Correlation of Oxidized-Low Density Lipoprotein (ox-LDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) with Framingham Risk Score (FRS) of Coronary Heart Disease (CHD)

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Abstract

Coronary Heart Disease (CHD) is the leading cause of death worldwide. CHD preceded by the atherosclerosis process. Ox-LDL, LDL, and HDL cholesterol may be more potent biomarkers for predicting and diagnosing CHD than those only conventional measurements of lipid levels with the Framingham Risk Score (FRS).

Purpose to determine the correlation between Ox-LDL, LDL, HDL levels and FRS in a population at risk of CHD

This study was an analytical descriptive with a cross-sectional research design. The research subjects were 143 (79 male and 64 female) subjects. Serum Ox-LDL, LDL, HDL levels were checked by Sandwich ELISA method. FRS includes gender, age, total cholesterol, HDL-cholesterol, systolic blood pressure, history of taking antihypertensive drugs, smoking, diabetes, and vascular disease (CHD, Stroke).

Ox-LDL and HDL levels had a very weak negative correlation to FRS ($p = 0.017$; $r = -0.199$; $p = 0.000$; $r = -0.305$) and had a very weak effect ($R=0.174$, $R2=3\%$; $R= 0.283$, $R2=8\%$). Levels of LDL and FRS were not correlated ($p = 0.558$; $r = -0.049$) and had no effect ($R=0.007$, $R2=0\%$).

Ox-LDL, HDL, and FRS levels were negatively correlated, meanwhile the LDL and FRS has no correlation. Besides, they all had a weak effect to FRS. The discrepancy of Ox-LDL, LDL, HDL and FRS levels is due to the limitations of FRS, which neglects other non-conventional CHD risk factors. Also, race differences may cause FRS might be unsuitable for Asian.

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Introduction

Cardiovascular Disease (CVD) or cardiovascular disease is the leading cause of death worldwide. There are 17.5 million people die from heart disease globally. Deaths from heart disease account for 80 percent of middle to low-income countries and are estimated to continue to increase, especially in developing countries.[1,2] CHD occurs preceded by atherosclerosis, namely the process of accumulation of cholesterol in the walls of blood vessels, causing stenosis or blockage.[2]

Atherosclerosis is considered a response to initial injury to the walls of blood vessels. Although some theories suggest that oxidative modification of the particle component of Low-Density Lipoprotein (LDL) makes it more susceptible to phagocytosis by macrophages, oxidative stress also underlies atherosclerosis. Besides, the production of growth factors by foam cells triggers the proliferation and migration of smooth muscle cells to the tunica intima, which in turn will form a fibrous cap. Oxidative stress facilitates stable plaque formation and creates conditions that make plaque more prone to rupture.

Clinicians have used biomarkers to assess patients' clinical conditions and identify

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patients at risk for CVD. With the presence of atherosclerosis biomarkers that reflect the pathophysiology of CVD, clinicians can diagnose subclinical atherosclerosis in subjects at risk for CVD.[3] Ox-LDL may be a more potent biomarker for predicting and diagnosing cardiovascular disease than conventional lipid measurement alone.[4] Ox-LDL is considered necessary in the atherosclerosis cascade and plays a role in the pathophysiology of cardiovascular disease. Ox-LDL acts as an antigen that is recognized by macrophages and will induce foam cell formation, atherosclerotic plaque development, apoptosis, cell death, and cytokine production.[5] According to a cohort study conducted by Koenig et al., there was an increase in Ox-LDL levels at 333 CHD patients. Ox-LDL has a positive correlation with the risk of the incidence of CHD. Ox-LDL is a marker of oxidative stress. Oxidative stress plays an essential role in the process of initiation and development of atherosclerosis.[6]

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LDL functions to transport cholesterol to various body tissues. Increased levels of LDL cholesterol have been recognized as a risk factor for cardiovascular disease.[5] LDL plays an essential role in oxidative stress through the oxidation mechanism of LDL to Oxidized-Low Density Lipoproteins (Ox-LDL). The formed ox-LDL will act as a chemoattractant for monocyte cells and cause an inflammatory reaction in the walls of arterial blood vessels. Later, it will impact the development of the atherosclerotic plaque phase.

