Original article

The Correlation between Polymorphism of β Fibrinogen Gene -455 G/A and Serum Fibrinogen Level with The Severity of Coronary Artery Stenosis In Coronary Artery Disease Patient

Taufik Indrajaya[1], Yudhi Fadilah[2], Mediarty Yuwono[3], Ali Ghanie[4]

Abstract

Background: Now a days coronary artery disease (CAD) becomes major cause of death. One among 7 deaths in America caused by CAD. CAD is an atherosclerosis process, which progresively develops into plaque that will lead to stenosis of coronary artery lumen. Several studies found that high serum fibrinogen level is an independent and significant to the severity of artery coronary stenosis. Serum fibrinogen level determined by genetic factor. Polymorphism of fibrinogen gene β -455 G/A seem plays an important role in plasma fibrinogen level. Although some studies showed a significant correlation between polymorphism and cardiovascular diseases, but some other studies report inversely. **Aim.** To evaluate the correlation between the polymorphism of fibrinogen gene β -455 G/A and serum fibrinogen level with the severity of artery coronary stenosis. ***Method:*** This is an analytic correlative study with prospective approach without comparison. Coronary angiography was performed in catheterization labor in the department of internal medicine, while DNA analysis and PCR done in the department of microbiology in General Hospital dr Muhammad Husin Palembang- Indonesioa, since July 2015 until Agustus 2016. Samples are CAD patient who undergo for coronary angiography and fulfield the criterias. The severity of stenosis in coronary artery determined by Gensini score. This study included 31 patient. ***Results.*** Among 31 CAD patients, this study found severe stenosis of coronary artery in 17 patients (53,1%), moderate in 5 patients (15,6%) and mild in 10 patients (31,2%). Genetic analysis showed that serum fibrinogen level was controlled by polymorphism of fibrinogen gene β -455 G/A, concecutively by genotipe AA in 15 patients (48,4%), genotipe GA in 12 patient (38,7%) and by genotipe GG in 4 patients (12,9%). *Chi Square* test showed a significant correlation between polymorphism gene fibrinogen β -455 G/A and serum fibrinogen level (p=0,039). *Spearman's rho* test found no significant correlation between serum fibrinogen level and severity of coronary artery stenosis based on Gensini score (r=0,142; p=0,447). And also this study found no significant correlation between polymorphism gene fibrinogen β -455 G/A with the severity of stenosis in coronary artery (p=0,512). ***Conclusion.*** Although this study succeded to prove that serum fibrinogen level was determined by polymorphism fibrinogen gene β -455 G/A, but there are no significant correlations between polymorphism fibrinogen gene β -455 G/A and serum fibrinogen level with severity of coronary artery stenosis in CAD patients. This study suggest to study other candidate gene to look for other cardiac risk beside this fibrinogen.

Keywords: Atherosclerosis; CAD; Gensini score; Polymorphism Fibrinogen Gen β -455 G/A

Bangladesh Journal of Medical Science Vol. 18 No. 02 April'19. Page : 379-384
DOI: https://doi.org/10.3329/bjms.v18i2.40711

Introduction

Coronary artery disease (CAD) is still being serious health problem caused by high morbidity and mortality rate. In 2008 there were 17,3 million death caused by cardiovascular diseases. More than 3 million of them died before the age of 60, therefore it can be prevented. Prevalence of CAD in Indonesia at 2013 was 0,5% or estimated 883.447 patients.[1,2]

CAD is a heart disease caused by the narrowing of the coronary arteries due to the process of atherosclerosis or spasm or a combination of both. Atherosclerosis is a chronic inflammatory progression disease. These interactions include pathological processes such as endothelial dysfunction, monocyte recruitment, inflammation, smooth muscle proliferation, accumulation and lipid oxidation, necrosis,

1. Taufik Indrajaya
2. Yudhi Fadilah
3. Mediarty Yuwono
4. Ali Ghanie

Departement of Internal Medicine-Medical Faculty, University of Sriwijaya - Indonesia,

Correspondence to: Taufik Indrajaya, Departement of Internal Medicine-Medical Faculty, University of Sriwijaya - Indonesia, E-mail: tfk_indrajaya@yahoo.com

calcification and thrombosis.[3-6]

Fibrinogen is a glycoprotein synthesized in the liver, present in platelets alpha granules and soluble in plasma. In addition to being a very important component of the clotting process, fibrinogen is an acute phase of proteins in which levels will increase in response to infection, inflammation, stress, surgery, trauma, necrotic tissue, as a result of elevated levels of fibrinogen will cause plasma viscosity and intense platelet aggregation. and also erythrocyte aggregation.[7-9]

