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Original Research Article

4G/5G Single Nucleotide Polymorphism of Plasminogen Activator Inhibitor-1 Geneis A Novel Diagnostic and Prognostic Marker in ST Elevation Acute Myocardial Infarction in Young Patients

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Abstract

Background: Prevalence of Acute Myocardial Infarction (AMI) in younger age is increasing in worldwide and it has been reported that 60% of AMI in young age is due to genetic causes. SNP inPAI-1 4G/5G plays major role and there is a need to authenticate its effectiveness and sensitivity. The aim of this study is to ascertain SNP in PAI-1 (4G/5G) as an independent biochemical marker for the South Indian young AMI patient's less than 45 yearsand to find its association with other risk factors of AMI. ***Methodology:*** This cross sectional study subjects includes40 Patients aged less than 45 years with AMI withtypical chest pain, ST Elevation in ECG and a rise in serum CK-MB, without any other known risk factorsand age and sex matched 40 control. ARMS-PCR was done with separated DNA. Remaining Serum was used to analyze Urea, HDL, CK-MB and other factors. Finally statistical analysisis done using SPSS-20.0. ***Result:*** Statistical analysis showed significant variation of SNP inPAI-1($P \leq 0.001$) between patients and controls. Further, positive significant changeswere observed between patients and controls in Serum Urea: $P \leq 0.001$, Serum Glucose: $P \leq 0.04$, Serum AST: $P \leq 0.001$, Serum CK-MB: $P \leq 0.001$. A significant inverse association was observed in SNP of PAI-1 and Serum HDL. ***Conclusion:*** This study confirms the independent association between STEMI and the 4G/5G allele polymorphism among South Indian euthenics and an inverse association of 4G/5G polymorphism with Serum HDL-cholesterol levels.

Keywords: PAI-1 4G/5G SNP, SNP in PAI-1, SNP in young AMI, Genetic Biomarkersin AMI.

INTRODUCTION

Coronary heart disease is the most important cause of mortality and morbidity all over the world and the ST Elevation Myocardial Infarction (STEMI) is the most common cause of death in developing countries like India. About 9% of new events occur in patients under 45 years of age; it is estimated that a genetic element is involved in some 20%-60% of these cases. Certainly, it is known that fibrinolytic activity is reduced in patients under 45 who suffers an Acute Myocardial Infarction (AMI) [1]. CHD will be the commonest single cause of death in developed countries over the next 20 years and will increase in frequency to become the commonest cause of disease-related disability in both developed and developing countries by the year 2020 [17]. According to WHO MI will be the leading cause of death in India and in most industrialized nations throughout the world by 2020. Cardiovascular death in Asian Indian population is expected to climb up 103% and 90 % in men and women by 2015. India is predicted to put up with the greatest CAD burden, according to the estimates from the Global Burden of Disease Study. In developing countries, during 1990 there were 9 million deaths due to CAD out of which 2.4 million(25%) occurred in India [2].

Genetic markers are variants in the DNA code (known as alleles) that, alone or in combination, are associated with specific disease phenotype. Markers whose presence confers a high degree of probability of disease (a "high predictive value") would be the most useful diagnostic tool or as predictors of prognosis or response to therapy. Even markers may provide important clues to disease pathophysiology or suggest new avenues of therapeutic intervention. Plasminogen activator inhibitor type 1(PAI-1) is the main inhibitor of both urinary-type (uPA) and tissue- type (tPA) Plasminogen activators. The increased plasma levels of PAI-1 causes reduction in plasma fibrinolytic activity, mainly, which is associated with coronary heart disease (CHD) [3] and recurrent myocardial infarction.

Endothelial injury can stimulate PAI-1 expression and facilitate thrombosis. Hereditarily determined variability in PAI-1 expression has been recommended as a risk factor for coronary atherogenesis and thrombosis. The human PAI-1 gene is present on chromosome 7q21.3-q22, and it is susceptible for several polymorphisms which have been described in various studies [4]. In spite of several polymorphism a single base (guanosine) insertion/deletion polymorphism (4G/5G) located in the promoter region seems to be functionally important [5].

