INTRODUCTION

Stroke ranks third after ischemic heart disease and cancer as a cause of lost disability in high-income countries and as a cause of death worldwide [1]. The incidence of stroke varies among countries and increases exponentially with age. In Western societies, about 80% of strokes are caused by focal cerebral ischemia, and the remaining 20% are caused by hemorrhages [2].

Pharmacogenetic determinants of beta-fibrinogen gene polymorphism in response to aspirin in patients with severe ischemic stroke

Kiking Ritarwan¹, Darwin Amir¹, Rosita J. Sembiring³, Ahmad H. Sadewa⁴, Aznan Lelo⁵

ABSTRACT

Objective: Carrier of the A allele in the −455 locus of the beta-fibrinogen gene’s promoter region has previously been shown to be associated with elevated fibrinogen levels. The relationship between fibrinogen gene polymorphism and outcome in subjects receiving aspirin therapy is not clear; because it is biologically plausible that a pro-thrombotic polymorphism may exert a differential effect across different ages, this study was classified into young and old aged participants. Materials and Methods: A cohort study design is comprising 136 consecutive patients was done. All patients with acute ischemic stroke who were admitted to Adam Malik Hospital were divided into two groups as below and above the age of 55 years. Subjects received anti-platelet aspirin for 3 months. Genomic DNA was extracted from peripheral blood lymphocytes using standard protocol. Plasma fibrinogen level and modified Rankin Scale (mRS) were determined, and cerebral imaging was performed. Results: Genotype distributions among the total population (n = 136) were 66.2% for GG, 27.2% for GA and 6.6% for AA; at young age, these distributions were found 30.1%, 15.4% and 6.6%, respectively. After administration of aspirin, plasma fibrinogen levels were found to be 248.65 ± 100.71 and 235.75 ± 82.01 mg/dl, respectively for young and old age; the cut-off value of plasma fibrinogen concentration was 268.05 mg/dl. In a logistic regression model, the −455 G/A locus genotype showed a significant interaction between age and fibrinogen level in mRS of day 0. The identified relative risk (RR) on age and fibrinogen level was 0.67 (0.30-1.49) and 0.78 (0.37-1.63), respectively. Conclusion: Plasma fibrinogen levels decreased after administration of either aspirin or polymorphisms according to age. Identification of stroke risk factors based on the RR of plasma fibrinogen levels are at higher degree in younger age.

KEY WORDS: Aspirin, beta-fibrinogen gene, polymorphism, severity, stroke
neglect. Symptoms and signs are unilateral, and consciousness is generally normal or impaired only slightly, except in the case of some infarcts in the vertebral basilar circulation [4]. In two large randomized trials, the use of aspirin (160 or 300 mg/day), initiated within 48 h after the onset of stroke and continued for 2 weeks or until discharge, led to reduced rates of death or dependency at discharge or at 6 months, probably by means of reducing the risk of recurrent ischemic stroke [5,6].

Prospective studies with large samples have suggested that the plasma fibrinogen level is an independent risk factor for coronary heart disease or stroke. Plasma fibrinogen levels are affected by genetic factors particularly beta fibrinogen genes. Carrier of the A allele in the −455 locus of the beta-fibrinogen promoter region has previously been shown to be associated with elevated fibrinogen levels. These have been found to confer susceptibility to thromboembolic disease [7,8]. However, the relationship between fibrinogen gene polymorphism and outcome in subjects receiving aspirin therapy is not clear. This study evaluated the association of beta-fibrinogen gene −455 G/A promoter polymorphism on modified Rankin Scale (mRS) with elevated fibrinogen levels. These have been found to confer susceptibility to thromboembolic disease [7,8]. However, the relationship between fibrinogen gene polymorphism and outcome in subjects receiving aspirin therapy is not clear. This study evaluated the association of beta-fibrinogen gene −455 G/A promoter polymorphism on modified Rankin Scale (mRS) treated with aspirin. Because it is biologically plausible that a prothrombotic polymorphism may exert a differential effect across different ages, the study was classified in to young and old aged participants.

MATERIALS AND METHODS

According to the criteria established by the National Survey of Stroke, ischemic stroke was defined as a focal neurologic deficit of presumably vascular origin lasting upper than 24 h and excluding primary hemorrhage on initial cerebral imaging. All patients with acute ischemic stroke who were admitted to Adam Malik Hospital were divided into two groups: Below and above the age of 55 years. Subjects received anti-platelet aspirin for 3 months. Exclusion criteria were uncooperative patients with systemic infection. Informed consent was obtained from each subject. The study was approved by the Ethics Committee of Medical Faculty Sumatera Utara University.

Genomic DNA was extracted from peripheral blood lymphocyte using standard protocol. The polymerase chain reaction (PCR) primers for DNA fragments in the promoter region of the fibrinogen gene −455 G/A polymorphism were:

Forward primer: 5'-GAACATTTTCGTTATGTGAATTACG-3'
Reverse primer: 5'-GAAGCTCCAAGAAACCATGC-3'

PCR reaction was performed with HaeIII restriction enzyme (Promega; Madison, WI, USA) in 50 μl with 50 μg of genomic DNA, 200 ng of each appropriate primer and reverse primer, 200 μmol/l of each deoxynucleotide triphosphate, and 1 U of DyNAzyme II DNA polymerase in ×1 reaction buffer (Finzymes Oy; Vantaa, Finland). Samples were incubated for 5 min at 95°C, followed by 34 cycles of 1 min at 95°C, 1 min at 72°C. PCR products (20 μl) were digested with 10 U of the HaeIII restriction enzyme and resolved in 2% agarose gel for determination of −455 G/A genotype. The amplification conditions were as follows: An initial denaturing step at 95°C for 7 s, followed by 35 amplification cycles of denaturation at 52°C for 45 s, 30 s at 72°C, 7 min at 72°C and 7 min at 16°C [7]. Subsequent digestion with the restriction endonuclease HaeIII resulted in fragments of 181 and 488 base pairs for a more common genotype GG, 488 and 669 base pairs for genotype GA, and 669 base pairs for genotype AA.

