The Role of Persistent Anticardiolipin Antibody as Risk Factor of Ischemic Stroke

Suzanna Immanuel*, Astuti Giantini*, Rahajuningsih S Darma*, Samino**

ABSTRACT

Aim: to determine the role of persistent ACA and hyperviscosity as risk factor of ischemic stroke

Methods: a study was conducted on 76 subjects whose age 40 to 70 years. Subjects consisted of 38 patients of post ischemic stroke and 38 controls with diagnosis other than stroke. Fresh blood samples were taken and mixed with EDTA for viscosity examination and serum for ACA IgM and IgG examination. The laboratory examination for persistent ACA IgM and IgG used ELISA method, while viscosity analysis was using viscometer. Statistical analysis used chi-square and multivariate analysis with logistic regression.

Results: In this study we found persistent ACA IgG in 25% of case group, and 2.63% in control group. Multivariate analysis showed persistent ACA IgG as risk factor for ischemic stroke with p < 0.05 and OR 14.11 (CI 95%; 1.64; 121.11). We found persistent ACA IgM in 2.78% of case group and 5.26% in control group. High blood viscosity was found in 15.79% case group and 10.53% in a control group. Statistical analysis showed no significant difference of viscosity (p = 0.740) and persistent ACA IgM (p = 1.000) between case and control group.

Conclusion: study showed that persistent ACA IgG in stroke ischemic was higher than in control subjects. Blood viscosity examination and persistent ACA IgM did not show significant difference. While persistent ACA IgG with OR 14.11 (CI: 1.64; 121.11) was the risk factor for ischemic stroke. Blood viscosity and persistent ACA IgM were not risk factors for ischemic stroke.

Key words: Ischemic stroke, ACA IgG, ACA IgM, blood viscosity.

INTRODUCTION

According to World Health Organization (WHO), stroke is neurologic deficit with sudden onset and persists for 24 hours or more and caused by vascular disorder.¹ Stroke was the 3rd leading cause of death in the world and responsible for mental and physical disability in productive age and elderly.²

There are still limited epidemiologic data on incidence of stroke in Indonesia. From Indonesia Health Survey 1998, stroke was the 1st leading cause of death in all hospital in Indonesia.³ From 1999 data there were 562 cases of stroke hospitalized with mortality rate 27.2%. In 2000, stroke was the most frequent case in neurology department in Cipto Mangunkusumo Hospital, Jakarta, with as many as 641 cases.⁴

Based on lesion characteristics, in general stroke is divided into 2 categories; ischemic and hemorrhagic stroke.⁴ The prevalence of ischemic stroke was 83% of all stroke cases.⁵,⁶ The number of patients hospitalized in Neurology Department Medical Faculty University of Indonesia in 1994 was 658 and the causes consecutively were cerebral thrombosis in 60.9% patients, cerebral emboli in 14.6%, intracerebral and subarachnoid bleeding in 24.5%.⁴

Ischemic stroke is frequently caused by vascular disorder, which is atherosclerosis, hypertension and thrombosis;⁷,⁸ Misbach et al found the causes of stroke in 28 hospitals in Indonesia were hypertension 73%, smoking 20.45%, ischemic heart disease 19.9%, diabetes mellitus 17.3% and hypercholesterolemia 16.4%.⁹

Other causes of ischemic were arterial thrombosis.¹⁰ Thrombosis may arise from vascular lesion, abnormal viscosity and blood flow.¹¹,¹² Vascular lesion may be caused by atherosclerosis. Viscosity abnormality is caused by the presence of ACA.¹³,¹⁴ Blood flow abnormality is caused by blood hyperviscosity.¹⁵,¹⁶ The aim of the study was to determine the role of persistent ACA and hyperviscosity as risk factor for ischemic stroke.
METHODS

Seventy six subjects from outpatient clinic at Neurology Department FKUI/RSCM were enrolled in this study whose age ranging from 40 to 70 years. Subjects consisted of 38 cases of stroke and 38 subjects in control group. Case group of stroke consisted of patients with 3 weeks post ischemic stroke and control group were patients with diagnosis other than stroke. Blood samples were taken from venous puncture. They were divided into 2 parts. First part was put in the tube containing EDTA for viscosity examination. The rest was put in vacuntainer without anticoagulant. The blood then was centrifuged for 15 minutes to obtain sera. The sera were frozen at 20 C for ACA IgG and IgM examination. Serum storage did not exceed 1 month. If the results of ACA IgG and IgM were positive, the examination would be repeated within 6 weeks after the first samples taken.