The homozygous or heterozygous carriage of 4G allele had been associated with higher PAI-1 levels and increased risk of CAD [6] although the relationship was not confirmed in other studies[4].This inconsistency is an expected one for a multi factorial disease like CHD. The effects of the polymorphisms on CHD risk may vary according to the presence or absence of other cardiovascular risk factors that affect PAI-1 concentrations (e.g., age, gender, smoking, and obesity) [7, 8]. Neale and Sham argued that this kind of association analysis was potentially problematic in the context of replication because a replication study might not provide supportive or negative evidence if only the associated allele from the initial study was examined. They suggested a gene-based approach in which all variants including single SNP and haplotype variants within a candidate gene are considered jointly [9].

In atherosclerosis, PAI-1 expression is correlated with the cellular replicative senescence of vascular SMC. SMC express high PAI-1 levels and up regulate PAI-1 synthesis in human umbilical vein endothelial cells through a secreted soluble factor, thus leading to a feed forward mechanism rendering the vessel wall antifibrinolytic. Increased levels of PAI-1, seen by several groups during the progression of atherosclerosis and restenosis, may represent an indirect marker for an ongoing proliferative tissue repair process, probably indicating progression of atherosclerosis and development of restenosis after percutaneous coronary interventions [10].

MATERIALS AND METHODS

Ethics

This clinical investigation was approved by the Ethics Committee at the K.A.P. Viswanatham Government Medical College, Tiruchirapalli, Tamil Nadu, India.

Participants

This cross-sectional study was conducted at Mahatma Gandhi Memorial hospital, Trichy, Study subjects includes40 Patients with acute myocardial infarction who had typical chest pain, shows electrocardiographic changes (ST Elevation in ECG) and a transient rise in cardiac enzymes to more than twice the upper limit, Age less than 45 years who had been admitted within 24 hours of chest pain. Exclusion criteria includes patients with any or combination of Renal Disease, Severe Neuropsychiatric Problems, Life Expectancy less than one year, Known Diabetic Patients, Known Hypertensive Patients, Known History of Thromboembolic Disorders, Smokers, Alcoholic, History of Previous Coronary Artery Disease and Obese Individuals. Control subjects were 40 healthy individual men and women who came with some patients and healthy volunteers of age less than 45 years, during May 2016 to March 2017. Ethical clearance obtained from K.A.P.V Government Medical College. Every individual completed a questionnaire concerning the presence of cardiovascular risk factors such as smoking and alcohol consumption. For patients, all questions referred to the period before their myocardial infarction. The Quetelet index was derived by dividing weight (kilograms) by squared height (m^2). Persons were considered obese if their Quetelet index exceeded 30 kg/m^2.

Sample Collection and Analysis

Under sterile condition, 6ml of peripheral venous blood was withdrawn using sterile disposable syringes from all the study subjects. Then 2ml of this blood was transferred to EDTA tube for SNP analysis and 4ml was transferred to another eppendorf tube, centrifuged at 2500 rpm for 20 minutes and plasma was separated and transferred to another eppendorf tube for the analysis of Blood Glucose, Urea, Creatinine, Total Cholesterol, Triacylglycerol, HDL, Alanine transferase (ALT), Aspartate transferase (AST), Serum Electrolytes, and Creatine Kinase-MB (CK-MB).

DNA was extracted from the EDTA whole blood cells using salting-out method. The separated DNA was stored at -20° C until ARMS- PCR was done. Amplification of a fragment of the PAI-1 gene was performed using the technique of Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS- PCR)

The amplification-refractory mutation system (ARMS) is a simple method for detecting any mutation involving single base changes or small deletions. ARMS are based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. Following an ARMS reaction, the presence or absence of a PCR product is diagnostic for the presence or absence of the target alleleusing HELINI Human SNP ARMS-PCR Kit. Kit components includes Red Dye PCR Master Mix 0.5ml, Primer mix – A allele 0.25ml and Primer mix – G allele 0.25ml, for PCR Product: for 270bp Forward Primer and Reverse Primer was '*ATCCCTTTTCCCCTTGTGTC'*, '*TCCGATGATACACGGCTGACT*'. For PCR Product 450bp Forward Primer and Reverse Primer was*'TTGAATCATCCCGAAACCAT''CGATGATACAC GGCTGACC'*. The kit was stored atminus 20°C until used.

Statistics

For standard statistical analysis of the data's, Statistical products and serviceSolutions (SPSS)-20 package were used.The biochemical parameters between Myocardial infarction cases and healthy controls were tested by using student's t-test. The frequency of Genotype distribution between cases and controls wereCompared by using Chi-square ($\chi 2$) test. Level of significance for p-value was set at point <0.05 p<0.001 strongly significant).Pearson correlation was used to compare PAI -1 with other parametersLevene's Test used to check Equality of Variances between patients and control.

