The Role of Lipid Profile as a Risk Factor Indicator for Ischemic Stroke at Cipto Mangunkusumo Hospital, Jakarta

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ABSTRACT

**Aim:** to determine the role of low HDL-cholesterol, and high total cholesterol, LDL-Cholesterol and triglyceride as risk factors for ischemic stroke at Dr. Cipto Mangunkusumo Hospital.

**Method:** a study was conducted on 76 patients with an age range of 40–70 years. Subjects consisted of 38 post ischemic stroke patients and 38 control subjects with a diagnosis other than stroke. The study sample consisted of serum for lipid profile assessment. Total cholesterol and triglyceride were assessed using enzymatic method, while HDL-cholesterol and LDL-cholesterol using direct homogenous enzymatic method. Statistical analysis was performed using chi-square and multivariate analysis using logistic regression.

**Results:** low HDL-cholesterol was found in ischemic stroke patients and demonstrated a significant difference compared to control subjects (p<0.05). The results of total cholesterol, triglyceride, LDL-cholesterol did not demonstrate a significant difference. The odds ratio (3.09; CI 95%: 1.04; 8.73) demonstrates that low HDL-cholesterol is a risk factor for ischemic stroke.

**Conclusion:** a low HDL-cholesterol level is a risk factor for ischemic stroke, with an odds ratio of 3.09, while total cholesterol, triglyceride and high LDL-cholesterol levels were not risk factors for ischemic stroke.

**Key words:** HDL-cholesterol, LDL-cholesterol, triglyceride, ischemic stroke.

INTRODUCTION

Ischemic stroke most commonly occurs due to blood vessel disorders, i.e., atherosclerosis, hypertension, and thrombosis. A study by Misbach et al. conducted in 28 hospitals in Indonesia, found that stroke is associated with hypertension (73%), smoking (20.45%), ischemic heart disease (19.9%), diabetes mellitus (17.3%), and hypercholesterolemia (16.4%).

Another cause of ischemic stroke is arterial thrombosis. Thrombosis can be caused by disorders on blood vessel, blood coagulation, and blood flow. Blood vessel wall disorders can occur due to atherosclerosis.

Atherosclerosis is a chronic inflammation that will manifest acutely when a plague detaches, causing thrombosis. Atherosclerosis may occur on all arterial segments, especially in medium and large blood vessels. This process starts in early childhood and continues until death. It is estimated that only 4% of the population that live until the eighth decade do not have atherosclerosis. It is presumed that atherosclerosis of the aorta already occurs in the first decade of life, followed by atherosclerosis of the coronary arteries in the second decade of life and cerebral arteries in the third decade of life. In males, cerebral atherosclerosis occurs 10 years earlier than in females.

From the many theories of the pathogenesis of atherosclerosis, the most followed is a combination of the endothelial damage theory and the lipid infiltration theory. This theory states that atherosclerosis occurs in several steps, initiated by blood vessel endothelial damage due to several causes, such as mechanical factors (hemodynamic and traumatic), endotoxin, and low density lipoprotein (LDL). These factors may cause a change in the endothelial membrane. The endothelial damage causes thrombocytic activation and attachment and increased lipid infiltration into the blood vessels.

An epidemiological study showed that the main risk factors of atherosclerosis are age, male sex, dyslipidemia, high blood pressure, smoking, diabetes mellitus, obesity, low activity, and family history. Woo et al reported the correlation of lipid profile and ischemic stroke. High levels of total cholesterol, LDL and Apo B were found in ischemic stroke. A research by the Honolulu Heart Program in Hawaii found that atherosclerosis of the small arteries of circle of Willis had a positive correlation with elevated triglyceride level. Tanne reported low HDL level was a risk factor of cortical ischemic stroke.