ACA IgG and IgM were determined using ELISA methods of Organon Teknika, while blood viscosity was measured using Viscometer apparatus Brookfield LVDV IIIcp40 with rotational methods.

Independent variables in this study were blood viscosity, ACA IgG and ACA IgM. Statistical analysis used chi-square test for categorical variables and logistic regression for multivariate analysis. After chi-square test, variable with p < 0.25 was included in multivariate analysis.

RESULTS

First ACA IgM examination in the case group was found to be negative in 30 subjects and indeterminate in 5 subjects. Moderate positive result was found in 2 subjects and high positive in 1 subject. Number of subjects in the case group was reduced because 2 patients dropped out and did not come back for repeated examination. Repeated examination on 3 subjects with positive ACA IgM revealed 1 subject with persistent ACA IgM, while the other 2 subjects were drop out. Thus, persistent ACA IgM in case group was 2.78% (1/36) (Table 1).

The first ACA IgG examination in case group we found to be negative in 24 subjects, indeterminate in 3 subjects and moderate positive in 11 subjects. Repeated examinations in subjects with moderate positive result of ACA IgG found highly positive in 1 subject; 8 remained moderate positive and 2 subjects were drop out. We found 25% (9/36) persistent ACA IgG in case group (Table 1).

First ACA IgM examination in control group revealed negative result in 25 subjects, indeterminate in 3 subjects and moderate positive in 11 subjects. Repeated examinations in subjects with moderate positive result of ACA IgG found highly positive in 1 subject; 8 remained moderate positive and 2 subjects were drop out. We found 5.26% (2/38) persistent ACA IgM in control group (Table 2).

First examination of ACA IgG in control group revealed negative result in 25 subjects, indeterminate in 3 subjects and moderate positive in 11 subjects. Repeated examinations in subjects with moderate positive result of ACA IgG found highly positive in 1 subject; 8 remained moderate positive and 2 subjects were drop out. We found 2.63% (1/38) persistent ACA IgG in control group (Table 2).

Table 1. Result of ACA Examination in Case Group

<table>
<thead>
<tr>
<th>Result</th>
<th>Examination I</th>
<th>Examination II*</th>
<th>Persistent ACA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACA IgM</td>
<td>ACA IgG</td>
<td>ACA IgM</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Moderate Positive</td>
<td>2</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Highly Positive</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Repeated examination only be done in certain subject with results of moderate or high positive on first examination; DO = Drop Out

Table 2. Result of ACA Examination in Control Group

<table>
<thead>
<tr>
<th>Result</th>
<th>Examination I</th>
<th>Examination II*</th>
<th>Persistent ACA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACA IgM</td>
<td>ACA IgG</td>
<td>ACA IgM</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Moderate Positive</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Highly Positive</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Repeated examination only be done in certain subject with results of moderate or high positive on first examination; DO = Drop Out
Table 3. Categorical Data Characteristics of Laboratory Parameter in Case and Control Group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>OR</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viskosity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>32 (84.22)</td>
<td>34 (89.47)</td>
<td>1</td>
<td>(0.41;6.17)</td>
<td>0.740</td>
</tr>
<tr>
<td>Increase</td>
<td>6 (15.78)</td>
<td>4 (10.53)</td>
<td>1.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA IgM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Persistent</td>
<td>35 (97.22)</td>
<td>34 (94.44)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>1 (2.78)</td>
<td>2 (5.56)</td>
<td>0.48</td>
<td>(0.04;5.60)</td>
<td>1.000</td>
</tr>
<tr>
<td>ACA IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Persistent</td>
<td>27 (75)</td>
<td>35 (97.22)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>9 (25)</td>
<td>1 (2.78)</td>
<td>11.48</td>
<td>(1.38;95.89)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Table 4. Multivariate Analysis with Logistic Regression Variable in The Equation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>sig</th>
<th>Exp(B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent ACA IgG</td>
<td>2.646</td>
<td>1.097</td>
<td>5.816</td>
<td>1</td>
<td>0.016</td>
<td>14.11</td>
<td>(1.64; 121.11)</td>
</tr>
</tbody>
</table>

8 subjects and moderate positive in 5 subjects. Repeated examination on 5 subjects with positive ACA IgG found 1 subject with persistent ACA IgG (2.63%) as shown in table 2.

