Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/7.1.20210111

Research Article
2021 | Volume 7 | issue 1 | Pages 32-37

ARTICLE INFO

Received
December 23, 2020
Revised
January 26, 2021
Accepted
March 4, 2021

***Corresponding Author**
Farkhanda Yasmin

E-mail
farkhandayasmin353@gmail.com

Keywords
Myocardial infarction
t-PA gene
I/D polymorphism
D/D polymorphism

How to Cite
Yasmin F, Rasheed M, Suleman S, Arif M, Farooq W, Hussain S, Khan J, Ali H. Association of Alu-repeat Polymorphism and Myocardial Infarction in Pakistani Population. Biomedical Letters 2021; 7(1):32-37.

Scan QR code to see this publication on your mobile device.

Open Access

Association of Alu-repeat Polymorphism and Myocardial Infarction in Pakistani Population

Farkhanda Yasmin[1,2*], Majeeda Rasheed[3], Saima Suleman[4], Maryam Arif[5], Waqas Farooq[6], Saghir Hussain[2], Jallat Khan[2], Habib Ali[3]

[1]National Institute for Biotechnology and Genetic Engineering, P.O. Box 577, Jhang Road, Faisalabad, Pakistan.
[2]Department of Biosciences Technology, Khwaja Fareed University of Science and Technology, Raheem Yar Khan, Pakistan.
[3]Department of life sciences, Khwaja Fareed University of Science and Technology, Raheem Yar Khan, Pakistan.
[4]Institute of Molecular Biology & Biotechnology, University of Lahore, Pakistan.
[5]Sheikh Zayed Hospital Lahore, Pakistan.
[6]Department of Biotechnology, Faisalabad Institute of Research Science and Technology, Faisalabad, Pakistan.

Abstract

Polymorphism of tissue plasminogen activator(t-PA), gene-induced myocardial infarction (MI) is not well-defined in patients suffering from high blood pressure. Plasminogen activator generates the active enzyme by limited proteolysis of zymogen plasminogen to plasmin. Plasmin then degrades the fibrin network of a clot to form soluble product in thrombi. This action of t-PA can be suppressed by plasminogen activator inhibitor type1(PAI-1). This study determined the potential insertion/deletion of polymorphism that may contribute to the development of MI in Pakistani population. The study analyzed blood samples originating from three hundred and fifty patients with MI, two hundred and fifty healthy individuals as controls, and hundred hypertensive study subjects. The genomic DNA was extracted from the blood of each individual, and a Polymerase Chain Reaction was carried out to study polymorphism of Tissue plasminogen Activator (t-PA) gene. The Chi-square method was used to reveal the demographic differences among the groups. Cholesterol's higher levels, triglyceride, LDL-cholesterol, and lower HDL-cholesterol levels had been investigated in cases/patients in contrast with controls. In some cases, the input allele frequency ("I") is higher with MI ($p = 0.0354$). Diabetes, high blood pressure, family history, and smoking had a strong association with MI ($p<0.01$). No significant association between myocardial infarction and Insertion/Deletion (I/D) and Deletion/Deletion (D/D) polymorphism of t-PA gene, significant association found between Insertion/Insertion(I/I) and MI, which supports the results of previous MI studies.

Introduction

Myocardial infarction (MI) is heart tissue death due to the formation of a blood clot in the coronary arteries [1]. It is the world's leading health complication, causing 8 million deaths a year [1]. Globally, 10% of coronary artery disease (CAD) patients are likely to develop MI [5]. Older age, diabetes mellitus, high blood pressure, hypercholesterolemia, smoking genetic factors and family lineage are significant determinants for MI patients are found to be linked with MI [3,4]. Regulated by clot formation and factors that cause lysis, the thrombosis-fibrinolysis system is directly linked to MI occurrence. Fibrinolytic gene polymorphisms and their contributions have been studied previously [6]. It has been shown in previous studies that genetic thrombosis-fibrinolytic polymorphisms may influence the etiology of the disease by disrupting the balance among these activities [7].