RESULT

The study revealed that there is a significant interaction between this polymorphism and plasma PAI-1 which increase the risk of AMI. The distribution of age of patients and control subjects detailed in Table-1 and values are Mean Age and SD of Patients 36.48 ±5.26 ,Mean Age of Controls: 32.33 ± 6.09, Minimum and maximum age for patients:18 and 43 years, Minimum and Maximum age for controls:19 and 42 years.

Table-1: Minimum age, Maximum age, Mean age and Standard Deviation of the patients and control groups

S.NO	Group	No of cases	Minimum Age	Maximum Age	Mean Age ± SD	P value
1	Patients	40	18.00	43.00	36.48± 5.26	
2	Control	40	19.00	42.00	32.33±6.09	≤ 0.61

A positive significant changes between patients and controls in SNP: P ≤ 0.001, Serum Urea: P ≤ 0.001, Serum Glucose: P ≤ 0.04, Serum AST: P ≤ 0.001, Serum HDL: P ≤ 0.008, Serum CK-MB: P ≤ 0. 001 was observed from Table-2.

Table-2: Mean, Standard Deviation and P value of Risk Factors of AMI

S. No	Variable	Group	Mean ± S. D	P value
1	AGE	Patients	36.484±5.2	≤ 0.61
		Control	32.35±6.09	
2	SBP	Patients	106.97±9.18	≤ 0.906
		Control	106.67±11.36	
3	DBP	Patients	74.24±8.3	≤ 0.871
		Control	74.55±6.6	
4	BMI	Patients	23.38±1.98	≤ 0.205
		Control	22.80±1.67	
5	UREA	Patients	36.91±6.19	≤ 0.001
		Control	31.18±5.73	
6	SUGAR	Patients	126.42±38.04	≤ 0.04
		Control	103.79±14.22	
7	CREATININE	Patients	0.942±0.16	≤ 0.443
		Control	0.973±0.14	
8	AST	Patients	122.09±52.44	≤ 0.001
		Control	31.94±10.44	
9	ALT	Patients	160.97±154.31	≤ 0.269
		Control	33.85±7.25	
10	APTT	Patients	25.33±6.17	≤ 0.511
		Control	24.58±2.45	
11	SODIUM	Patients	138.79±3.40	≤ 0.327
		Control	135.69±17.68	
12	POTASSIUM	Patients	3.967±0.43	≤ 0.547
		Control	3.912±0.28	
13	CHOL	Patients	168.39±29.00	≤ 0.240
		Control	176.39±25.67	
14	TGL	Patients	169.03±59.07	≤ 0.532
		Control	158.03±81.46	
15	VLDL	Patients	33.80±11.81	≤ 0.532
		Control	31.60±16.29	
16	LDL	Patients	95.89±24.36	≤ 0.320
		Control	103.06±33.07	
17	HDL	Patients	38.70±3.869	≤ 0.008
		Control	41.73±3.843	

18	CKMB	Patients	138.58±108.01	≤ 0.001
		Control	133.24±192.76	
19	PT	Patients	16.94±13.193	≤ 0.085
		Control	12.89±1.651	

Table-3: Serum CK-MP shows negative correlation with SNP (P ≤ 0.828) which is indicated in

S. No	Variables	Group	4G/4G n=14(P) n=2(C)	4G/5G n=11(P) n= 2(C)	5G/5G n=7(P) n=3(C)	Normal genotype n =8(P) n=33 (C)	P-value
1	CK-MB	Patients	175.71 ± 50.49	119.90 ±15.33	91.57±20.04	72.5±35.29	≤ 0.8
		Controls	29.5 ±7.7	22.5±3.5	27±1.01	30.42±5.9	

Table-4: 4G/5G Genotypic distribution, Allelic frequencies and their association with Acute Myocardial Infarction among South Indian Patients and Control Subjects

S. No	Genotypes	Patients (n=40)	Controls (n=40)	P value
1	4G/4G	14 (35%)	2 (5%)	≤ 0.001
2	4G/5G	11 (27.5%)	2 (5%)	
3	5G/5G	7 (17.5%)	3 (7.5%)	
4	Normal Genotypes	8 (20%)	33 (82.5%)	

DISCUSSION

The possible association between 4G/5G polymorphism (rs1799889) and the risk of cardiovascular disease has been studied in this work. The study revealed that there is a significant interaction between this polymorphism which increases the risk of AMI. Table-4 shows distribution in percentage of each allelic frequencies of 4G/5G polymorphism among patients and controls. As per Table-1 Mean Age and SD of Patients 36.48 ± 5.26, Mean Age of Controls: 32.33 ± 6.09, Minimum and maximum age for patients: 18 and 43 years, Minimum and Maximum age for controls: 19 and 42 years.