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The fact that stroke remains the primary cause of death in all hospitals in Indonesia, as stated in the Indonesian Health Profile in 1999, encouraged us to conduct this research. The prevalence of ischemic stroke is 83% of all strokes. In the United States, 500,000 out of 3 million living patients suffered recurring stroke. Therefore, the prevention of stroke recurrence is especially important and to do this, it is essential to know the risk factors of stroke. The purpose of this research is to determine the role of lipid profile as a risk factor indicator for ischemic stroke in Dr. Cipto Mangunkusumo Hospital.

METHODS

A case control study was conducted on 76 subjects with the age ranging from 40 to 70 years old, visiting outpatient clinic of Neurology Department, Medical Faculty University of Indonesia, Cipto Mangunkusumo Hospital. The subjects were divided into 38 persons in the case group and 38 in the control group. The case group consisted of third-week post-ischemic stroke patients, whereas the control group consisted of patients with non-stroke diagnosis. The inclusion criteria of the case group were third-week post-ischemic stroke patients who were willing to participate in the research and signed the informed consent. The exclusion criteria were previous history of stroke and those taking lipid-lowering agents. The inclusion criteria of the control group were patients with same age range and sex as the case group and willing to participate in the study and sign an informed consent form. The exclusion criteria were patients who had suffered from stroke and had a family history of stroke. Subjects in the control group were matched based on age and sex.

Subjects were required to fast 12-16 hours before sample taking. The research material was 5mL venous blood without anticoagulants, left to coagulate, centrifuged 1500x for 15 minutes to get the serum. The serum was used for total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride level measurements.

Total cholesterol and triglyceride were measured by enzymatic method, while HDL and LDL cholesterol were measured by direct homogenous enzymatic method. Lipid profile measurement was done using the Humalyzer 2000.

Data from the sensitivity and specificity tests of each parameter were entered into the main table. The mean, standard deviation, coefficient of variation, and deviation from target values were calculated.

The results were recorded using the Statistical Product and Service Solutions (SPSS) version 10. Data were presented descriptively and analysed by chi-square test, with independent variables as below:

- Total cholesterol < 200 mg/dL = 1, > 200 mg/dL = 2
- Triglycerides < 150 mg/dL = 1, >150 mg/dL = 2
- LDL-cholesterol < 130 mg/dL = 1, > 130 mg/dL = 2
- HDL-cholesterol < 40 mg/dL = 1, > 40 mg/dL = 2

The dependent variable was ischemic stroke.

To evaluate the correlation between the independent variables and a categorically scaled dependent variable, double logistic regression multivariate analysis was performed. The double logistic regression was conducted in steps, starting with the univariate analysis to obtain the frequency of the variable tested. The next step was bivariate analysis done by crosstab and Chi square for 2x2 tables with continuity correction and Pearson for tables larger than 2x2. Of the parameters tested if Chisquare p <0.25 it became a candidate for multivariate analysis. Double logistic regression multivariate analysis was done several times by each time excluding a candidate with greater non significant correlation (p>0.05). The last analysis done produced the significant parameter (p<0.05) with odds ratio (OR).

RESULTS

Before the sample measurement, we did within-run and between-day sensitivity tests and specificity test using normal control and high control materials. For the within-run sensitivity test, the Humalyzer 2000 was used to test lipid profile using normal control material (Serodos) from Human, resulting the coefficient of variation (CV) of total cholesterol 0.5%, triglycerides 0.43%, LDL 0.71% and HDL 2.03%. (Table 1)

In the within run sensitivity test, the Humalyzer 2000 was used to measure lipid profile using high control material (Humatrol P) from Human. The results were total cholesterol CV 0.24%, triglycerides 0.20%, LDL 0.38%, HDL 0.55%. (Table 1)

The results of the between day sensitivity test of the lipid profile measurement device with normal control were total cholesterol CV 0.55%, triglycerides 0.28%, LDL cholesterol 0.71%, and HDL cholesterol 1.93%. (Table 1)

In the between day sensitivity test, the Humalyzer 2000 was used using high control material. The total cholesterol CV was 0.28%, triglycerides 0.33%, LDL-cholesterol 0.38% and HDL-cholesterol 0.46%. (Table 1)