Several studies have shown that High-Density Lipoprotein (HDL) levels have a negative correlation with the incidence of CHD. Therefore, HDL is known as an anti-atherosclerosis factor.1 HDL cholesterol has a protective function against vascular endothelial cells. Low HDL levels are associated with the presence of lipoprotein oxidation and endothelial dysfunction. HDL is an anti-atherogenic lipoprotein that plays a role in cholesterol transport, anti-inflammatory effects, and protects LDL from oxidative damage. HDL's ability to inhibit LDL oxidation is due to ApoA1 and several enzymes in HDL particles, such as paraoxonase 1 (PON1) and lecithin-cholesterol acyl-transferase (LCAT). The LDL oxidation process will reduce HDL's ability to inhibit LDL oxidation.[5]

One of the most widely used risk assessment methods to predict cardiovascular disease risk is the Framingham Risk Score (FRS). FRS is applied in conjunction with other biomarkers of oxidative stress, or with any biomarker of atherosclerosis to assess the risk of cardiovascular disease. The FRS is a risk assessment method widely used to predict the presence of CVD risk in the next ten years. This method is recommended by the National Cholesterol Education Program (Adult Treatment Panel III) and has been widely used in various studies.[7] The score includes risk factors such as gender, age, total cholesterol, High-Density Lipoprotein (HDL) cholesterol, systolic blood pressure, history of consumption of antihypertensive drugs, smoking, diabetes, and vascular disease (CHD, Stroke). The score does not include a family history of CVD, oxidative stress, inflammation, and genetic factors. However, when FRS is applied together with the examination of biomarkers of oxidative stress or atherosclerosis, FRS can be used to assess the risk of CVD. This study was conducted to analyze the correlation between levels of Ox-LDL and FRS in populations at risk of CHD.

Materials and methods

This research is descriptive-analytic with cross-sectional research design and was conducted on the community in the Kelurahan Kebalen Wetan Malang City during the period November 2019 - March 2020. Measurement of Ox-LDL, LDL, and HDL levels using the Sandwich ELISA method was carried out at the Bioscience Laboratory of Brawijaya University. After that, a descriptive analysis was carried out. A total of 143 patients consisting of 79 male and 64 female subjects were involved in this study. The inclusion criteria for CHD risk samples are samples that have the following risk factors:

gender, age, total cholesterol, HDL cholesterol, systolic blood pressure, history of taking antihypertensive drugs, smoking, diabetes, and vascular disease (CHD, Stroke). The risk factors for CHD were analyzed using the FRS, and the total FRS score was calculated for each study subject. The exclusion criteria in this study were subjects diagnosed with atherosclerotic cardiovascular disease, and subjects who had a history of taking cholesterol-lowering drugs.

Data analyzed using SPSS 23.0 for Windows. The Kolmogorov-Smirnov test was used to see the normality of the data. One way ANOVA / Kruskal Wallis difference test was used to see the mean difference in the two groups. In statistical tests, p <0.05 was considered significant. Regression tested to determine the effect of serum Ox-LDL levels on FRS scores in populations at risk of CHD.

Results

This study comprised 143 research subjects who had met the inclusion and exclusion criteria and signed informed consent. The general characteristics of patients in this study can be seen in Table 1.

Characteristic		N	%	Mean Ox-LDL (ng/mL)	Mean LDL (ng/mL)	Mean HDL (mg/dL)	FRS Score Mean
Gender	Male	79	55.24	0.099	117.7848	39.7468	20.015
	Female	64	44.76	0.099	137.3281	47.6563	13.254
	p-value			**0.135**	**0.135**	**0.006***	**0.000***
Age	≤ 49 y.o	32	22.38	0.099	131.6563	39.5000	9.084
	50-69 y.o	90	62.94	0.099	126.5444	44.6222	18.602
	70-89 y.o	21	14.68	0.099	118.6667	43.3333	22.123
	p-value			**0.635**	**0.635**	**0.450**	**0.106**

*Significant different if p<0.05

Table 1. Characteristics of Research Subjects and independent T-test results.

Table 1 presents the characteristics of research subjects based on gender and age; it was found that the 55.24% were male, and the rest 44.76% was female subjects. Besides, there was a significant difference FRS score based on age characteristics (p = 0.000). The highest mean FRS scores were found in male patients and patients 70-89 years old.

Ox-LDL levels was found higher in female patients and patients 70-89 years old. The highest mean of Ox-LDL levels was found in females and 70-89 years old subjects. The correlation test for Ox-LDL and FRS levels showed a significant correlation (p = 0.017). The Spearman correlation (R=0.199) indicates a negative correlation with weak correlation strength. The results of the regression test for Ox-LDL levels with FRS obtained the R=0.174. This result shows that there is a weak relationship between levels of Ox-LDL and FRS. The results of the analysis of the coefficient of determination (R2) show that the percentage effect of Ox-LDL levels on FRS is 3%. In comparison, the remaining 97% is influenced by other variables not included in this study. The significance value test (Sig) of ANOVA is 0.038 (P<0.05), which shows a simultaneous influence between Ox-LDL levels on the FRS score.

