Association of High Blood Homocysteine and Risk of Increased Severity of Ischemic Stroke Events

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Abstract

Stroke is the leading cause of death and disability in the world as well as in Indonesia. Initial stroke severity is an important factor that affects short- and long-term stroke outcomes. This cross-sectional study was conducted in Cipto Mangunkusumo Hospital from July 2017 to January 2018 to investigate the factors that affect stroke severity. A total of 77 acute ischemic stroke patients were divided into three groups, which include low blood homocysteine levels ($< 9 \mu\text{mol/L}$), moderate blood homocysteine levels ($9\text{--}15 \mu\text{mol/L}$), and high blood homocysteine levels ($> 15 \mu\text{mol/L}$). The acquired data were analyzed using Kruskal–Wallis test and a significant difference of initial National Institute of Health Stroke Scale (NIHSS) and blood homocysteine levels ($H = 13.328$, $p = 0.001$) were seen, with a mean rank of 25.86 for low blood homocysteine levels, 33.69 for moderate blood homocysteine levels, and 48.94 for high blood homocysteine levels. The patients were then divided into two groups based on the NIHSS (≤ 5 and > 5) to calculate the risk correlation of blood homocysteine levels and NIHSS by using regression. We found that patients with high blood homocysteine levels had 14.4 times higher risk of having NIHSS > 5 compared with those with low blood homocysteine levels ($p = 0.002$, 95% confidence interval [CI] [2.714–76.407]), and 3.9 times higher risk compared with those with moderate blood homocysteine levels ($p = 0.011$, 95% CI [1.371–11.246]). We concluded that homocysteine is a risk factor for a higher stroke severity. Future studies to evaluate the usefulness of homocysteine-lowering therapy in stroke patients are recommended.

Keywords

- ▶ acute ischemic stroke
- ▶ homocysteine
- ▶ NIHSS
- ▶ stroke severity

Stroke is the leading cause of death and disability in the world as well as in Indonesia.^{1,2} Every year, 16 million people suffer a stroke for the first time worldwide.¹ The prevalence of stroke survivors is 62 million people. Stroke causes 9.7% of deaths worldwide.¹ These figures are expected to increase to 23 million new stroke cases per year and a death rate of 7.8 million per year by 2030.¹ In adults aged 45 to 64 years, stroke is a major cause of disability. In Indonesia, based on Riskesdas 2013, the prevalence of stroke (age > 15 years) is 1.21%. The highest prevalence is found at age above 75 years (6.7%).²

The burden of stroke depends on the acute phase management, rehabilitation, and functional outcomes of stroke patients.³ A wide variety of factors influence stroke outcome, including stroke severity, which can be determined by neurological examination.⁴ Generally, strokes with severe initial neurological impairment have poorer outcomes compared with strokes with mild impairment.⁴ Stroke severity can be quantitatively measured by using scoring systems. The most commonly used scoring system is the National Institute of Health Stroke Scale (NIHSS). Several studies have

showed that NIHSS can predict stroke outcome although it has some limitations such as inability to capture all stroke symptoms.^{4,5}

Acute stroke severity is affected by several factors. Factors that affect the risk of stroke occurrence can also affect severity of stroke in different ways. For example, stroke in diabetic patients were found to be similar in stroke severity to nondiabetic patients.⁶ Extremely low and high blood pressure at the time of admission is known to influence stroke severity and outcome.^{7,8}

Although homocysteine (Hcy) has been known to be a risk factor of vascular diseases,⁹ study results about the association between blood Hcy levels and ischemic stroke severity are not yet convincing.¹⁰ Hcy is an amino acid that can, independently or together with other factors such as low-density lipoprotein (LDL), induce endothelial damage by transforming into Hcy-thiolactone.¹¹ Hyperhomocysteinemia is a probable cause of cryptogenic stroke.¹²