In control group we found median viscosity of 4.1 mPa.s, minimum value of 3.2 Pa.s and Maximum value 5.4 Pa.s. High blood viscosity was found in 10.53% (4/38). In case group we found median blood viscosity was 4.2 mPa.s, minimum value 3.4 mPa.s, and maximum was 5.4 mPa.s. High viscosity blood was found in 15.79% (6/38). Statistical analysis showed no significant difference of viscosity (p=0.740) and ACA IgM (p=1.000) between case and control group (Table 3).

We conducted logistic regression analysis tests between independent and dependent variables. After chi-square test, variable of persistent ACA IgG with p<0.25 (p=0.010) was included in multivariate analysis. Multivariate analysis showed persistent ACA IgG as risk factor for ischemic stroke with p < 0.016 and OR 14.11 (CI 95%: 1.64; 121.11) (Table 4).

DISCUSSION

WHO definition of stroke is sudden onset of neurologic deficit that persists for 24 hours or more caused by vascular disorder.¹

Prevalence of ischemic stroke is 83% of all stroke.² Recurrent stroke in US reached 500,000 patients from 3,000,000 surviving patients. Therefore, it is very important to prevent the recurrence of stroke by identifying the risk factor.¹⁷,¹⁸

According to Sacco, risk factor of stroke is categorized into modifiable and non modifiable risk factors. Nonmodifiable risk factors include age, sex, race/ethnicity and genetics. Modifiable risk factors include hypertension, cardiac disorder such as a trial fibrillation, diabetes mellitus, smoking habit, alcoholism, and hyperlipidemia.¹⁷,¹⁹

Stroke occurs when blood clotting blocks cerebral blood flow. Ischemic stroke caused by blood clotting in artery is called thrombotic stroke. If blood clotting originates from other part of body, it is called embolic stroke.²⁰

Based on lesion characteristics, stroke is divided into 2 groups: ischemic and hemorrhagic stroke.⁴ Ischemic stroke occurs due to inadequate cerebral oxygen perfusion caused by temporary or permanent occlusion of intracranial and extracranial feeding arteries. This condition may cause irreversible cell damage due to insufficient oxygen and nutritional supply. On the other hand, hemorrhagic stroke is frequently related to hypertension and may occur spontaneously due to the rupture of abnormal vascular tissue such as aneurysm and artery vena malformation (AVM) or arterioles in cerebral tissue.⁵

Ischemic stroke is mostly caused by vascular disorder such as atherosclerosis, hypertension and thrombosis.⁷ Main cause of atherosclerosis is dyslipidemia.²¹ Risk factors are related to ischemic stroke include ACA ¹³,¹⁴ and increased blood viscosity.²²

Virchow stated that 3 factors having important role
in pathophysiology of thrombosis are vascular lesion, blood flow changes and coagulation. In arterial thrombosis, main factor is vascular lesion due to atherosclerosis. Changes in blood flow may be caused by hyperviscosity or turbulence flow.  

Changes in coagulation or hypercoagulable state may be caused by acquired or genetic disorder. Acquired disorder related to thrombosis are activated protein-C resistance, protein C deficiency, protein S deficiency, anti thrombin III (AT III) deficiency, hyper/dysfibrinogenemia, hyperhomocysteinemia and increased fibrinolytic inhibitor plasminogen activator inhibitor (PAI-1). Acquired disorder which is the most frequently cause of thrombosis is antiphospholipid antibody (APA). There are several theories regarding mechanism of thrombosis in ACA-thrombosis syndrome: ineffective anticoagulation, increased platelet activation, decreased fibrinolytic activity and activated coagulation system. Ineffective coagulation is caused by inhibition of thrombomodulin by which is needed for protein C activation, lack of active protein C, F Va, F VIII which all would result in hypercoagulable state. Besides, fibrinolytic activity is declined and active protein C has role to increase fibrinolysis.  

