients with coronary artery stenosis.[13,14] Once significant negative correlation between sd-LDL and the atherosclerosis percentage was found, the finding was then analyzed further (post hoc) using Mann-Whitney test as shown in Table 4. The

results showed that there were significant differences between the mean of ratio of LDL-C/Apo-B and group I and II, I and III, II and III, II and IV and III and IV. However, there were no significant differences between group I and IV, and group II and IV. This might be related to lipid-lowering treatment which was greater in group IV, namely two (2) kinds compared with other groups (one kind) as well as the distribution of samples which was uneven.

The research conducted by Toft-Petersen *et al* in 2011[15] found that compared with patients with non-CAD, patients with CAD had a higher sd-LDL ratio significantly (40.0% and 50.1%, respectively; p <0.001).

Table 4. The difference in the mean of ratio of LDL-C/Apo-B based on the group of the atherosclerosis

Atherosclerosis group	n	Ratio of LDL-C/Apo-B	p
		mean±SB	
I	12	1.34±0.10	0.009*
II	15	1.20±0.14	
I	12	1.34±0.10	0.000*
III	21	1.08±0.12	
I	12	1.34±0.10	0.151
IV	6	1.25±0.13	
II	15	1.20±0.14	0.000*
III	21	1.08±0.12	
II	15	1.20±0.14	0.424
IV	6	1.25±0.13	
III	21	1.08±0.12	0.004*
IV	6	1.25±0.13	

Remarks: Group I: atherosclerosis 0% (normal), group II: atherosclerosis between 1−50%, group III: atherosclerosis between 51−99%, group IV: atherosclerosis 100% (CTO: *Chronic Total Oclusion*). Mann Whitney test, significant if p(*)<0.05

Small dense low-density lipoprotein is a significant predictor of CAD, matched by invasive CAG (p<0.001), not affected by the difference in age, sex, smoking and waist size or LDL cholesterol. There was a significant difference in sd-LDL in non CAD patients compared with CAD patients matched by CT CAG (39.5% and 45.8%, respectively p=0.029). Small dense low-density lipoprotein is a significant predictor of CAD, matched by CT CAG affected by the difference in age, sex, smoking and waist size or anything related to cholesterol or LDL (p=0.03).[4]

CONCLUSION AND SUGGESTION

The study concluded that there was a significant moderate negative correlation between the size of sd-LDL and increase in the atherosclerosis percentage by angiography. Suggestion for further research is that small dense low-density lipoprotein can be used as one of the considerations in assessing atherosclerotic lesion area, especially in conditions before getting a lipid-lowering treatment.

REFERENCES

1. American Heart Assocation. International Cardiovascular Disease Statistics. Statistical Fact Sheet Populations 2004; 1–14.
2. Grodstein F, Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. Prog Cardiol Dis. 1995; 38: 199–210.
3. Topol LJ, Califf RM, Prystowsky EN, Thomas JD, Thompson PD. Textbook of Cardiovascular Medicine, 3rd Ed., Philadelphia, Lippincott Williams & Wilkins. 2007; 2–10.
4. Hirano T, Ito SY, Koba S, Toyoda M, Ikejri A, Saegusa H, *et al*. Clinical Significance of Small Dense Low Density Lipoprotein Cholesterol Levels Determined by the Simple Precipitation Method. Arterioscler Thromb Vasc Biol. 2004; 24: 558–63.
5. Bidgood W.D., *et al.*, Understanding and Using DICOM, the Data Interchange Standard for Biomedical Imaging. JAm Med Inform Ass. 1997; 4(3): 199–212.
6. Anonim. CD Medical Release 2. Operator's Manual. Copyright Philips Medical Systems Nederland B.V. Q-Station User Manual 2001; 1–29.
7. Himpunan Kimia Klinik Indonesia (HKKI) & Perhimpunan Dokter Spesialis Patologi Klinik Indonesia (PDS PATKLIN). Panduan Pemantapan Mutu Laboratorium Klinik. Jakarta, 1995; 1–8.
8. Kafonek SD, Derby CA, Bachorik P. Biological Variability of Lipoproteins and Apolipoproteins in Patients Referred to a Lipid Clinic. Clin Chem.1992; 38(6): 864–72.
9. Pope B. Angina Pectoris. Available from: www.Blackwellpublishing.com. (Accessed at: February 13, 2011).
10. Koba S, Hirano T, Ito Y, Tsunoda F, Yokota Y, Ban Y, Iso Y, Suzuki H, Katagiri T. Significance of small dense low-density lipoprotein-cholesterolconcentrations in relation to the severity of coronary heart diseases. Atherosclerosis 2006; 189: 206–14.
11. Mohan V, Deepa R, Velmurugan K, Gokulakrishnan K. Association of Small Dense LDL with Coronary Artery Disease and Diabetes in Urban Asian Indians-The Chennai Urban Rural Epidemiology Study (CURES*). Diabetes Care, 2004; 27(8): 1962–7.
12. Tokuno A, Hirano T, Hayashi T, Mori Y, Yamamoto T, Nagashima M, *et al*. The Effects of Statin and Fibrate on Lowering Small Dense LDL-Cholesterol in Hyperlipidemic Patients with Type 2 Diabetes. J Atheroscler thromb. 2007; 14(3): 128–32.
13. Kwon SW, Yoon SJ, Kang TS, Kwon HM, Kim JH, Rhee J, Lee SJ, Park JK, Lim JY, Yoon YW, Hong BK. Significance of small dense low-density lipoprotein as a risk factor for coronary artery disease and acute coronary syndrome. Yonsei Med J. 2006; 47(3): 405–14.
14. Gohari LH, Ghassab RK, Firoozray M, Zavarehee A, Basiri HA.The association between small dense low density lipoprotein, apolipoprotein B, apolipoprotein B/apolipoprotein A1 ratio andcoronary artery stenosis. Medical Journal of the Islamic Republic of Iran 2009; 23(1): 8–13.
15. Toft-Petersen AP, Tilsted HH, Aaroe J, Rasmussen K, Christensen T, Bruce A, *et al*. Small dense LDL particles - a predictor ofcoronary artery disease evaluated by invasiveand CT-based techniques: a case-control study. Lipids in Health & Dis. 2011; 10: 21.