Two PCR reactions were run per sample (1 for A allele and one for G allele). Each allele-specific primer and downstream primer was amplified. The band size of 270-bpfor the 4G allele and a 450-bp for the 5G allele was noticed evidently in the submarine gel electrophoresis (Figure 1 and 2). The 4G/4G homozygote was demonstrated at 270-bp, 4G/5G heterozygote demonstrated 270- and 450-bp bands for both reactions, while 5G/5G homozygote demonstrated a 450-bp. Further, a positive significant changes between patients and controls in SNP: P ≤ 0.001, Serum Urea: P ≤ 0.001, Serum Glucose: P ≤ 0.04, Serum AST: P ≤ 0.001, Serum HDL: P ≤ 0.008, Serum CK-MB: P ≤ 0. 001were also observed from Table III. As per Table II, Serum CK-MP shows negative correlation with SNP (P ≤ 0.828). Some authors have reported an association between 4G/5G polymorphism in the promoter region of PAl-1 and the development of AMI but the outcome of others were unsuccessful to corroborate this [11]. The review of literatures showed that no study so far done in south Indian population to evaluate the association of 4G/5G polymorphism in STEMI in patient's ≤45 years of age. In the greater part of populations around the world, the 4G allele appears with greater frequency than the 5G allele. The allelic frequency of 4G/4G among the control subjects was just 6 % in the present study and it is one of the lowest among the similar studies indicated for the African American [11] (25%) and Japanese (30%) [12] Populations. The low frequency of the 4G allele of control group in the present study agrees with the findings of Ruiz-Quezada *et al*., [13]. In agreement with previously reported findings the present results show that the 4G allele is a risk factor for STEMI. Inconsistency in PAI-1 plasma concentrations has been reported in different ethnic groups around the world. Festa *et al*., [14] reported the ethnic differences in the distribution of 4G/5G polymorphism to be a determining factor in the plasma concentration of PAI-1

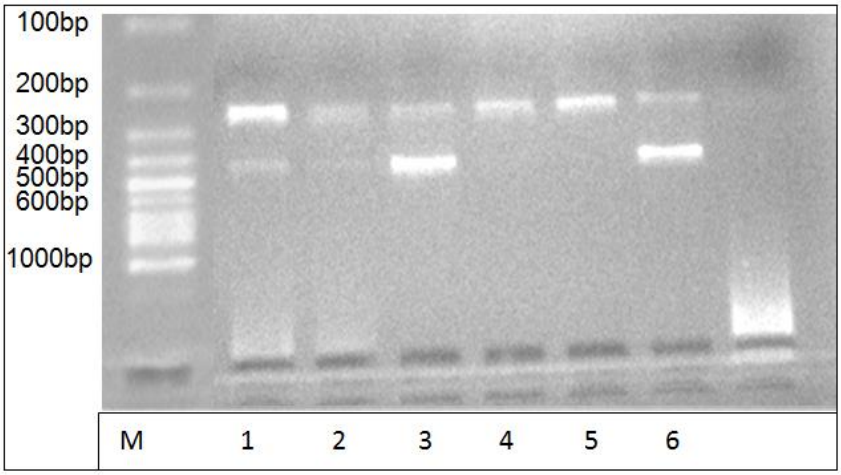

Fig-1: Submarine agarose gel electrophoretic analysis of the 4G/5G polymorphic region of PAl-1. M represent 100 bp molecular weight marker; lines 1, 3, 6, represent the 270 and 450bp 4G/5G polymorphic fragment; lines 2, 4, and 5 represents the fragments corresponding to the genotype 4G/4G after amplification by ARMS-PCR