Endothelial cells generate the tissue plasminogen activator (t-PA), a serine proteinase. Transforming plasminogen into plasmin, it significantly participates in fibrinolysis by breaking down fibrin clot [8]. The t-PA is fitted with the PLAT (plasminogen tissue activator type) gene. Hyper-fibrinolysis is caused by the increased activity of the enzyme t-PA, which causes intemperate bleeding, and is responsible for thrombosis when it is less active. In this model, a polymorphism of the intron 8 Alu element is incorporated with various plasma levels of t-PA gene [12 - 14]. The t-PA-antigen shows the presence of confounders like high blood pressure, the resistance of insulin, heart failure, or ischemic heart disease, which may lead to endothelial dysfunction and vascular damage [9]. A polymorphism of tissue type plasminogen activator(T-PA) has been found, locus on chromosome 8 of this gene which consists of the presence or absence of a 311bp Alu sequence in intron 8 types [8, 10, 11]. The homozygous insertion of 8 Alu is known to cause a 100% increase in MI risk, while heterozygous I/D is linked to a 50% more risk of MI [10], indicating a link between polymorphism of Alu disease repeats and heart disease [5, 12]. With this background, the study was conducted to assess whether I/I or I/D polymorphism in the t-PA gene is associated with MI in Pakistan's population or not.

Materials and Methods

Sampling

The study included 350patientswithstandard cardiac catheterization for heart disease and myocardial infarction (MI), visiting the Punjab Institute for Nuclear Medicine (PINUM) hospital and control group consisting of 250 healthy and 100 hypertensive volunteers were included in this study. Diagnosis of ischemic heart disease and MI was performed by cardiologists based on patients' complaints and specific cardiac investigations (electrocardiographical abnormality, MI history), according to the WHO's standard procedure. Following official written approval, demographic and control subjects were recorded. Cardiovascular risk factors included a history of hypertension, smoking, hypercholesterolemia, and diabetes were recorded. The participant of this study had high blood pressure (systolic blood pressure above 140 mm Hg and diastolic blood pressure above90 mm Hg). The research committee of the participating hospital and NIBGE(National Institute of Biotechnology and Genetic Engineering) Faisalabad, Pakistan approved this study. Blood (5 ml) was drawn from each of the individuals using disposable syringes by venipuncture and was immediately transferred to tubes containing an anticoagulant (K-EDTA/heparin) and stored at 20 ° C for continued use.

Biochemical analysis

Lipid analysis was performed on patients and controls in no more than 24 hours from the collection. Enzymatic colorimetric methods estimated total plasma cholesterol (TC), triglyceride (Tg), and glucose levels. Similarly, enzymatic colorimetric assay estimated total cholesterol levels, HDL-plasma cholesterol, and triglycerides as per manufacturer's instruction.

Genetic analysis

A standard organic phenol-chloroform method was used to extract genomic DNA from peripheral blood [18]. PCR amplification of I / D polymorphism, indicating the presence or absence of Alu-repetitive in-intron 8 of t-PA, was performed using primers (5'CCGTAACAGGACAGCTCA3 '5' and ACCGTGGCTTCAGTCATGGA3') as described earlier (Falk et al., 1995; Van der Bom et al., 1997).

A gel of 1.8% was used to visualize the fragments. The reaction yielded specific products for the t-PA allele (656 bp (D), 967 bp (I), or both (656/967(I/D)). These results were further confirmed by using Restriction Fragment Length Polymorphism (RFLP).

Statistical analysis

With the help of direct gene counting method, frequencies of s and genotype were calculated. A student's t-test was used to compare lipid and plasma serum concentrations. A Chi-square test was applied to differentiate between patients and controls about the presence or absence of t-PA polymorphism.