The highest mean of cLDL levels was found in female subjects and ≤ 49 years old. Based on the analysis of the characteristics of the research subjects, it was found that there were significant differences in the mean cLDL levels and FRS scores based on gender characteristics (with a significant p-value 0.017 and 0.000, respectively). The Spearman correlation value of -0.049 shows a weak correlation. The results of the correlation test analysis between cLDL and FRS levels showed no significant correlation between cLDL levels and FRS (p = 0.558). The results of the regression test for cLDL levels with FRS obtained the R= 0.007. This result shows that there is a weak relationship between cLDL levels and FRS. The results of the analysis of the coefficient of determination (R2) show that the percentage effect of cLDL levels on FRS is 0%. In comparison, it is influenced by other variables not included in this study. The significance value test (Sig) of ANOVA is 0.935 (P<0.05). This result shows that there is no simultaneous effect between cLDL levels on the FRS score.

The analysis result showed significant differences between HDL levels mean and FRS scores based on gender characteristics (with significant p values of 0.006 and 0.000, respectively). The highest mean levels of cHDL were found in females and subjects aged 50-69 years old. The results of the Spearman correlation test showed a significant correlation between cHDL and FRS levels (p = 0.000). The Spearman correlation 0.305 indicates a negative correlation with weak correlation strength. The results of the regression test for HDL levels with FRS showed that the R=0.283. This result indicates that there is a weak relationship between HDL levels and FRS. The coefficient of determination (R2) showed that the percentage

effect of HDL levels on FRS is 8%, while the remaining 92% is influenced by other variables not included in this study.The significance value test (Sig) of ANOVA is 0.001 (P<0.05), it showed that there is a simultaneous influence between cHDL levels on the FRS score.

Discussion

The results of the analysis of the correlation test for Ox-LDL and FRS levels showed a significant correlation between levels of Ox-LDL and FRS (p = 0.017). The Spearman correlation value of 0.199 indicates a negative correlation with a weak correlation strength. The results of the correlation test analysis of LDL and FRS levels showed no significant correlation between LDL and FRS levels (p = 0.558), shows that there is no correlation between LDL levels and FRS scores. According to a study by Silverman et al., there is a relationship between a decrease in LDL levels and a reduced risk of cardiovascular disease, where each 38.7 mg / dL (1 mmol / L) reduction in LDL levels will reduce the relative risk of cardiovascular disease by 23%.[8] The significant correlation between HDL and FRS levels (p = 0.000), followed by Spearman correlation value of 0.305, indicates a negative correlation with weak correlation. This study shows that the higher the Ox-LDL level so that the lower the FRS score will. This negative correlation result indicates a discrepancy with the theory. A person who has a high risk of developing atherosclerosis and cardiovascular disease has higher levels of Ox-LDL and LDL, also have lower HDL than those with low risk. But,the negative correlation between HDL and FRS was also consistent with research by Ryoo et al., which showed that HDL levels were negatively correlated with FRS (r = -0.14, p <0.001).[8]

The results of the regression test for Ox-LDL levels with FRS obtained the R=0.174. This result shows that there is a weak relationship between levels of Ox-LDL and FRS. The results of the analysis of the coefficient of determination (R2) show that the percentage effect of Ox-LDL levels on FRS is 3%. In comparison, the remaining 97% is influenced by other variables not included in this study. The significance value test (Sig) of ANOVA is 0.038 (P<0.05), which shows a simultaneous influence between Ox-LDL levels on the FRS score.

Ox-LDL levels are elevated in subclinical atherosclerosis and are a more reliable predictor of coronary heart disease than standard lipid levels or other conventional risk factors. The ox-LDL buildup will be recognized and will interact with the two scavenger receptors in macrophages. After interacting with scavenger receptors, macrophages are activated and uptake Ox-LDL (8). Scavenger receptors have a high affinity for Ox-LDL and cause intracellular lipid accumulation to form foam cells. Furthermore, the foam cell develops into a fat core and has a fibrous cap, that triggers the atherosclerosis process.[9] Ox-LDL is reported to be able to differentiate between coronary heart disease patients and healthy people. Ox-LDL can also be used as a predictor of myocardial infarction in patients with unstable CAD. [10] Also, according to a study by Holvoet et al., it was stated that CHD patients had higher levels of Ox-LDL when compared to the control group and has sensitivity 76% to detect CHD cases.[11] The inconsistency between Ox-LDL levels and the FRS score could be due to the FRS scoring system's limitations. FRS is a risk assessment system used to predict the risk of CHD. However, there are limitations to the FRS scoring system, namely ignoring other unconventional CHD risk factors for young individuals (<55 years old).

