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POLYMORPHYSM BETA FIBRINOGEN GENE -455 G TO A IN ISCHEMIC STROKE : ASSOCIATED WITH BARTHEL INDEX

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Abstract

Objective. 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. The relationship between fibrinogen gene polymorphism and outcome subjects with aspirin therapy is not clear. Because it is biologically plausible that a prothrombotic polymorphism may exert a differential effect across different ages, we classified study as young and old ages.

Methods. A cohort study design comprises 136 consecutive patients was done. 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. Genomic DNA was extracted from peripheral blood lymphocyte using standard protocol. Level fibrinogen plasma, mRS scale and cerebral imaging were available.

Results. Genotype distributions were 66.2% for GG, 27.2% for GA and 6.6% for AA. At a young age, genotype distributions were 30.1% (GG), 15.4% (GA) and 6.6% (AA). After administration of aspirin, plasma fibrinogen levels was 248.65(100.71) mg/dl 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 Barthel Index (BI) day 0 ($p<0.25$).The identified relative risk on age was 0.67 (0.30-1.49) and fibrinogen level was 0.78 (0.37 – 1.63).

Conclusions. Plasma fibrinogen levels decreased after administration of either aspirin or polymorphisms according to age. Identification of stroke risk factors based on the relative risk of plasma fibrinogen levels are high degree in young age.

Introduction

Stroke ranks third after ischemic heart disease and cancer 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] Ischemic brain injury is thought to result from cascade of events from energy depletion to cell death. Intermediate factors include an excess of extracellular excitatory amino acids, free-radical formation, and inflammation.[3,4]

Acute stroke is typically characterized by sudden onset of a focal neurologic deficit. Common deficit include dysphasia, dysarthria, hemianopia, weakness, ataxia, sensory loss, and neglect. Symptom and signs are unilateral,

and consciousness is generally normal or impaired only slightly, except in the case of some infarcts in the vertebra basilar circulation.[4] In two large randomized trials, the use of aspirin (160 or 300 mg per day), initiated within 48 hours 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 has been found to confer susceptibility to thromboembolic disease.[7,8] However, the relationship between fibrinogen gene polymorphism and outcome subjects with aspirin therapy is not clear. This study evaluated the association of beta fibrinogen gene -455 G/A promoter polymorphism on Barthel Index (BI) treated with aspirin. Because it is biologically plausible that a prothrombotic polymorphism may exert a differential effect across different ages, we classified study as young and old ages.

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 hours 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 5'-GAACATTTTACCTTATGTGAATTAAGG-3' (forward primer) and 5'-GAAGCTCCAAGAAACCATCC-3' (reverse primer). PCR reaction were performed with Hae III Thermo in 50 micro liter reaction with 50 microgram of genomic DNA, 200 ng of each appropriate primer and reverse primer, 200 micromol/L of each deoxynucleotide triphosphate, and 1U of Dynazyme II DNA Polymerase in 1 x reaction buffer (Finzymes OY). Samples were incubated for 5 minutes at 95° C, followed by 34 cycles of 1 minute at 95°C, 1 minute at 72°C. PCR Products (20 micro liters) were digested with 10 U of the HaeIII restriction enzyme (Promega Corp) 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 second, followed by 35 amplification cycles of denaturation at 52°C for 45 second, 30 second at 72°C, 7 minute at 72° C and 7 minute at 16°C.[7] Subsequent digestion with the restriction endonuclease HAEIII resulted in fragments of 181 base pair and 488 base pair for the more common genotype GG, 488 base pair and 669 base pair for genotype GA and 669 base pair for genotype AA.

Computed 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 (Precil C2000-4). Coefficient variation intra-assay and inter-assay are less than 4% and 3-6% (respectively).

Data analysis includes determining the differences in plasma fibrinogen levels according to genotype used paired T test. To determine changes in outcomes and plasma fibrinogen levels before and after aspirin use paired T test and Mc Nemar test. Also 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 RRs were calculated using SPSS software to carry out statistical analysis. P value less than 0.05 was significant.

Results

We included 136 patients with median age 70 years old (for old age) and 48 years old (for young age). A total of 136 scans showed infarct in right side (47.8%) and left side (52.2%). Main characteristics of genotype -455 G/A beta fibrinogen were shown in Table 1.Of 136 sample genotype distribution of the -455 G/A locus were 66.2% for GG, 27.2% for GA and 6.6% for AA.

Table 1. Main characteristics of Patients in Genotype Data

Genotype-455 G/A	Young age N (%)	Old age N (%)	Total N(%)
GG	41 (30.1)	49 (36)	90 (66.2)
GA	21 (15.4)	16 (11.8)	37 (27.2)
AA	6 (4.4)	3 (2.2)	9 (6.6)

Plasma fibrinogen levels before administration of aspirin were 301.44 ± 96.34 mg / dl and after administration of aspirin were 240.08 ± 90.76 mg/dl for allele G. In the other hand, plasma fibrinogen levels before administration of aspirin were 362.70 ± 110.95 mg / dl and after the administration of aspirin were 272.04 ± 105.69 mg / dl for allele A. The comparisons of characteristics between before and after treatment aspirin was shown in Table 2.

Table 2. Scoring BI according polymorphism

Genotip	BI 0		BI14		BI 90	
	Better n(%)	Worse n(%)	Better n(%)	Worse n(%)	Better n(%)	Worse n(%)
GG	39(26.7)	51(37.5)	46(33.8)	44(32.4)	45(33.1)	45(33.1)
GA	18(13.2)	19(14)	15(11)	22(16,2)	24(17.5)	37(27.2)
AA	5(3.7)	4(2.9)	4(2.9)	5(3.7)	5(3.7)	9(6.6)

Discussion

Role of genotype in the 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 of platelet aggregation and pulled into the vessel wall or sub endothelial collagen.[7,17,18] Beta fibrinogen genes as predisposing to further atherothrombotic cerebral vascular circulation

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 observed in the first 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 use of anti platelet agents for prevention of atherothrombotic events is now well established. In this study, young age subjects had lower fibrinogen levels more significant than old age subjects. Our findings showed that aspirin has a significant effect on the fibrinogen, especially in young age which 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 lyses structure. Hence, this structure associated with lower risk of stroke.[19]

In this study, the -455 G/A locus genotype showed a significant interaction age with fibrinogen level at BI day 0. 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 being involved in the pathogenesis process.

Another explanation is there being an interaction between environmental and genetic factors, which contributes to the phenotypic heterogeneity of cerebral infarction.

Conclusion

Plasma fibrinogen levels decreased after administration of either aspirin or polymorphisms according to age. Identification of stroke risk factors based on the relative risk of plasma fibrinogen levels are high degree in young age.

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Conflicts of interest. None

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