Results

Demographic data

Patients, related to hypertension (n = 100), controls (n = 250) and MI (n = 350) were screened for polymorphism in gene encoding t-PA and genotyped by molecular assay. Samples were analyzed to include variables such as age, gender, smoking, high blood pressure, duration of the disease, treatment, and previous hospital admissions, cardiovascular complications, and exposure to risk factors (Table 1). No remarkable differences were observed between controls and patients regarding age and HDL (P = 0.8 and 0.004, respectively). History of smoking over the past five years (OR: 8.0; 95% Cl: 4.3 to 15, P = 0.01), high blood pressure (OR: 2.3; 95% Cl: 1.3-4.1, and diabetes (OR: 3.4; 95 %Cl; 1.6 to 7.3, P = 0.0014), familial history and gender (male) were dependent variables but age and hypercholesteremia weren't dependent variables (**Table 1**).

t-PA analysis

Genotypic analysis of t-PA disclosed that 380 MI patients (21%) were I/I homozygous, 75 (49%) were D/D homozygous, and 46 (30%) were I/D heterozygous. For controls, there were35 (22%) I/I homozygous,85(52%) D/D homozygous, and 42 (26%) were I/D heterozygous. Any significant dissimilarity wasn't observed in genotype division between both groups [P >0.05, X^2 = 0.680, **Table 2**). The frequency of I allele in MI cases was 54%, while in controls, it was 53%and no considerable differences were noticed between them (P >0.05, X^2 = 0.20, OR = 0.936 (Figure 2) (95% CI = 0.699-1.252). Also, the best and the most popular types of t-PA genotypes didn't show any significant association with MI (OR = 1.31, 95% CI = (0.783-2.193),P>0.05,Table 2).The diffusion of genotype data, as well as gender sequence figures, revealed no noticeable relationship between male and female MI patients having any type of allele or genotype (**Fig. 2**) (P>0.05, **Table 1**).

The distribution of specific symptoms of cases and controls of MI is elaborated in Table 1. Diabetes frequency, high blood pressure, and family history showed significant differences in the occurrence of myocardial infarction (p <0.01). Compared to controls, the cases had an increased extent of TC, TG, LDL-C, and decreased concentration of HDL-C (Table 1). In the cases and controls, different t-PA gene genotypes were identified (**Fig. 1**). Whereas, allele frequency (p = 0.90) didn't express considerable dissimilarity in genotype frequency between both groups (p = 0.65) (**Table 2**). Hence, for myocardial infarction cases, the genotype frequency was 21 %homozygotes I/I, 49%homozygotes D/D, and 34% heterozygotes I/D; while for controls, it was 35% I /I, 85% D/D, and 421% I/D. Concerning each patient's MI number, the fraction of the PLAT gene allele" I" frequency division is shown in Table 2. The rate of allele "I" rate is recorded more in cases with higher MI (p = 0.903).

Discussion

The current study identified the character of polymorphism of t-PA gene in the succession of MI infections in Punjabi Pakistanis.

Table 1: Demographic features and Prevalence of risk factors for myocardial Patients with Cardiac disease and controls

Indicators	Controls (n=380)	Cases (n=250)	Odds Ratio (95 % Cl)	*P Value*
Age (y)*	73.4±7.6	73.6±8.1	-	0.1
Females	30 (30)	40 (44.44)		
Males	70 (70)	50 (55.55)	1.9 (1.0 –3.4)	<0.039
Family history MI	50 (60)	70 (77.77)	2.3 (1.2-4.4)	<0.015
Hypertension	65 (15)	60 (55.55)	2.3 (1.3-4.1)	<0.01
Diabetes mellitus	70 (10)	80 (11.11)	3.4 (1.6-7.3)	<0.014
Smoking	40 (20)	80 (66.66)	8.0 (4.3-15.0)	<0.012

Note: *Expression of age as a mean ±1 SD

Table 2: Genotype and polymorphism of rate of occurrence of allele (frequency) of t-PA gene Ins/Del in MI patients and unaffected controls.