A study by Waśkiewicz et al showed that people with Hcy levels above 8.2 $\mu\text{mol/L}$ had a 1.5 times higher risk of death from cardiovascular diseases (including stroke).¹³ In individuals with Hcy levels above 10.50 $\mu\text{mol/L}$, this risk increased to 4.2-fold.¹³ In another study by Vollset et al, it was found that blood Hcy levels were a strong predictor of death from cardiovascular diseases. People with Hcy levels of 9 to 12 $\mu\text{mol/L}$ had a 1.25 times higher risk of death from cardiovascular disease compared with those with lower Hcy level ($< 9 \mu\text{mol/L}$).¹⁴ Every increase of 5 $\mu\text{mol/L}$ in Hcy level increases the risk of death from cardiovascular disease by 1.65 times, and by 1.99 times specifically for cerebrovascular disease.¹⁴ A meta-analysis of prospective observational studies by He et al demonstrated the relative risk of ischemic stroke in the high Hcy group compared with low group is 1.69 times.¹⁵ Hcy is more strongly associated with small blood vessel/lacunar infarct disorders compared with other stroke types.¹⁶

This study was done to investigate the associations between these factors and acute ischemic stroke (AIS) severity. We conducted this study at Cipto Mangunkusumo Hospital, the top referral hospital for stroke in Jakarta, in hope of gaining data which are representative to the Indonesian population.

Methods and Statistics

All AIS patients who came to Cipto Mangunkusumo Hospital between July 1, 2017, and January 31, 2018, were enrolled in this study if they were admitted within 72 hours after stroke onset. Patients were excluded if the diagnosis was not ischemic stroke (e.g., hemorrhagic, transient ischemic attack [TIA]), the stroke onset was more than 72 hours, the patient had been having an infection before admission, the patient had chronic kidney disease, or if the patient underwent intravascular intervention. All stroke patients who came to Cipto Mangunkusumo Hospital underwent standard stroke risk factor assessment (hypertension, diabetes, etc.) and NIHSS assessment. Patients recruited for this study were additionally checked for blood Hcy levels. Hypertension was defined as previous diagnosis of hypertension or if the patient was under

antihypertensive treatment. Diabetes mellitus (DM) was defined as fasting blood sugar $\geq 126 \text{ mg/dL}$, previous diagnosis of DM, or if the patient was under hypoglycemic agent and/or insulin treatment. The patients' blood Hcy levels were divided into normal ($< 9 \mu\text{mol/L}$), moderate (9–15 $\mu\text{mol/L}$), and high ($> 15 \mu\text{mol/L}$). For determining statistical significance, the NIHSS data were used both as continuous and categorical data for different purposes.

Data were analyzed using SPSS version 17. Normality of the data was first tested using the Kolmogorov-Smirnov test. Kruskal-Wallis test was used to determine if there were any statistically significant differences of NIHSS scores between the three Hcy groups. Mann-Whitney test was used where the independent variable consisted of two groups (hypertension, diabetes). Odds ratios (ORs) between groups of patients for having a moderate-severe (> 5) NIHSS score instead of mild (≤ 5) NIHSS score were calculated using logistic regression.

The study was approved by the Research Committee at the Faculty of Medicine, University of Indonesia, Jakarta. All subjects provided informed consent to participate in the study.

Results

There were a total of 77 AIS patients that fulfilled our criteria from July 1, 2017 until January 31, 2018, with demographical characteristics as seen in ►Table 1.

The mean age of our study population was 57.6 years, with 57.1% of them below 60 years and 42.9% of them 60 years of age or more. There were slightly more females than males (39:38 [50.6%:49.4%]). The prevalence of hypertension in our subjects was 76.6%, which was, although quite high, still typical of stroke patients especially in Asia.^{17,18} Meanwhile, the prevalence of diabetes in our subjects was 33.8% (►Table 2).

Table 1 Demographic characteristics of study participants

Sample characteristics	Overall (n = 77)
Demographic characteristics	
Age, y, mean (SD)	57.57 (11.94)
Age, y, n (%)	
≥ 60	33 (42.90)
< 60	44 (57.10)
Gender, n (%)	
Male	38 (49.4)
Female	39 (50.6)
Hypertension, n (%)	
Yes	59 (76.6)
No	18 (23.4)
DM, n (%)	
Yes	26 (33.8)
No	51 (66.2)

Abbreviations: DM, diabetes mellitus; SD, standard deviation.