Computerized tomography (CT) is widely used for early evaluation of acute strokes. Most importantly, CT excludes acute hemorrhage or other diseases mimicking ischemia. Therefore, CT is the main imaging examination in patients with brain ischemia and when antithrombotic agents are being considered. Each team of doctors was blind to all clinical information except symptom side and blind to follow-up imaging and outcome information.

Plasma fibrinogen levels were determined with Clauss method (Preci C2000-4 four channel coagulation analyzer; Beijing, PR China). Coefficient variation intra-assay and inter-assay are <4% and 3-6%, respectively.

The mRS is a global outcome rating scale ranging from 0 to 6: 0, no impairment; 1, no impairment but with symptoms; 2, mild disability; 3, moderate disability; 4, moderate to severe disability; 5, bedridden, incontinent, requiring constant nursing care and attention; and 6, fatal outcome. Scales 1-2 have a good outcome and scale 3-6 have a bad outcome [9].

Data analysis includes determining the differences in plasma fibrinogen levels according to genotype using paired t-test. To determine changes in outcomes and plasma fibrinogen levels before and after aspirin paired t-test and McNeMar’s test was used. Furthermore, we performed a forward stepwise analysis for variables of age, genotype and mRS scale on days 0 and 90. For each study, exact 95% confidence intervals were calculated for the respective outcome. Pooled estimates for the event rates and relative risks (RRs) were calculated. SPSS software (SPSS Inc, Chicago, IL, USA) was used to carry out statistical analysis P < 0.05 were considered as significant.

RESULTS

We included 136 patients with median age of 70 years for old age and 48 years for young age. A total of 136 scans showed infarct in the right (47.8%) and left hemisphere (52.2%). The fibrinogen level was impaired from the 1st day of ischemic stroke patients treated with aspirin and 90 days after treatment. The baseline characteristic of fibrinogen levels was shown in Figure 1.

Main characteristics of beta-fibrinogen −455 G/A genotype were shown in Table 1. Of 136 samples’ genotype distribution the −455 G/A locus were 66.2% for GG, 27.2% for GA and 6.6% for AA.

Plasma fibrinogen levels before administration of aspirin were 301.44 ± 6.34 mg/dl and after administration of aspirin were 240.08 ± 90.76 mg/dl for allele G. On the other hand, plasma fibrinogen levels for allele A were 362.70 ± 110.95 mg/dl before
and 272.04 ± 105.69 mg/dl after administration of aspirin. The comparisons of characteristics between before and after aspirin treatment were shown in Table 2.

The −455 G/A locus genotype showed a significant interaction between age and plasma fibrinogen level. The identified RR on age and fibrinogen was 0.67 (0.30-1.49) and 0.78 (0.37-1.63), respectively [Table 3].

**DISCUSSION**

In previous studies, fibrinogen has emerged as a risk factor for stroke [10]. Plasma fibrinogen level is an independent factor for stroke [11,12], especially in non-lacunar stroke [13]. The fibrinogen levels above average (>3 g/l) raises the risk of ischemic stroke and these especially common in young and middle-age [14]. In this study, impaired fibrinogen levels were observed in the 1st days of ischemic stroke patients treated with aspirin, and 90 days after treatment. This reflects the tendency of atherosclerosis especially in young and middle-age subjects.

The role of genotype in fibrinogen level adjustment is still controversial. Lately, many studies mainly focused on the increased levels of beta-fibrinogen [15,16]. Mutation G to A at −455 promoters will increase the levels of blood fibrinogen. Rising levels of fibrinogen will increase blood viscosity and the formation of fibrin. Hence, these cause platelet aggregation and pull into the vessel wall or sub-endothelial collagen [7,17,18].

The use of anti-platelet agents for prevention of atherothrombotic events is now well established. In this study, young age subjects had significant lower fibrinogen levels than old age subjects. Our findings showed that the aspirin has a significant effect on the fibrinogen level, especially in young age that is more predisposed to atherothrombotic events. Aspirin alters the phenotype of fibrin clot leading to the formation of fibrin characterized by increased fiber thickness and enhanced lysis structure; hence, this structure is associated with lower risk of stroke [19].

In this study, the −455 G/A locus genotype showed a significant interaction of age with fibrinogen level at mRS day 0. In the literature, some results were consistent with this study, but others were different [20-22]. These may be explained by the fact that cerebral infarction is a polygenic disease, and many candidate genes are involved in the pathogenesis process. Another explanation is a possible interaction between environmental and genetic factors, which contributes to the phenotypic heterogeneity of cerebral infarction.

In conclusion plasma fibrinogen levels decreased after administration of either aspirin or polymorphisms according to age. Identification of stroke risk factors based on the RR of plasma fibrinogen levels are at higher degree in younger age.

**ACKNOWLEDGMENTS**

We thank our staff in Clinical Laboratories (Prodia, Biochemistry lab), Hibah Bersaing Fund (Ministry of Health and Education, Republic of Indonesia) and all persons helping our research.

**REFERENCES**


© AKAY; licensee AKAY. This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.

Source of Support: Nil, Conflict of Interest: None declared.