Studies by Gothenburg, NPHS, Framingham, PROCAM, GRIPS reported that fibrinogen is a risk factor for CAD occurrence and they proved that elevated fibrinogen levels are associated with CAD. The PROCAM (Prospective Cardiovascular Munster) studyshowed that with an increase in fibrinogen levels associated with LDL cholesterol, the risk of CAD became 6-fold.[10]*Ahmed* et al reported high fibrinogen levels as an independent and significant factor with the severity of coronary artery stenosis.[11]*De Luca* et al and *Zhang* et al report the same with *Ahmed* et al.[12,13]

Atherosclerosis in CAD is evaluated using an invasive examination known as coronary angiography or cardiac catheterization. Cardiac catheterization is a technique used as the best and most accurate technique to detect coronary blockage.[14] Coronary artery stenosis may occur in part or total of one or more coronary arteries and their branches. Stenosis is significant if the stenosis is more than 50% stenosis, and will decrease blood flow if stenosis is more than 70%. Cardiac catheterization (coronary angiography) can see and measure the degree of coronary artery stenosis visuallyhow much percent the dfecrease diameter compare to adjacent of normal segment. There are several methods might measure coronary artery stenosis, among others GRACE score and TIMI score, but in this study using Gensini score.[15] Gensini score measurement has many benefits because it is easy and practical in examining the degree of coronary artery by examining the degree of coronary artery stenosis and the location of the anatomy of the artery.

Synthesis *ß*chain is involved in the fibrinogen maturation process due to limitating of mature fibrinogen. Increased fibrinogen level will induce hypercoagulability that will promote the progression of atherosclerosis. The polymorphism of the ß fibrinogen gene contributes to fibrinogen serum level. Substitution of G by A at -455 gene position ß fibrinogen seems the most responsile to determine of fibrinogen serum level.[16]*Imran* et al. reported an significant association between the polymorphism of fibrinogengene with serum fibrinogen level. Serum fibrinogen levels are higher in patients with ischemic stroke.[17]Inversely,*Study of Myocardial Infarction Leiden*reported high fibrinogen is not a risk for myocardial infarction service.[18,19] Also, *Koch* et al reported on relationship halotype gene chains α, ß, and ɣ with myocardial infarction in 2 large control case studies.[20]

Untill now, scientific publications about the polymorphism of the ß-455 G/A fibrinogen gene and serum fibrinogen levels and their effects on coronary artery stenosis are not present in Palembang-Indonesia. Based on the above description, the researcher is interested to conduct a research between polymorphism of fibrinogen gene and fibrinogen level on coronary disease with severity of stenosis associated with gensini score.

Materials and Study Methods

This study used correlative analytic design with prospective approach without comparing group, was held in cardiac catheterization laboratory of internal medicine department and in microbiology department of General Hospital Muhammad Husin (GHMH) Palembang, starting July 2015-August 2016. The population of this study are all CAD patients undergoing cardiac catheterization and age > 30 years. This study excluded people with chronic kidney disease, malignancy, infection, pregnancy and lactation, and who are taking anticoagulant drugs. Based on the formula this study needs minimum sample is 31orang. The selection of samples was done by concecutive samplingtechnique.

Gensini score is one of the scoring methods to examine the degree of coronary artery stenosis based on the results of coronary angiography. The calculation of these scores is based on the location of the anatomy of the arterial segment undergoing stenosis and the degree of narrowing of the affected lumen of the coronary artery. The degree of stenosis is divided into mild stenosis (0-15), moderate stenosis (16-30), and severe stenosis (31-72).

A 7 ml of blood sample was taken through radial or femoral artery shortly before the initiation of coronary angiography. Two cc of them was put into

tube containing ethylene diamine tetra acetic acid (EDTA) for DNA extraction and for PCR, then DNA stored at -20 °C before the DNA isolation procedure is performed. The rest was not given anticoagulant for blood chemistry including fibrinogen. Serum fibrinogen levels were examined by ELISA (normal value: 150-450 mg / dl).