Genotype	**Male controls (n = 162)**	**Male patients(n=153)**	**P(χ^2)**	**P (z-test)**	**OR (95% CI)**	**P of OR**
Ins/Ins	35(22%)	32(21%)	>0.05 (0.685)	0.881(0.51)	1.036 (0.585-1.834)	0.903
Ins/Del	42(26%)	46(30%)		0.413(0.82)	1.241(0.737-2.090)	0.416
Del/Del	85(52%)	75(49%)		0.540(0.61)		
Genotype	**Female controls (n = 55)**	**Female patients(n=76)**	**P(χ^2)**	**P (z-test)**	**OR (95% CI)**	**P of OR**
Ins/Ins	12 (22%)	17(22%)	>0.05 (0.020)	0.940(0.07)	1.012 (0.412-2.488)	0.979
Ins/Del	18(33%)	24(32%)		0.890(0.14)	0.952 (0.429-2.115)	0.905
Del/Del	25(45%)	35(46%)		0.946(0.07)		
Allele	**Controls (n=380)**	**Patients (n=250)**	**P(χ^2)**	**P (z-test)**	**OR (95% CI)**	**P of OR**
Ins	200 (53%)	190 (54%)	>0.05 (0.200)	0.654(0.45)	0.936 (0.699-1.252)	0.654
Del	180 (47%)	160 (46%)		0.000(4.19)		
Allele	**Controls (n=113)**	**Patients (n=122)**	**P(χ^2)**	**P (z-test)**	**OR (95% CI)**	**P of OR**
Ins	65 (58%)	62 (51%)	>0.05 (1.061)	0.302(1.03)	1.31 (0.783-2.193)	0.303
Del	**48 (42%)**	**60 (49%)**		**0.302(1.03)**		

The t-PAs assist as a regulatory part in thrombus degradation that digests fibrin clot by converting plasminogen into plasmin [14,15]. The release of t-PA influenced by natural factors such as diabetes is more pronounced [22, 27]. According to previous studies, MI is not caused by a single accident. Both genes and environmental factors are involved in the progression of the disease. The serum t-PA level was found to be consistent with I/D polymorphism of the t-PA gene. High risk of MI was associated with a homozygous I/I genotype of the t-PA gene [16]. The relationship between different risk factors and MI was observed in the present study. Most of MI patients were found to be heavy smokers in contrast with controls. It is consistent with more prevalence of MI in Pakistani patients (52%) with a smoking history.

Significant factors, such as diabetes and familial history [22], were more common in MI patients than control studies. These observations are consistent with an earlier report by Ismail et al. 2004. The MI patients with lesser age and smoking were compared with an observation (Joshiet al.2007, Van der Bom et al.1997) found that the I/I genotype increased in cases of group outcomes associated with our results [13]. however, it contradicts the conclusions from other surveys. According to the findings of Steed et al. (1994), the cases and controls were alike in proportion to the persistence of the "I" allele (21% vs.22%, respectively). I/D polymorphism was noted to not correlate with MI (p> 0.05). Also, Hooper et al. (2000) investigated the "I" allele intervals ranged from 37% and 44% in cases and controls, respectively.

In addition to this, in an advanced study investigating the correlation of t-PA I/D polymorphism and MI, no MI-related interactions were observed [19].On the other hand, in this research, we got to know that frequency of I/I and I/D had a more significant effect on patients at high risk of MI (p = 0.309), that was conceivably described by the effect of two types of genotypes on t-PA enzymatic action. Speaking of extent of lipid, plasma TC, LDL-C, and TG are elevated in the disease group, and HDL-C is lower. Interestingly, like other countries, total plasma cholesterol didn't distinguish among cases and controls [20]. Besides, we have found statistically significant associations, among other things like diabetes, hypertension, smoking, and family history with MI. The conclusion corroborates a preceding organizational study in which t-PA manifestation was evaluated to be controlled by the environmental constituents [7], [18], [23].

The current survey indicated that MI is a multifaceted disease that may be the consequence of "genetic" environmental interactions. Our present study helps to understand the association of t-PA gene polymorphism with hypertension and atherothrombotic Stroke.

