Polymorphism in beta fibrinogen -455 g/a gene was associated with diabetic in severe ischemic stroke patients

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Abstract. There is an association of polymorphism in the promoter region of the beta fibrinogen gene -455 G/A with enhancement plasma fibrinogen level. Diabetes mellitus is a risk factor for early neurologic deterioration in acute ischemic stroke. The prothrombotic fibrinogen protein is frequently elevated in patients with diabetes and may be association with poorer prognosis. This study evaluated the association of beta fibrinogen gene -455 G/A promoter polymorphism on modified Ranking Scale of Ischemic Stroke patients treated with diabetic and nondiabetic group. In a Cohort study design comprises 200 consecutive patients diabetic and a nondiabetic who, three months using completed a detailed outcome stroke. Of 200 samples genotype distribution were 27.1% for GG+GA and 0% for AA with diabetic and than 4.4% for GG+GA and 0.05% diabetic patients. Fibrinogen levels were higher in diabetic than nondiabetic group patients (307.7 + 106.3 vs 278 + 84 gr/dl, p=0.002). Fibrinogen level was found to be an independent predictor for diabetic patients. On Genotype GG+GA were associated with diabetic and nondiabetic group patients. Modified Rankin Scale on day 90 were found associated with diabetic and nondiabetic patients.

Conclusion: Elevated fibrinogen level is dose-dependently associated with 90 days outcome severity stroke with diabetic following ischemic stroke

Keywords: beta fibrinogen promoter gene, diabetic-fibrinogen level, disease progression

1. Introduction
Diabetes Mellitus (DM) and hyperglycemia negatively affect the outcome and pathophysiology of acute ischemic stroke [1]. Stroke ranks third after ischemic heart disease and cancer as a cause of lost disability-adjusted life-years 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 due to arterial occlusion, and the remaining 20% are caused by hemorrhages [2,3].

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, though some patients have a stepwise or gradual progression of symptomatic. Common deficit includes 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 posterior circulation [4].

Prospective studies with large samples have suggested that the plasma fibrinogen level is an independent risk factor for coronary heart disease, diabetes mellitus or stroke [5]. Fibrinogen is a glycoprotein and associated with increased risk of arteriosclerosis disease in humans. G/A variability in the -455 locus of the beta fibrinogen promoter region, especially the carrier status of the A allele has previously been shown to be associated with elevated fibrinogen levels and to increase the risk of cardiovascular disease and ischemic stroke [3,6,7,8].

This study evaluated the association of beta fibrinogen gene -455 G/A promoter polymorphism on modified Rankin Scale of Ischemic Stroke patients treated with diabetic and nondiabetic group.

2. Materials and Methods
2.1 Subjects
All patients with acute ischemic stroke who were admitted to Adam Malik Hospital after Head CT scans were divided into two groups: diabetic and non diabetic patients for three months outcome. The study was approved by the ethics committee of Medical Faculty of Universitas Sumatera Utara. The study design was fully explained, and written information was offered to the patients; if they agreed to participate, the signed a written consent form.

2.2 Genetic Analysis
Genomic DNA was extracted from peripheral blood lymphocyte using a 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 was performed with Hae III Thermo in 50 micro liter reaction with 50 micrograms 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 minutes 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 [3,5,6]

2.3 Outcome Stroke
Prior knowledge of the expected outcome after stroke and its predictor is important for selection of appropriate instruments and analysis of clinical trials in the stroke field. In the clinical trials, the most widely used are modified Rankin Scale (MRS). The MRS is a global outcome rating scale ranging from 0 (no impairment) to 5 (bedridden, incontinent, requiring constant nursing care and attention) and 6 (fatal outcome). This study was divided scale one until two from MRS has good outcome and than scale three until 6 has bad outcome [3,9,10,11,13,15]
2.4 Data analysis
Data analysis include to determine the differences in plasma fibrinogen levels according to diabetic and non diabetic group used Paired T test and to determine changes in outcomes and plasma fibrinogen levels Chi-Square Test, Student T test and Regression analysis test. \( P < 0.05 \) was considered statistically significantly.

3. Results
Of 200 sample genotype distribution of the -455 G/A locus was 71% for GG+ AA, 29% for G/A. At a young age (< 55 y.o) was 50.5%, male was 45.5% and Hyper fibrinogen 0 day was 55% and than hyper fibrinogen 90 days was 38%. Baseline characteristics of Patients gene -455 G/A beta fibrinogen are shown in table 1.

| Table 1. Baseline Characteristic of the subjects (n=200) |
|--------------------------------------------------------|
| Variable | n (%) |
| Age : < 55 years old | 101 (50.5%) |
| >55 years old | 99 (49.5%) |
| Sex (Male) | 91 (45.5%) |
| Genotype GG + AA | 142 (71%) |
| GA | 58 (29%) |
| Fibrinogen 0 day | |
| Hipo fibrinogen ( < 268.05) | 90 (45%) |
| Hiper fibrinogen (> 268.05) | 110 (55%) |
| Fibrinogen 90 day | |
| Hipo fibrinogen (< 272.7) | 60 (30%) |
| Hiper fibrinogen (> 272.7) | 76 (38%) |

| Table 2. Main characteristics of Patients in Diabetic and Nondiabetic Patients data According to gene -455 G/A beta fibrinogen |
|---------------------------------------------------------|
| DM (n=166) | Non DM (n=34) | \( P \) |
| Age | 54.3 ± 12.6 | 63.5 ± 12.1 | 0.001 |
| Sex (Male) | 78 (85.7%) | 13 (6.5%) | 0.796 |
| Fibrinogen 0 | 307.7 ± 106.3 | 278.5 ± 84 | 0.092 |
| Fibrinogen 90 | 235.1 ± 94.8 | 214 ± 65.5 | 0.002 |
| Genotype | |
| GG+GA | 45 (27.1%) | 155 (4.5%) | 0.005 |
| Genotype AA | 0 (0) | 2 (0.05) | 0.367 |
| BMI | 34.2 ± 36 | 22.2 ± 2.1 | 0.001 |
| MRS 0 | 64 (38.5%) | 10 (29.4%) | 0.994 |
| MRS 90 | 32 (19.2%) | 6 (17.6%) | 0.003 |