DNA isolation was performed by using Chelex-100 DNA extraction method using Phosphate Buffer Saline (PBS) pH 7.4; 0.5% Safonin in PBS; and chelex 20% in *dd* H2O pH 10.5. Detection of gene polymorphism by cutting the primmer PCR product by using the restrictive enzyme *Hae*III (MBI, Glen Burnie, MD, USA), where the 10 μl β-fibrinogen gene (amplicon) product is inserted into a digestive tube containing 0.2 μl enzyme *Hae*III in 1,2μl 1/10 buffer 1 buffer buffer solution, then pure water (*dd*H2O) until the mixed volume in the tube reaches 12 μl, and then incubated at 37 °C in water bath for 4 hours. To determine whether the restriction process is working properly or not, it is also used in the internal control of PCR products from individual samples known to have polymorphism. After incubation the restriction was separated electrophoresis in 2% agarose gel. Primmer for PCR is intended to identify the following fragments: Genes β-fibrinogen -455G/A

*Forward*GAA CAT TTT ACC TTA TGT GAA TTA AGG and

*Reverse*GAA GCT CCA AGA AAC CAT CC.

The amplicon length for the β-fibrinogen -455G/A gene is 669 bp. The amplicon fragments was cut with the *Hae*III retrictive enzyme (New England Biolabs, Ipswich MA, USA).

Processing and data analysis using *SPSS for windows* program. Data are presented in tables and graphs. The data is tested whether the distribution is normal or not, if the normal distribution then used *Pearson correlation* test, if the data distribution is not normal *Spearman correlation* test used.

Ethical Clearance: This study was approved by ethics Committee of University of Sriwijaya – Indonesia.

Results

A correlative analytic study with a non-comparison prospective approach to see the correlation between the polymorphism of the fibrinogen β chain gene with serum fibrinogen level with the severity of coronary heart disease stenosis on 31 patients was obtained as follows.

Table. 1. Baseline Characteristics

Characteristic	n = 31
Sex, n (%)	
• Male	20 (64,5%)
• Female	11 (35,5%)
Age, year,mean ± SD	56,52 ± 8,76
Age groups, n (%)	
• 30-39 yo	2 (6,5%)
• 40-49 yo	2 (6,5%)
• 50-59 yo	14 (45,2%)
• ≥ 60 yo	13 (41,8%)
Smoking habits, n (%)	
• Smoking	13 (41,9%)
• Not smoking	18 (58,1%)
Body Mass Index, n (%)	
• Underweight	1 (3,2%)
• Normoweight	11 (35,4%)
• Overweight	19 (61,4%)
Diabetes Melitus	
• Yes	4 (12,9%)
• No	27(87,1%)
Hypertension	
• Yes	11 (35,5%)
• No	20 (64,5%)

The CAD subjects in this study were more male (64.5%) with age above 50 years (87%). Nearly half of them were smokers (41.9%) and about 61.3% were overweight. By using *Shapiro-Wilk* normality test, the probability value of serum fibrinogen level and gensini score was<0,05 which means the data was not normarlly distributed, and so then calculation countinued to see the Mean value of both variable.

Table 2. Distribution of serum fibrinogen level and Gensini score

Correlation	N	Median (min- max)	Mean ± SD
Serum fibrinogenlevel	31	288-711	420,16 ± 100,65
Gensini score	31	2.5-90	32,03 ± 26,72

Electroporesis result of Gen β Fibrinogen promoter - 455 G / A

Fragments generated from electrophoresis are genotype -455 G/G homozygote resulting in fragments 488 and 181 bp, heterozygote -455G/A yields fragments 669, 488, 181 bp. Homozygote -455 A/A produces fragments 669 bp (uncut) (figure.1 below)

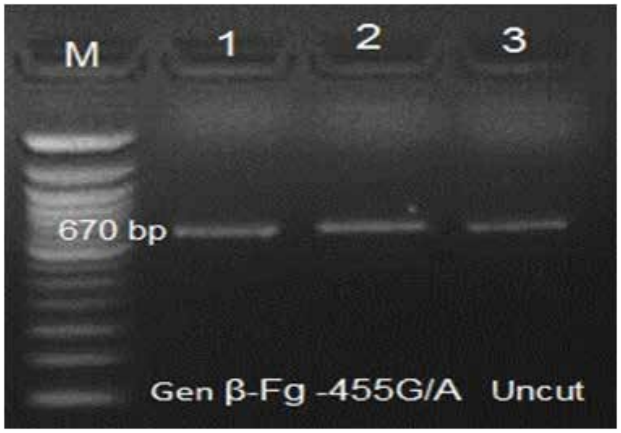

Figure. 1. Ampliconof Gen β-Fg-455G/A with size about 670 bp Homozygote
wildtype G/G. M is a Marker. No 1, 2 and 3 are sample numbers.