ACA will enhance platelet activation. Interaction between ACA and phospholipid membrane of platelet will cause platelet activation. ACA will reduce the synthesis of prostacyclin inside the endothel. Thus, it will reduce platelet aggregation inhibition.  

ACA may disturb fibrinolytic activity due to circulating plasmin. Plasmin is derived from activation of plasminogen by plasminogen activator. ACA will disturb plasminogen activator so that the conversion of plasminogen to plasmin will be declined. ACA activates coagulation because tissue factor synthesis by endothelial cells will increase and thus, enhance coagulation activation via extrinsic pathway.  

Viscosity of the fluid is defined as flowing intrinsic resistance. This resistance is induced by internal friction between molecules and particles in the fluid. Viscosity measurement is an in vitro method for evaluating overall intrinsic resistance of blood flowing in the big vessel. In some diseases, blood viscosity is increased and cause complications. Hyperviscosity may occur due to changes in blood component which influence the blood flow. Factors affecting blood viscosity are cellular and non cellular factors. Cellular factors include amount of erythrocytes, leucocytes and platelets. It is also influenced by erythrocyte rigidity and morphology. In addition, soluble substance such as plasma protein and lipid may also influence blood viscosity. Some reports have mentioned that blood viscosity increased in patients with thrombosis. High viscosity in patients with ischemic stroke will increase shear stress so that vessel wall of the arteries become more permeable to protein to lipid and protein. Hyperviscosity influences thrombus formation on the atheromatous surface or in the area where there is turbulence flow through platelet activation and coagulation factor. Thus, the cerebral blood flow will be hampered and ischemic stroke occurs. 

Diagnosis of ischemic stroke in this study was made based on clinical symptoms and brain CT scan. Time of blood sample taken was 1 week after acute phase of stroke. Acute phase of stroke is a period between onset of stroke attack and 2 weeks after. The reason to take blood samples in the 3rd week post stroke was to avoid influence of acute phase because lipid profile would decrease while blood glucose and fibrinogen would increase. 

In this study we found that high viscosity in the case group was 15.79% (6/38). Table 3 showed no significant difference between the case and control group. A study by Ernst revealed high blood and plasma viscosity in post ischemic stroke patients. In this study we found high median value of viscosity in the case group compared to control but there was no significant difference. This might happen because of time difference in taking the blood samples. Ernest did not explain when he took the blood samples of post ischemic stroke or whether there was influence of acute phase of stroke. In the acute phase of stroke we usually find increased concentration of fibrinogen. Study by Haliman showed increased hematocrit and fibrinogen would increase blood viscosity.  

In this study, we called persistent ACA if there were increased concentration of ACA IgG > 20 GPL and IgM > 20 GPL in two different examinations within 6 weeks interval. Persistent ACA IgM found in case group was 2.78% (2/38). Statistical analysis between case and control group found no significant difference with p = 1.0000. In this study we found higher proportion in control group compared to case group. This could happen because ACA IgM was only temporary and rarely caused complication. ACA IgM is usually found in infection, use of certain kind of drugs or even in healthy person. In this study, persistent ACA IgG in case group was 25 % (9/36), while in control group was 2.63% (1/38). Statistical analysis showed that persistent ACA IgG in case group was significant higher than control group with p = 0.010. According to Harris, increased ACA IgG was related to incidence of thrombosis and might cause...
ischemic stroke. Kushner found 29% positive ACA IgG in patients with ischemic stroke compared to 5% positive ACA IgG in patients with other neurologic disorders. A study by Roelftgen found that prevalence of positive ACA IgG in patients with stroke was 6%. In this study, we found similar result with those done by Kushner which was 25% (9/36). Multivariate analysis showed that OR of persistent ACA IgG was 14.11.

CONCLUSION

It was concluded from this study that persistent ACA IgG showed significant difference between group with ischemic stroke and control. While persistent ACA IgG with OR 14.11 (CI 95%: 1.64; 121.11) was the risk factor for ischemic stroke. Blood viscosity and ACA IgM were not risk factors for ischemic stroke.

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