The lipid profile examination specificity test used normal control and high control materials. Deviations found were as follows: total cholesterol 2.7%; 2.05%, triglycerides 1.95%; 1.09%, LDL-cholesterol 1.07%; 1.37% and HDL-cholesterol 2.32%; 1.41%. (Table 1)

All of the results were still within the WHO and the
In the control group, the total cholesterol median value was 228.65 mg/dL, minimum value was 157.20 mg/dL, and maximum value 341.60 mg/dL. High total cholesterol level was found in 65.79% (25/38). In the case group the total cholesterol median value was 229.40 mg/dL, minimum value was 155.10 mg/dL, maximum value 474.70 mg/dL. High total cholesterol level was found in 86.84% (33/38) as shown in table 2 and 3. The significance test revealed that there was no significant difference between the case and the control group, p = 0.060.

In the control group the triglyceride median value was 137.65 mg/dL, the minimum value was 51 mg/dL, maximum value 386.40 mg/dL. High triglyceride level was found in 47.37% (18/38). In the case group the triglyceride median value was 180.45 mg/dL, the minimum value was 99.90 mg/dL, maximum value 580.40 mg/dL. High triglyceride level was found in 65.79% (33/38) as shown in table 2 and 3. There was no significant difference between the case and the control group, p = 0.165.

In the control group the LDL cholesterol median value was 111.25 mg/dL, the minimum value was 52.70 mg/dL, maximum value 275.80 mg/dL. High LDL cholesterol level was found in 34.21% (13/38). In the case group the LDL cholesterol median value was 133.95 mg/dL, the minimum value was 53.10 mg/dL, maximum value 273.80 mg/dL. High LDL cholesterol was found in 65.79% (25/38) as shown in table 2 and 3. The significance test revealed that there was no significant difference between the case and the control group, p = 0.110.

In the control group the HDL cholesterol median value was 47 mg/dL, the minimum value was 28.10 mg/dL, maximum value 80 mg/dL. Low HDL cholesterol level was found in 21.06% (8/38). In the case group the HDL cholesterol median value was 42.15 mg/dL, the minimum value was 25 mg/dL, maximum value 86.70 mg/dL. Low HDL cholesterol level was found in 39.48% (15/38) as shown in table 2 and 3. There was no significant difference between the case and the control group (p = 0.134).

Double logistic regression test of the independent variables to the dependent variable was done. From the chi square test, variables with p<0.25 were entered into the multivariate analysis, i.e. total cholesterol (p 0.060), triglyceride (p 0.165), HDL cholesterol (p 0.134), and LDL cholesterol (p 0.110) (Table 3). The results of the multivariate test with logistic regression 1 are shown on table 4. The results of the multivariate test with logistic regression 2 showed that HDL cholesterol was a risk factor for ischemic stroke with p = 0.041 and odds ratio 3.09 (CI 95%: 1.04; 8.73) (see Table 5).

**DISCUSSION**

Ischemic stroke occurs most commonly due to blood vessels disorders, i.e, atherosclerosis, hypertension, and thrombosis. The main cause of atherosclerosis is dyslipidemia.

Dyslipidemia is a lipid metabolism disorder marked by increased or decreased plasma lipid fraction. Dyslipidemia may occur due to hereditary (genetic) or
Acquired disorders are caused by diabetes mellitus, nephrotic syndrome, biliary obstruction, or hypothyroidism.19