In Figure 2 genotypes βFg-455G/A genotypes were cut with restrictive enzyme HaeIII resulted in A/A mutant Homozygote fragments of 490 bp and 180 bp (sample no 1). Fragments Heterozygote G/A 670 bp, 490 bp and 180 bp (samples no 2 and 3).

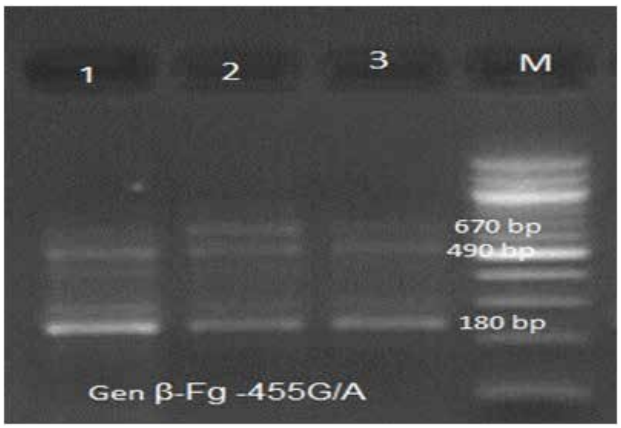

Figure.2. Amplicon Gen βFg-455G/A is cut with the restriction enzyme HaeIII, M is the Marker.

Serum fibrinogen levels in this population were determined by genotype AA, GA and GG, respectively. CAD patients with elevated fibrinogen levels are most commonly found in the AA genotype (48.4%), see table below.

Table 3. Distribution of Fibrinogen B Polymorphism Promoter -455G/A

Characteristic	Serum fibrinogen level		Total
	Increase	Normal	
Genotype			
• AA	12	3	15
• GA	4	8	12
• GG	3	1	4
Total	19	12	31

Study Result Analysis

Analysis of the results of the study begins by looking for correlations between the polymorphism of the fibrinogen β chain gene with serum fibrinogen levels. Using *Chi-Square* test, there was a significant correlation between the two variables p = 0.039. (see table 4.4).

Table 4. The correlation between the polymorphism of the fibrinogen β chain gene with serum fibrinogen levels

Characteristic	Serum fibrinogen level		Total	*p value*
	Increase	Normal		
Genotype				
• AA	12	3	15	**0,039**
• GA	4	8	12	
• GG	3	1	4	
Total	19	12	31	

The analysis then continued to look for correlation of serum fibrinogen level with degree of coronary artery stenosis based on gensini score of CAD patients, the results can be seen in table.4.5 below.It is already known that serum fibrinogen levels and Gensini score in this study were not normally distributed, so then the analysis is continued using Spearman / s rho test. The results of the analysis were found no significant correlation between serum fibrinogen level and coronary artery stenosis degree based on gensini score (p = 0,447), see table below.

Table 5. Correlation of serum fibrinogen levels with Gensini score

Correlation	N	Median (Min-Max)	Mean ± SD	*p*
Serum fibrinogen	31	288-711	420,16 ± 100,65	0,447*
Gensini score	31	2.5-90	32,03 ± 26,72	

**Uji Spearman's rho*

The effect of serum fibrinogen level on gensini score was then analyzed by linear regression test to see any correlation between the two variables, found that the effect of serum fibrinogen level on gens score was only 4%.

In Chi Square test there was no significant correlation between polymorphism of fibrinogen β chain gene with degree of coronary artery stenosis (p = 0,512) (see table 4.6).

Table 6. Correlation between β Fibrinogen Chain Gene polymorphism with Gensini score

Characteristic	Degree of Stenosis			Total	*p value*
	Severe	Moderate	Mild		
Genotype					
• AA	6	3	6	15	
• GA	7	1	4	12	0,512
• GG	3	1	0	4	
Total	16	5	10	31	

Discussion

This study was aimed to evaluate the correlation between polymorphism of β fibrinogen gene and serum fibrinogen level with severity of coronary artery stenosis in CAD patients who undergone coronary angiography at GHMH Palembang Indonesia.