Table 2 Descriptive statistics of blood homocysteine level and NIHSS

Hcy, μmol/L, mean (SD)	15.5 (7.21)
< 9, n (%)	14 (18.2)
9–15, n (%)	29 (37.7)
> 15, n (%)	34 (44.2)
NIHSS, median, mode	5, 4
> 5, n (%)	37 (48.1)
≤5, n (%)	40 (51.9)

Abbreviations: NIHSS, National Institute of Health Stroke Scale; SD, standard deviation.

The prevalence of hyperhomocysteinemia (using 9 μmol/L as cut-off) in our study population was 81.8%, which was higher than the prevalence of hypertension. Among our study population, 34 (44.2%) patients had Hcy > 15 μmol/L and 29 (37.7%) had Hcy of 9 to 15 μmol/L. Most of the patients (51.9%) had NIHSS score of 5 or lower.

A Kolmogorov–Smirnov test revealed that our NIHSS data were not normally distributed ($p < 0.001$, $p = 0.011$, and $p = 0.003$, for each Hcy group); therefore, we used nonparametric tests to determine statistically significant differences between groups. A Kruskal–Wallis test showed that the difference in NIHSS between the Hcy groups was statistically significant ($H = 13.328$, $p = 0.001$), with a mean rank of 25.86 for normal blood Hcy levels, 33.69 for low–moderate blood Hcy levels, and 48.94 for high Hcy. Mann–Whitney tests revealed that existence of hypertension and diabetes did not significantly affect stroke severity ($p = 0.827$ and $p = 0.458$, respectively) (►Table 3).

By categorizing the NIHSS into two groups, we calculated the OR of AIS patients to have NIHSS > 5 instead of ≤5. Patients with high (> 15 μmol/L) blood Hcy level had 14.4 times higher risk of having NIHSS > 5 compared with those with normal (< 9 μmol/L) blood Hcy level ($p = 0.002$, 95% confidence interval [CI] [2.714, 76.407]), and 3.9 times higher risk compared with those with moderate (9–15 μmol/L) blood Hcy level ($p = 0.011$, 95% CI [1.371, 11.246]). The difference between moderate and low group was not statistically significant (OR, 3.67, $p = 0.128$, 95% CI [0.687, 19.563]).

Table 3 Contingency table of homocysteine and NIHSS

		NIHSS		Total
		≤5	> 5	
Homocysteine	< 9	12	2	14
	9–15	18	11	29
	> 15	10	24	34
Total		40	37	77

Abbreviation: NIHSS, National Institute of Health Stroke Scale.

Using logistic regression, we also excluded other risk factors, namely, hypertension ($p = 0.548$), DM ($p = 0.403$), age ($p = 0.086$), and gender ($p = 0.762$). We found that Hcy is a risk factor for increased stroke severity, which is independent of other risk factors we included in the analysis.

Discussion

Hcy is a nonprotein amino acid that is homologous to cysteine. It is not obtained from food directly, but biosynthesized from methionine through several steps. Hcy can be recycled back to methionine or converted to cysteine.¹¹ Hcy metabolism pathways have several steps that require pyridoxine, folic acid, or methylcobalamin as coenzymes. Vitamin B deficiency is one of the possible hindrances that can affect Hcy metabolism and cause hyperhomocysteinemia.¹¹

In hyperhomocysteinemia, Hcy tends to be converted to Hcy-thiolactone. Hcy-thiolactone is formed by methionyl-transfer ribonucleic acid synthase.¹¹ Hcy-thiolactone is a reactive intermediate compound that can cause N-homocysteinylation of proteins. Hcy-thiolactone induces apoptosis in vascular endothelial cells, premyeloid HL-60 cells, placental trophoblasts, and inhibits insulin signaling, at lower concentrations than Hcy (0.05 μmol/L compared with 3 μmol/L)¹¹; thus, it is theorized that Hcy toxicity occurs after conversion to Hcy-thiolactone.