The main lipid fraction disorders in dyslipidemia are raised total cholesterol, LDL cholesterol, and triglyceride levels, and decreased HDL cholesterol level.20 The degree and progressivity of carotid atherosclerosis is directly related with increased total cholesterol, LDL cholesterol, and triglyceride levels, and decreased HDL cholesterol level.21 Patients who were given LDL lowering agents for 17 months had 38% reduced carotid plaque.22 The scandinavian simvastation survival study group research found a significantly decreased stroke incident as much as 30% with simvastation treatment.23 A clear connection between cholesterol and stroke was commonly not found. According to the Copenhagen City Heart, as quoted by Widjaya, cholesterol was a risk factor of ischemic stroke when the level >310 mg/dL. Some researchers stated that there is no fixed connection between cholesterol and stroke. This statement might be due to the fact that not all strokes are caused by atherosclerosis.24

| Table 3. Characteristics of Case-Control Laboratory Parameter Categorical Data |
|---------------------------------|------------------|------------------|----------------|----------------|------------------|
| CASE (%)                        | CONTROL (%)      | OR               | CI 95%         | p               |
| Total cholesterol <200 mg/dL    | 5(13.16)         | 13(34.21)        | 1              | (1.08;10.89)    | 0.060            |
| >200 mg/dL                      | 33(86.84)        | 25(65.79)        | 3.43           | (0.85;5.39)     | 0.165            |
| Triglyceride <150 mg/dL         | 13(34.21)        | 20(52.63)        | 1              | (0.94;5.99)     | 0.110            |
| >150 mg/dL                      | 25(65.79)        | 18(47.37)        | 2.14           | (0.89;6.75)     | 0.134            |
| LDL-Cholesterol <130 mg/dL      | 17(44.74)        | 25(65.79)        | 1              | (0.94;5.99)     | 0.110            |
| >130 mg/dL                      | 21(55.26)        | 13(34.21)        | 2.38           | (0.94;5.99)     | 0.110            |
| HDL-Cholesterol >40 mg/dL       | 23(60.52)        | 30(78.94)        | 1              | (0.89;6.75)     | 0.134            |
| <40 mg/dL                       | 15(39.48)        | 8(21.06)         | 2.45           | (0.89;6.75)     | 0.134            |

F = free variable coefficient, SE = standard error for F coefficient, Wald = wald statistics if df = 1. df = degree of freedom from Wald statistic, sig = significance point. Exp (F) = odds ratio, CI = confidence interval

| Table 4. Results of the Multivariate test with 1 Variable Logistic Regression in The Equation |
|---------------------------------|------------------|------------------|----------------|----------------|------------------|
| Parameter                       | F    | SE   | Wald | df | sig   | Exp (F)         | 95% CI          |
| Total cholesterol               | 0.753| 0.671| 1.262| 1  | 0.261 | 2.1124          | (0.571;7.909)   |
| Triglyceride                    | 0.198| 0.571| 0.12 | 1  | 0.729 | 1.219           | (0.398;3.728)   |
| LDL-Cholesterol                 | 0.531| 0.552| 0.923| 1  | 0.337 | 1.7             | (0.576;5.018)   |
| HDL-Cholesterol                 | 0.765| 0.604| 1.603| 1  | 0.205 | 2.15            | (0.657;7.029)   |

| Table 5. Results of the Multivariate Test with 2 Variable Logistic Regression in the Equation |
|---------------------------------|------------------|------------------|----------------|----------------|------------------|
| Parameter                       | B    | SE   | Wald | df | sig   | Exp (B)         | 95% CI          |
| HDL-Cholesterol                 | 1.105| 0.542| 4.158| 1  | 0.041 | 3.09            | (1.044;8.731)   |
The statement that triglyceride is a risk factor for ischemic stroke is still debatable. Alam et al, however, found a significant correlation between carotid artery stenosis, examined by carotid ultrasound, with elevated triglyceride level. A recent research stated that high triglyceride level was atherogenic to the external carotid artery.

The role of lipid in atherosclerosis begins in the fatty streak build up, the LDL entrance into the arterial wall. Artery released products which oxidises LDL, trapping LDL in the subendothelial segment. Oxidised LDL is phagocytised by macrophages through scavenger receptors and becomes foam cells. In the macrophages ester cholesterols are accumulated, the foam cells will necrotize due to the cytotoxic ox-LDL and the direct effect of free radicals. The foam cells will develop to fatty streak.