Neither the Gensini score nor the serum fibrinogen levels were found to be abnormally distributed. In this study the average score of Gensini is 32.03 ± 26.72 in a range of 2.5 to 90. A total of 17 people (53.1%) obtained with severe stenosis degree, this number is more than those with mild degrees of stenosis and mild *ie* each of 5 people (15,6%) and 10 people (31,2%). In this study average serum fibrinogen level of CAD patients was 420.16 ± 100.65 mg/dl. Results of statistical analysis showed no significant correlation between serum fibrinogen level and coronary artery stenosis degree based on Gensini score (p = 0.45). Unlike the study results of *Ahmedet al* (2014) that reported a significant independent association between serum fibrinogen levels and the degree of coronary artery stenosis, and that the higher serum fibrinogen levels, the more severe the coronary artery stenosis. This study included only 31 people, unlike *Ahmed et al* who recruited 210 samples and divided them into 3 groups: fibrinogen levels <400 mg/dl, 400-600 mg/l and>600 mg/dl. In this study there was only 1 person with levels of 711 mg/dl (>600 mg/dl) and indeed with severe stenosis of the coronary arteries.[11]

Fibrinogen is a plasma soluble glycoprotein composed of 1.482 amino acids with molecule weight (MW) of 340 kDa, 2 carbohydrate clusters with BM 10 kDa, and several Ca+ bonds with high affinity. Fibrinogen plays a role in physiopathological processes in the body, including inflammation, atherogenesis and thrombogenesis. Low fibrinogen levels can cause hemostasis disorders such as bleeding, while high levels affect the function of hemereologi. In atherotrombogenesis, fibrinogen infiltration into the walls of blood vessels, increases blood viscosity, increases tombocyte aggregation and forms thrombus. Fibrinogen also acts as an acute phase protein that promotes degranulation of platelet.[21] Its levels will increase two to tenfold in response to stimuli.

Chi Square test result obtained significant correlation between polymorphism of fibrinogen β chain gene with serum fibrinogen level (p = 0,039). The results of this study are in accordance with the research of *Liu* in China which proves the existence of polymorphism of beta fibrinogen -455 G/A gene with increased levels of fibrinogen. *Dogen's* research found the same result in which there is a fibrinogen content relationship with polymorphism of fibrinogen genes. Studies by *de Maat* and *Behaque*found that the polymorphism of the -455G/A fibrinogen gene led to an increase in fibrinogen levels on which coronary atherosclerotic progression was based.

Polymorphism is a gene mutation that does not cause changes in protein structure but results in variation in the function of the protein. Polymorphism can result from a change of base on a promoter, an exon or an intron. Polymorphism does not manifest clinically but can determine susceptibility to disease. Polymorphisms are found in populations with frequencies greater than 1%. Several studies have revealed the influence of genetic factors on plasma fibrinogen levels, genetic influences varying from 20% to 25% on plasma fibrinogen levels. The influence of genetic control supports the view that high plasma fibrinogen levels are a primary risk factor for atherothrombotic disorders. This study proved the existence of polymorphisms of Fibrinogen gene -455 G/A, A/A and G/G.

This study concluded there was no significant relationship between polymorphism of fibrinogen β chain gene with degree of coronary artery stenosis which obtained p = 0,512 (p> 0,05). *Dogen's* research found the same thing where there is no influence between polymorphism of fibrinogen genes and the risk of myocardial infarction. In contrast to the *Tosetto* study showing that the polymorphesis of the -455 G/A gene represents an initial risk of atherosclerosis seen in carotid artery plaque.*Bozdemir* proved that the beta-fibrinogen gene polymorphism -455G/A plays a role in the occurrence of left atrial thrombus and spontaneous echo contras.Similar results with this study also found in the *Blake* study, mutations of the fibrinogen gene have no effect as risk factors for cardiovascular disease. *Koch's* study found no association of variations in fibrinogenic genes with myocardial infarction. Several other risk factors play a role as risk factors for coronary heart disease. This

is the case in *Hastuti* et al., And *Molagheghi's* study that risk factors such as age, sex, lipid profile, smokers and diabetes play a greater role than fibrinogen.

Conclusion

Although this study succeded to prove that serum fibrinogen level was determined by polymorphisme fibrinogen gene β -455 G/A, but there are no significant correlations between polymorphisme fibrinogen gene β -455 G/A and serum fibrinogen level with severity of coronary artery stenosis in CAD patients. This study suggest to study other candidate gene to look for other cardiac risk beside this fibrinogen.

Conflict of interest statement

We declare that we have no conflict of interest.