The results of the regression test for cLDL levels with FRS obtained the R= 0.007. This result shows that there is a weak relationship between cLDL levels and FRS. The results of the analysis of the coefficient of determination (R2) show that the percentage effect of cLDL levels on FRS is 0%. In comparison, it is influenced by other variables not included in this study. The significance value test (Sig) of ANOVA is 0.935 (P<0.05). This result shows that there is no simultaneous effect between cLDL levels on the FRS score.

The discrepancy between LDL levels and the FRS score can be caused because the LDL level measured in this study was the cLDL level, not the direct measurement of LDL or apoB particles. Cholesterol is an essential component of cell membranes and is a precursor to bile acids and steroid hormones. Cholesterol is carried to peripheral cells mostly by plasma lipoproteins containing apoB. In most people, LDL particles constitute 90% of the lipoprotein containing apoB in the fasting blood sample. Each LDL particle contains a single molecule

apoB. In general, plasma LDL levels are not measured directly but are estimated from total cholesterol levels. The plasma LDL level is known as cLDL, a measurement of the total amount of cholesterol in LDL particles. In general, cLDL levels have a strong correlation with the number of LDL particles so that plasma cLDL levels can be used as a substitute for LDL particle levels. However, under certain conditions (metabolic syndrome, diabetes, and hypertriglyceridemia), the plasma levels of cLDL, and LDL particles can be mismatched due to the predominance of small dense LDL. Small dense LDL has a low affinity for the LDL receptor so that it has a longer half-life in the circulation. The plasma cLDL levels might be inaccurate in describing LDL particle levels and their effect on cardiovascular risk. In these conditions, direct measurement of the LDL particle level or the apoB level should be carried out to describe the causal effect of LDL on cardiovascular risk accurately.

The study of FRS in the risk of CHD increased markedly in patients with HDL levels below 40 mg / dL. Meanwhile, according to the Quebec Cardiovascular study, every 10% decrease in HDL levels will increase the risk of CHD by 13%.[11] The regression test results between cHDL levels and FRS showed that there was a weak relationship (R = 0.283) between cHDL levels and FRS by 8%, followed by the significance value of ANOVA was 0.001 (P <0.05). It can be concluded that there is a weak influence between cHDL levels and FRS scores, and it has a weak correlation.

Several epidemiological studies have shown a strong negative correlation between cHDL levels and the risk of cardiovascular disease. Several mechanisms can explain this, namely the stimulation of reverse cholesterol transport from foam cells in coronary plaque to the liver, protection of vascular endothelial cells through activation of the endothelial pathway Nitric Oxide Synthase (eNOS), and inhibition of LDL oxidation. HDL can increase eNOS activation and NO release, causing vasodilation of blood vessels.[12] HDL cholesterol has a protective function against vascular endothelial cells. Low HDL levels are associated with the presence of lipoprotein oxidation and endothelial dysfunction. HDL will directly inhibit the production of ICAM-1 and VCAM-1 by Ox-LDL on endothelial cells' surface. The determinants of HDL metabolism are HDL-related enzymes, paraoxonase and LCAT. Paraoxonase prevents LDL oxidation by hydrolyzing lipid peroxidase, cholesterol linoleate hydroperoxide, and hydrogen peroxidase. Meanwhile, LCAT plays a role in preventing the accumulation of oxidized lipids in LDL.[13]

Conventional risk factors such as dyslipidemia, hypertension, diabetes mellitus, and smoking cannot explain CHD's cases in young individuals. Thus there are other unconventional risk factors associated with atherosclerosis in young individuals. Based on the research of Huang et al., Ox-LDL is an independent risk factor for CHD in young individuals after including other risk factors such as the ApoB / ApoA1 ratio. Male gender, smoking, triglyceride levels, ApoB / ApoA1 ratio, and Ox-LDL levels are independent risk factors for CHD in young individuals. Ox-LDL is more atherogenic than native LDL.[14] In this study, 32 patients were under 49 years old. The FRS score for individuals under 49 years old can ignore other non-conventional CHD risk factors such as triglyceride levels, ApoB / ApoA1 ratio, Ox-LDL levels. So, the FRS score obtained can be lower than it is and may affect statistical results. This condition does not reflect the accurate prediction of CHD risk in individuals under 49 years old. There are other independent risk factors such as triglyceride levels, ApoB / ApoA1 ratio, and Ox-LDL levels, which were not taken into account when assessing the FRS score.

Conclusions

Ox-LDL, HDL, and FRS levels were negatively correlated, meanwhile the LDL and FRS has no correlation. Besides, all of them had a weak effect to FRS. HDL levels are inversely proportional to FRS because HDL functions protectively against vascular endothelial cells. The discrepancy of Ox-LDL, LDL, HDL and FRS levels is due to the limitations of FRS, which neglects other non-conventional CHD risk factors.

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Declaration of Interest

The authors report no conflict of interest.

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