Hcy-thiolactone is a reactive intermediate that causes protein N-homocysteinylation through the formation of amide bonds with ε-amino groups of protein lysine residues. N-homocysteinylation by Hcy-thiolactone causes protein damage. For example, N-homocysteinylation of fibrinogen can lead to fibrin clots that are abnormally resistant to lysis.^{19–21} N-homocysteinylation of proteins, such as myoglobin, fibrinogen globulins, trypsin, and transferrin are susceptible to multimerization and undergo structural changes that lead to their denaturation and precipitation.¹¹ N-homocysteinylation may also have negative effect on LDL function. Additional thiol groups in N-Hcy-LDL may protect LDL from oxidation.²² N-Hcy-proteins are also cytotoxic and can induce autoimmune response.¹¹

Our finding in this study is supported by several studies we mentioned earlier.^{13–16} Combined with our finding, we can conclude that blood Hcy effect on stroke severity and outcome starts to manifest at 8 to 10 μmol/L, although this value should be adjusted further to other factors, particularly age and gender, in a larger study with a greater number of samples. In this cross-sectional study, we did not directly observe the outcome of ischemic stroke. However, since the link between stroke severity and stroke outcome is strong,^{4,5} this study result is very promising and a prospective study will very likely confirm our assumption. Further studies should be conducted to find out if lowering blood Hcy in acute stroke ischemic patients with hyperhomocysteinemia helps improve stroke outcomes.

Hypertension plays an important role in the pathogenesis of atherosclerosis, which in turn causes ischemic stroke due to thrombotic arterial occlusion or embolism. In chronic hypertension, local changes in the endothelium, interaction

between endothelium and blood cell, and release of substances that can influence vascular tone and/or permeability, such as endothelin, nitric oxide, cytokines, and free radicals, are damaging to the endothelium.²³ Endothelial damage can enhance leucocyte adhesion and cause local thrombi formation. Hypertension also accelerates the arteriosclerosis process which usually starts in the larger extracerebral arteries. An embolus from plaques in larger arteries, such as the carotid and vertebral arteries, can cause TIAs and brain infarction. All this increases the likelihood for an ischemic stroke to happen.²³ However, our data showed that the mere presence of chronic hypertension may not be enough to predict the severity and outcome of AIS patients. Instead, other studies concluded that the specific blood pressure value at the time of admission is a good predictor of stroke severity and outcome.^{7,24} Extremely high or low blood pressure tends to increase stroke severity.^{7,24}

It seems to be the same case with diabetes. Hyperglycemia supposedly contributes to the pathogenesis of macrovascular complications and, therefore, ischemic stroke, through several possible pathways including the generation of reactive oxygen species which leads to endothelial dysfunction, formation of advanced glycosylation end products that accelerates the atherosclerotic process, and the diversion of glucose into the aldose reductase pathway and the activation of one or more isozymes of protein kinase C.²⁵ These changes lead to the chronic state of low-grade inflammation, endothelial dysfunction, hypercoagulability, dyslipidemia, and insulin resistance.²⁵ However, until now there are no evidence to show that stroke prevention will be improved by intensive glucose-lowering treatment, in people with either type 1 or type 2 diabetes.²⁶ Diabetes also does not seem to predict the severity of AIS. However, blood glucose and glycemic control status at the time of admission are more predictive of AIS severity.²⁷

Conclusion

Hyperhomocysteinemia is an independent factor that affects initial stroke severity measured by NIHSS. Since initial stroke severity is highly predictive of stroke outcome, we predicted that blood Hcy is also an important factor in determining AIS's short- and long-term outcome. Hyperhomocysteinemia is strongly dominant in determining AIS outcome. Further studies to look into the effect of Hcy-lowering therapy on stroke severity and outcome can be beneficial to improve the management of ischemic stroke. The simple presence of hypertension and diabetes did not seem to affect stroke severity. The more important factors that affect stroke severity are the specific values (blood pressure, blood glucose, etc.) at the time of admission and should be considered in future studies.

Note

This study has been approved by Ethics Committee of Faculty of Medicine, Indonesia University.

Source of Support

None.

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