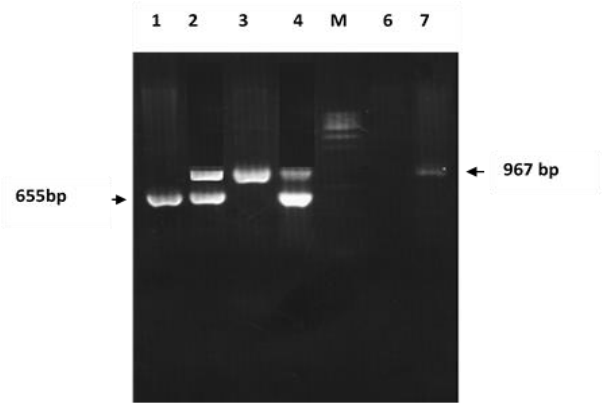

Fig. 1: Electrophoresis band pattern to detect TPA I/D polymorphism: Analysis of 967 bp insertion and 655 bp deletion allele of the t-PA gene on 1.8% agarose gel electrophoresis of cardiac patients, hypertensive and control subjects. Lane1: Homozygous for deletion polymorphism; Lane 2 & 4: Heterozygous for I/D polymorphism, Lane 3 & 7: Homozygous for Insertion polymorphism, Lane6: Negative control for I/D polymorphism while Lane 5:1 kb DNA ladder.