HDL functions in transporting cholesterols from the periphery to the liver. In dyslipidemia, decreased HDL level causes excessive cholesterols inside the arterial wall. This, in turn, causes enlarged plaque and decreased plaque stability, making it easily tearable.

The diagnosis of ischemic stroke in this research was established based on clinical symptoms and brain CT scan. The blood samples were taken 1 week after the acute phase of stroke. The acute phase of stroke starts from the beginning of stroke to 2 weeks after. The 3 weeks after stroke sample taking was done to avoid the changes in acute phase of stroke. In such phase lipid profile will decrease whilst glucose and fibrinogen levels will increase.

In this research, high total cholesterol levels were found in 86.84% (33/38) of the case group and 65.79% (25/38) of the control group. Table 3 showed the result of significance test between the case and control group. There was no significant difference, p = 0.060 (p>0.05). Hachinski et al found a significant difference of elevated total cholesterol level (p = 0.03). In a cohort study of Japanese Hawaiian by Takeya et al, total cholesterol was stated as a primary causing factor of stroke on account of raised total cholesterol level precipitates the development of atherosclerosis. Tanne et al stated total cholesterol level as a fatal cause of ischemic stroke with a percentage of 5.5%. Tell et al performed autopsies and found a correlation between elevated total cholesterol and atherosclerosis of large arteries of the circle of Willis, while raised triglyceride level had a correlation with atherosclerosis of small arteries. This research did not find a significant difference. This result is in accordance with a research by Ramli which did not find a significant correlation between cholesterol and stroke. The stroke might be due to other risk factors.

In the case group high triglyceride level was found in 65.79% (25/38), in the control group 47.37% (18/38). Table 3 showed that the triglyceride levels in the case group were not significantly different compared to the control group, p=0.165. The role of triglycerides as a risk factor for ischemic stroke is still debatable. However, a research by Alam et al succeeded in proving a significant correlation between carotid artery stenosis (assessed by carotid ultrasound) and elevated triglycerides, p = 0.01 and r = 0.678. Reed et al and Tell et al also proved that atherosclerosis of the small blood vessels was related with elevated triglycerides. Lu et al considered triglycerides as atherogenic, reasoning that triglycerides are taken by macrophages forming foam cells and therefore precipitates the process of atherosclerosis. Lu quoted the result of the systolic hypertension in the elderly program (SHEP) research of 4736 nondiabetic patients, which found elevated triglyceride levels above 400 mg/dL could be used as a predictor of cerebrovascular disease (CVD). The National Cholesterol Education Program (NCEP) stated that elevated triglyceride level was an independent risk factor for coronary heart disease on account of triglycerides are part of VLDL, which is atherogenic.

This research did not find a significant difference and this is in accordance with the belief that its role as a risk factor for ischemic stroke remains debatable.

In the case group 55.26% (21/38) had high LDL cholesterol levels, in the control group 34.21% (13/38). Table 3 showed the results of the significance test. There was no significant difference between the case group and the control group, p=0.110. LDL cholesterol is an important risk factor for atherosclerosis, which influences ischemic stroke. This research found no significant correlation between LDL cholesterol levels and ischemic stroke. The stroke might be due to other risk factors besides atherosclerosis.

There were 39.48% (15/38) in the case group who had low HDL cholesterol levels and 21.06% (8/38) in the control group. The multivariate test between the case group and the control group found a significant difference, p = 0.041 (Table 4 and 5). Based upon the result of the multivariate test we obtained the odds ratio for low HDL cholesterol of 3.09. This indicates that subjects with low HDL cholesterol have 3.09 x risk compared to subjects with low HDL level. The result is in accordance with Eckardstein et al which stated that HDL functions by transporting cholesterols from the periphery back to the liver. Low HDL level leads to excess of cholesterols in the arterial wall, which may cause atherosclerotic plaque build up.
CONCLUSION

Low HDL cholesterol level is a risk factor of ischemic stroke with odds ratio of 3.09. High levels of total cholesterol, triglycerides, and LDL cholesterol are not risk factors for ischemic stroke.

REFERENCES