Authors' Contribution:

Data gathering and idea owner of this study: Taufik Indrajaya, Yudhi Fadilah

Study design: Taufik Indrajaya, Yudhi Fadilah, Mediarty, Yuwono, Ali Ghanie

Data gathering: Taufik Indrajaya, Yudhi Fadilah, Mediarty, Yuwono,

Writing and submitting manuscript: Taufik Indrajaya, Yuwono, Ali Ghanie

Editing and approval of final draft: Taufik Indrajaya, Yudhi Fadilah, Mediarty, Yuwono

Refferences:

1. Riset Kesehatan Dasar 2013. Badan LitBangKes RI, Profil Kesehatan Indonesia 2103. Kementerian kesehatan RI, 2014.
2. Heart Diseas and Stoke Statistic 2015 update. A report from American Heart Association. Circulation 2015, 131; e29-e322
3. Libby P. Inflammation in Atherosclerosis. Arterioscler Thromb VAsc Biol 2012 ; 32; 2045 - 205.
4. Tabas I, Cardena GG, Owen GK. Recent Insight into the elluler Biology of Atherosclerosis . J Cell Biol 2015 ; 209 (1) 13-22.
5. Papageorgiou N, Tousoulis D, Siasos G, Stefanadis C. Is fibrinogen a Marker of Inflamation in Coronary Artery Disease ?. Hellenic J Cardiol 2010; 51: 1-9.
6. Czaja M, Kabat M, Wasilewski J. Hemorheological Parameters and Fibrinogen in Atherosclerosis. Journal of cardiology and Therapy 2014 December 10, 1(9):206-211
7. Pulanic D, Rudan I. The Past Decade : Fibrinogen. Coll. Antropol 29. 2005; 1: 341-349.
8. Davalos D, Akassoglou K. Fibrinogen as a Key Regulator of Inflammation in Disease. Semin Immunopathol, January 2012; 34(1) 43-62.
9. Kilicarslan, Uysal, Roach. Acute Phase Reactants, . Acta Medica 2013; 2: 2-7
10. Marezca G, Di Blasio A, Marchioli R, Di Minno G. Measuring Plasma Fibrinogen to Predict Stroke and Myocardial Infarction. Arterioscler Thromb Vasc Biol. 1999: 1368-1377.
11. Ahmed M et all. High Fibrinogen Level is An Independent Predictor of Presence and Severity of Coronary Aretery Disease. Cardiovasc. Journal 2014; 7 (1):4-10.
12. De Luca G, Verdoia M et al. High Fibrinogen Level is an Independent Predictof of Presence and extent of Coronary Disease among Italian Population. Journal of Thrombosis and thrombolyisis Mei 2011, 31(4) ; 458-463.
13. Zhang Y, Gang Zhu C e all. Higher Fibrinogen Level is Independently Linked with the Presence of New Onset Coronary Atherosclerosis among Han Chinese Population. Plos one 2014, Nov 26; 9(11):1-14.
14. Anddrson RD, Pepine CJ. Coronary Angiography Is it Time to Reassess. Circulation 2013; 127; 1760-1762.
15. Barbosa CE et al. Accuracy of the GRACE and TIMI Score in Predicting the Angiographic Severity of Acute Coronary Syndrome. Arq Bras ardiol. Agt 2012; 99 (3): 818-824.
16. Ritarwan K, Amir D et al. Outcome of Ischemic Stroke and -455 G/A Beta Fibrinogen Gene Polymorphism,International Journal of Pharma Research and Health Science, 2014; 2(6) : 420-424.
17. Imran I, Lamsudin R et al. Association of ß-Fibrinogen Promotor Gene Polimorphism (-148C/T), Hyperfibrinogenemia and Ischemic Stroke in Young Adult Pasients. The Egyptian Journal of Medical Human Genetic. 2015; 16 : 11-17.
18. Iacoviello L, Vischetti M, Zito F, Donati MB. Genes Encoding Fibrinoen and Cardovascular Risk. Hypertension 2001; 38: 199-1203.
19. Doggen, Bertina, Cats M, Rosendaal. Fibrinogen Polymorphisms are not Associated with the Risk of Myocardial Infarction. British Journal of Haematology 2000; 110 : 935-938
20. Koch W, Hoppmann. Fibrinogen Genes and Myocardial Infarction: a Halotype Analysis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008; 28: 758-763.
21. Mackie IJ, Kitchen S, Machin SJ, Lowe GD. Guideline on Fibrinogen Assays. British Journal of Haematology 2003; 123 : 396-404