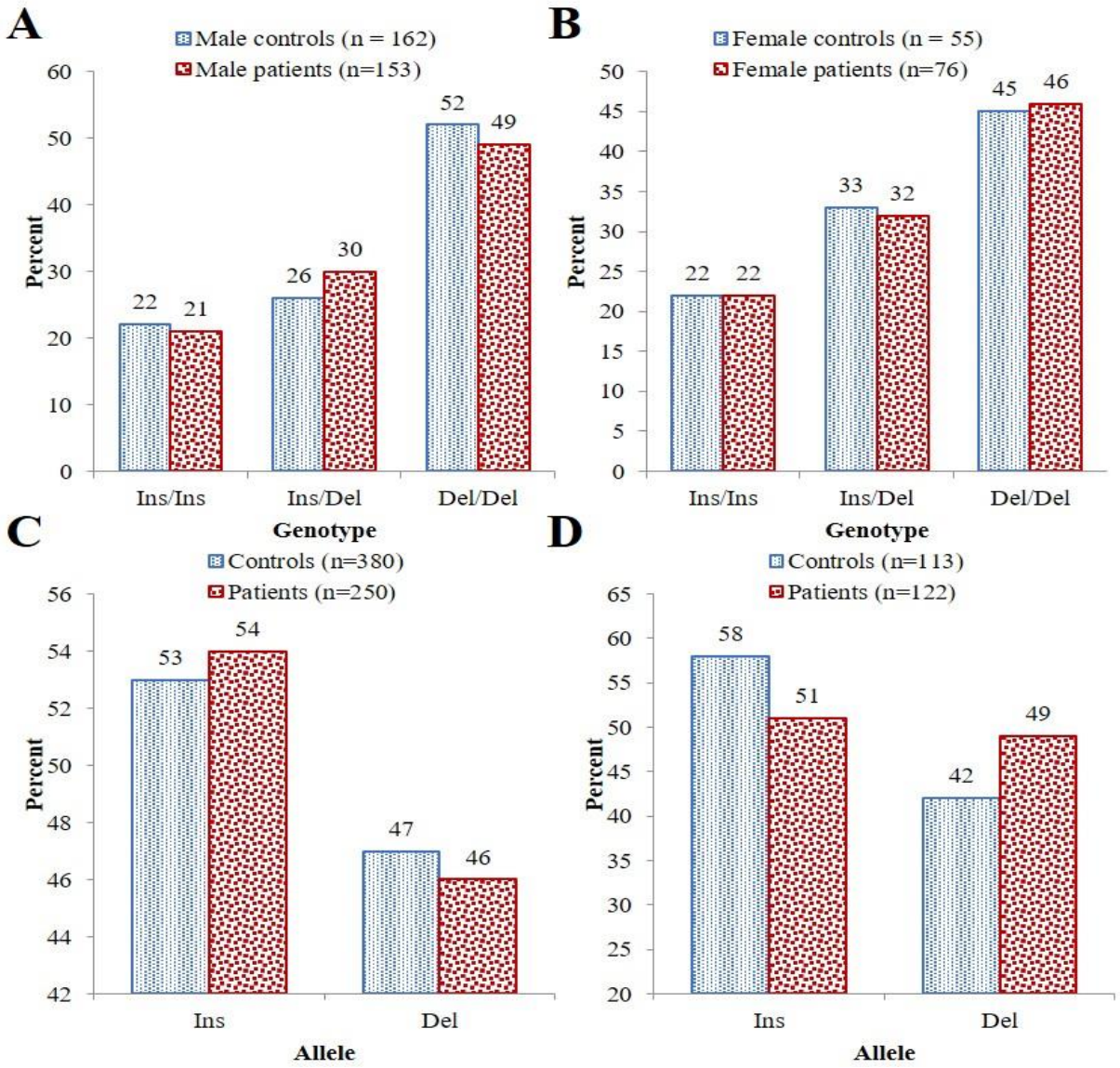

Fig. 2: Comparisons between male control and male patients using bar graph blue graph indicating male control red indicating male patients, Comparison between female control& female patients, Comparison between total percent of Insertion and Deletion genotype in control male group and male patient group, Comparison between total percent of Insertion and Deletion in female control and patients' group of females.

Conclusions

It is evident from our results that the frequency of allele I/I and I/D genotype results in amplification of the number of patients having two MI alleles in contrast with the patients who have one MI allele, as the "I" allele was discovered to be partly linked with MI. Also, the most significant correlation is found between territorial factors and MI. The study results will be helpful in the treatment of cardiac patient of local population of Pakistan.

Acknowledgements

We would like to extend thanks to the Department of Punjab Cardiology and the Punjab Institute of Nuclear Medicine, Pakistan Science Foundation (PSF). Cardiologists, neurologists, and radiologists from PINUM, Faisalabad, Pakistan are thanked for their skilled help. Some chemicals were purchased from funds made available by the United States Agency for International Development under PSTC proposal 6-163 and Pakistan Science Foundation.

This work is funded in part by the IAEA grant number PK6034 for I. Rajoka and in part by PAEC. Some of the chemicals purchased from the funds were made available by the United States Agency for International Development under Recommendation 6-163 with Pakistan.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Bang C, Park H, Ahn M, Shin M, Hwang K, and Hong S. 4G/5G polymorphism of the plasmoginogen Activator Inhibitor-1 gene and insertion/deletion polymorphism of the tissue–type plasminogen activator gene in atherothrombotic sroke. Cerebrovascular Disease.2001; 11: 294-299.

[2] Burzotta F, Castelnuovo A, Amore C, Dorazio A, Donati MB, and Iacoviello L. The role of 4G/5G polymorphism in the regulation of plasma levels of PAI-1: a model of interaction between genetic and environmental factors. Cardiologia. 1998; 43:83-88.

[3] Chambers C, Shinichi E, Basset P, Karim Y, Simon T, Gallimore R. C-Reactive protein, insulin resistance, central obesity and coronary heart disease risk in Indian Asians from the United Kingdom compared to European whites. Circulation. 2001; 104,145-50.

[4] Eriksson P, Kallin B, Hooft M, Baven-holm P, and Hamsten A .Allele specific increase in basal transcription of the plasminogen activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci. 1995; 92: 1851-1855

[5] Festa A, Agostino R, Howard G, Mykkänen L, Tracy P, Haffner M.Chronic subclinical inflammation as part of the insulin resistance syndrome. Circulation. 2000; 102:42-47.

[6] Gardeman A, Lohre J, Katez N, Tillmann H, Hehrlen, W, and Haberbosch W. 4G/4G genotype of the plasminogen activator inhibitor 4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk of this disease. ThrombHaemost. 1999; 82 :1121- 1126.