There were 166 diabetic and 34 non diabetic patients. In DM Population, age (54.3 ± 12.6 yo vs 63.5 ± 12.1, \( P = 0.001 \)), fibrinogen 90 (235.1 + 94.8 vs 214 + 65.5%, \( p = 0.002 \)), Genotype GG + GA (27.1% vs 4.5%, \( p = 0.005 \)), BMI (34.2 ± 36 vs 22.2 ±2.1, \( p = 0.001 \)), and outcome Modified Rankin Scale 90 days (19.2 % vs 17.6%, \( p = 0.003 \)). There were significant differences in the genotype GG + GA but not significantly for allele A.
Table 3 shows a further regression analysis in the matched population including: age, sex (male), Diabetes Mellitus, Fibrinogen and MRS for 0 days and 90 days and fibrinogen did not prove to be associated with Outcome MRS 0 days (p=0.355). Only fibrinogen 90 days proved to be associated with outcome MRS 90 days (p<0.05). Body Mass Index proved to be associated with early neurologic patients with outcome ischemic stroke 90 days (p<0.005). On Regression analysis, DM (OR.1.10, 95% CI 0.91 – 1.25, p =0.210) and Body Mass Index (OR.1.07, 95% CI 1.05 – 1.08, p =0.001).

Table 3. A Logistic Regression model including potential factors associated with early neurologic patients with Outcome ischemic stroke 90 days.

|          | Odd’s Ratio (95% Confidential Interval) | P    |
|----------|----------------------------------------|------|
| Age      | 0.726 (0.393 – 1.340)                  | 0.304|
| Sex (Male) | 1.170 (0.634 – 2.159)                  | 0.878|
| DM       | 1.10 (0.91 – 1.25)                     | 0.210|
| Fib0 MRS 0 | 1.010 (0.546 – 1.866)                  | 0.355|
| Fib90 MRS 90 | 1.417 (0.752 – 2.670)                 | 0.042|
| BMI      | 1.07 (1.05 – 1.08)                     | 0.001|

4. Discussion

In previous studies, fibrinogen has emerged as a risk factor for stroke, ischemic heart disease, Myocardial Infarction, venous thrombosis, and PAD [3,5,9,10,11]. In this study, impaired fibrinogen levels from the first day of ischemic stroke patients treated with aspirin and 90 days after treatment.

At a young age (< 55 yo) was 50.5%, male was 45.5%. According Ritarwan et al, in the 136 sample taken, the genotype distribution of the -455 G/A locus was young age (< 55 years old) and significantly young and old age (p = 0.001) [3]. Imran found age for ischemic stroke 42.2 ± 6.53 yo and p =0.076. This study found that the level of fibrinogen was significantly higher in ischemic stroke group than control (419.2 mg/dl vs 351.1 mg/dl, p ≤ 0.000) [5]. In this study, fibrinogen 90 days were significantly from diabetic patients (p = 0.002).

In this cohort study, genotype distributions was GG + AA were 71 % and 29 % for GA. The -455 G/A fibrinogen polymorphism may aid in the development and progression of cerebral arteriosclerosis [10,14]. Nishiuma et al. [16] showed significant association (OR. 2.05; p = 0.05) between fibrinogen genotype and ischemic stroke. Martikainen et al. [10] showed The SAM Cohort, genotype distributions were 64.9% (GG), 31.8% (GA) and 3.35 (AA). Researchers from Korea, Liu et al. [17] found the average age based on beta-fibrinogen -455 G / A genotype GG was at 11.6 years, and GA genotype was 64.39 years and AA genotype was 65.09 ± 10.6 years. And found no significant differences between genotypes GG and GA and AA genotype (p> 0.05).

In this a study, based on forwarding stepwise a logistic regression model, the -455 G/A locus genotype showed a significant interaction fibrinogen level at modified Rankin Scale (MRS) day 90, where p=0.042. Identification of stroke risk factors based on the relative risk of age found was 1.417 times according to DM and plasma fibrinogen levels according to the MRS scale on day 90 was 1.10 times. Blake et al. [18] report prospective study, the distribution of the C148T beta fibrinogen polymorphism was similar among those subsequently developed cardiovascular events compared with those who did not. Specifically, the adjusted relative risk associated with the presence of T allele were 0.95 (95% CI 0.75-1.19) for all cases, 1.10 (0.83-1.45) for myocardial infarction, 0.93 (0.65-1.33) for stroke, and 0.71 (0.47-1.1) for venous thrombo-embolism.
This is a study to link diabetic hyperfibrinogenemia to a higher frequency of early neurologic deterioration after ischemic stroke. Epidemiological study has established that elevated fibrinogen levels are strongly and independently correlated with the risk of coronary disease (CAD) and peripheral arterial disease. Of note, elevated fibrinogen levels in our group patients with diabetic may be linked to antiplatelet resistance [3,10,12,17]

**Conclusion**
Elevated fibrinogen level is dose-dependently associated with 90 days outcome severity stroke with diabetic following ischemic stroke

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