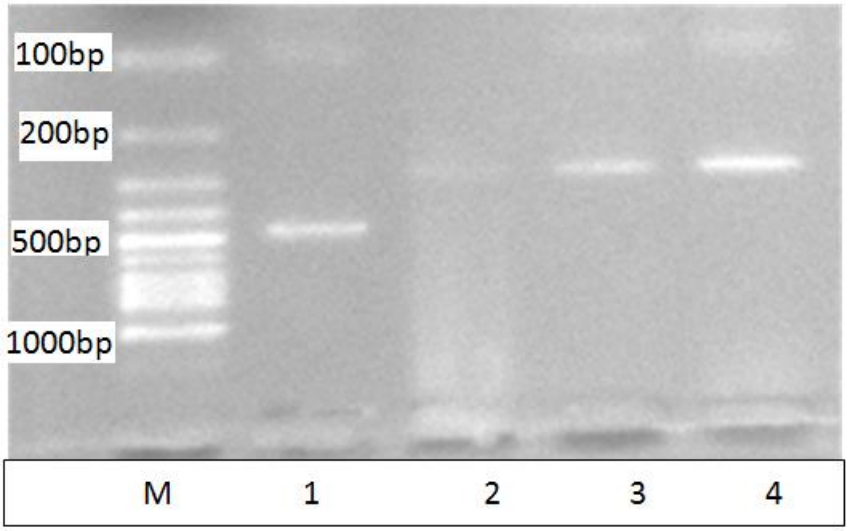

Fig-2: Submarine agarose gel electrophoretic analysis of the 4G/5G polymorphic region of PAl-1. M represent 100bp molecular weight marker; lines 1 represent 450bp 5G/5G polymorphic fragment; lines 3 and 4 represents the fragments corresponding to the genotype 4G/4G after amplification by allele specific primer

In some case plasma PAI-1 appears to be governed by 4G/5G polymorphism whereas environmental factors such as smoking along with other components of metabolic syndrome such as obesity and the insulin resistant and dyslipidemia [15] or the interaction between smoking and this syndrome [7] also contribute. However, interaction with other, traditional risk factors is certainly involved in the development of a STEMI and it is important to identify them if primary prevention from early life is to be improved. Plasma PAl-1 concentrations will behigh in those subjects with a 4G allele, as reported by Serrano Rios et al in patients with metabolic syndrome. Such increases in PAl-1 have been associated with AMI as Panahloo *et al*., report that they can remain high for six months. These findings have the same opinion with the proposal of Sobel *et al*., [16] that the over expression of PAI-1 leads to a reduced smooth muscle fiber content in atherosclerotic plaques, inducing a reduction in the amount of collagen and extracellular matrix proteins, a reduction in resistance to atheroma, the development of a vulnerable plaque, and its eventual breakage and consequent AMI. Further, an increased concentration of PAI-1 favors a state of hypo fibrinolysis via the inhibition of tPA and therefore a reduction in the transformation of Plasminogen into plasmin, a key enzyme in the regulation of the fibrinolytic system. It might, therefore, be hypothesized that the 4G allele is associated with high concentrations of PAI-1 and accordingly with two mechanisms that favor the onset of an AMI: the formation of vulnerable plaques and a reduction in fibrinolysis. This could be of particular interest in explaining the pathophysiological mechanisms behind STEMI in young patients. Studies have shown an association between such polymorphism and an increased risk for STEMI. PAI-1 might play an important role in the pathogenesis of CAD and gives

rise to 2 alleles 4G and 5G which differ in the regulation of the concentration of PAl-1.

The results concludes that there is an independent association between STEMI and the 4G allele, 5G allele polymorphism among south Indian ethnics. It also shows an inverse association of 4G/5G polymorphism with the HDL-cholesterol levels. Hence, it is concluded that the 4G/5G polymorphism in the PAI-1 gene is associated with the risk of AMI. The study confirmed that there is no positive association between this polymorphism levels in control subjects, whereas, there is a positive association between PAI-1 polymorphism and AMI patients.

CONCLUSION

This study confirmed that the polymorphism of PAI-1 4G/5G is an important cause of AMI, along with the traditional risk factors, the 4G allele is an independent risk factor for the occurrence of STEMI in patients under 45 years of age among south Indian ethnics. In addition the polymorphism of PAI-1 4G/5G coexists with increased serum Urea, Glucose and reduced HDL. However, it doesn't show any significant connection with Sex, Serum AST, ALT, Creatinine, PT, APTT Serum sodium and Potassium levels and Serum CK-MB levels. The detection of this allele along with other risk factors will be useful in primary prevention of AMI among south Indian ethnics. This will be helpful to avoid social burden of the young AMI patients and prevention of sudden death of young individual due to AMI in south Indian population.

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