[7] Gialeraki A, Politu M, Rallidis L, Merkouri E, Markatos C, Kremastinos D, and Travlou A. Prevalence of prothrombotic polymorphisms in Greece. Genet. Test. 2008;12: 541-547.

[8] Grenett E, Khan N, Jiang W, and Booyse M. Identification of the Hind III polymorphic site in the PAI-1 gene: analysis of the PAI-1 Hind III polymorphism by PCR. Genet 2000; 4(1): 65-68.

[9] Heit A. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost. 2005; 3: 1611-1617.

[10] Hooper C, Lally C, Austin H, Renshaw M, Dilley A. The role of the t-PA I/D and PAI-1 4G/5G polymorphisms in African American adults with a diagnosis of myocardial infarction or venous thromboembolism. Thromb Res 2000; 99: 223-230.

[11] Ismail J, Jafar H, Jafary H, White F, Faruqui M. Risk factors for non-fatal myocardial infarction in young South Asian adults. Heart.2004; 90: 259-263.

[12] Joshi P, Islam S, Pais P, Reddy S, Dorairaj P. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA. 2007; 297: 286-294.

[13] Iacoviello L, Burzotta F, Castelnuovo A, Zito F, Marchioli R, and Donati B. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of mycocardia infarction: a meta-analysis. Thrombhaemost. (1998); 80,1029-1030.

[14] Ladenvall P, Wall U, Jern S, and Jern C. Identification of eight novel single nucleotide polymorphisms at human tissue type plasminogen activator (t-PA) locus: association with vascular t-PA release in vivo. Thromb Haemos. 2000; 84:150- 155.

[15] Onalan O, Balta G, Oto A, Kabakci G, Tokgozoglu L, Aytemir K, Altay C, Gurgey A, and Nazil N. Plasminogen activator inhibitor -1 genotype is associated with myocardial infarction but not with stable coronary disease. J. Thromb Thrombolysis. 2000; 26: 211-217.

[16] Ossei N, Mansfield W, Stickland MH, Wilson J, and Grant J. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. Arterioscler Thromb Vasc Bio. 1997; l17: 33-37.

[17] Raji A, Seely W, Arky A, and Simonson C. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Clin Endocrinol Metab. 2001. 86:5366-71.

[18] Ridker M, Hennekens H, Stampfer J, Miletich P. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. Circulation. 1997; 95:49-62.

[19] Steeds R, Adams M, Smith P, Channer K, Samani J. Distribution of tissue plasminogen activator insertion/deletion polymorphism in myocardial infarction and control subjects. Thromb Haemost. 1998; 79:980-984.

[20] Stegnar M, Uhrin P, Peternel P, Mavri A, Salobir-Pajnic B, Stare J, and Binder R .The 4G/5G sequence polymorphism in the promoter of plasminogen activator inhibitor -1 (PAI-1) gene: relationship to plasma PAI-1 level in venous thromboembolism. Thromb. Haemost. 1998; 79, 975 -9.

[21] Tsantes E, Nkkolopoulos K, Bagos G, Bonnovas S, Kopterides P, and Vaiopoulos G. The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk. Thromb Res. 2008; 122: 736-742.

[22] vander G, Knijff P, Haverkate F, Bots L, Meijer P. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. Circulation. 1997. 95: 2623-2627.

[23] Wang B, Zhou X, Dang A, Liu G, and He R. Alu-repeat polymorphism in the gene coding for tissue-plasminogen activator and the risk of hypertension in a Chinese Han population. 2002; Hypertens Res.25: 949-953.

[24] Ahmed W 1, Malik M, Saeed I, Khan A, Sadeque A, Kaleem U, Ahmed N, Ajmal M, Azam M, Qamar R. Role of tissue plasminogen activator and plasminogen activator inhibitor polymorphism in myocardial infarction. Mol Biol Rep.2011; 38(4):2541.