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Original article

# Impact of PAI-1 4G/5G and C > G polymorphisms in acute ST elevation myocardial infarction and stable angina patients: A single center Egyptian study

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ABSTRACT

*Background:* Many genetic factors, including polymorphisms in the genes regulating blood coagulation and fibrinolysis have been proposed as risk factors for coronary artery disease (CAD). PAI-1 is the chief inhibitor of tissue plasminogen activator and urokinase plasminogen activator. PAI-1 has a crucial role in regulation of fibrinolysis.

*Aim of the study:* Is to investigate the association between Plasminogen activator inhibitor-1 (PAI-1) 4G/5G, PAI-1C/G polymorphisms and CAD. In addition, studying the relation of these polymorphisms to the level of active PAI-1 in Egyptian patients presenting to a single tertiary center in Cairo.

*Subjects and methods:* One hundred and forty-four patients were included in this study: 42 STEMI (ST elevation myocardial infarction) patients, 63 stable angina patients, and 39 as a control group. Detection of PAI-1 4G/5G and C > G polymorphisms was done using allele specific polymerase chain reaction and restriction fragment length polymorphism (RFLP) respectively. Plasma plasminogen activator inhibitor-1 activity was detected using enzyme linked immunosorbent assay (ELISA).

*Results:* In the studied CAD patients, PAI-14G/5G polymorphism showed 31.7%, and 68.3% for 5G/5G, and (4G/5G + 4G/4G) respectively; however for the control group, 5G/5G, and (4G/5G + 4G/4G) were detected in 21.6%, and 78.4% respectively (p value 0.59). The genotypic frequencies for PAI-1C/G in CAD patients accounted for 27% (CC), 73% (CG + GG); while in the control group these frequencies were 35.3%, and 64.7% respectively (p value 1.43).

*Conclusion:* No significant association between PAI-1 4G/5G and C > G polymorphisms and the risk of coronary artery disease or the activity level of PAI-1 among the studied Egyptian population sample. However, STEMI patients showed significant presence of combined mutant allele of both genes more frequently.



## 1. Introduction

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in the modern world. Despite the improvement in diagnosis and treatment options, its prevalence is still high [1]. Nuclear cardiac imaging has been extensively used as a diagnostic and prognostic tool for CAD. Negative stress myocardial perfusion study on maximal exercise correlated with normal coronary angiographies. Furthermore, it carried excellent prognosis with a sensitivity approaching 98%. Patients showing positive scans on exercise were previously categorized as Stable Angina patients in the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial [2]. A number of risk factors have been linked to CAD, among these factors: hyperlipidemia, hypertension, diabetes, smoking, family history and obesity [3,4]. These classical predisposing factors could explain about half of the cases of coronary artery disease. Moreover, studies on twins and family history had explored a possible role of genetic alterations as contributors to CAD [5–7].

Genes involved in blood coagulation and fibrinolysis processes, lipid metabolism and blood pressure regulation have been implicated as genetic risk factors for CAD. [8].

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The dynamic balance between coagulation and fibrinolysis is an important factor in determining the severity of CAD. CAD may range from unstable angina, associated with the non-occlusive thrombus to more severe form of disease; ST-elevated myocardial infarction (STEMI) accompanying the occlusive thrombus formation [9].

Myocardial infarction (MI) may result as a consequence of atherosclerotic plaque rupture, which stimulates blood coagulation resulting in occlusive thrombus and cardiac tissue necrosis [9,10]

Plasminogen activator inhibitor (PAI-1) has a pivotal role in regulation of fibrinolysis. The PAI-1 encoding gene is located on the short arm of chromosome 7 and consists of 9 exons [11]. PAI-1 is synthesized in the endothelial cells, liver, spleen and adipose tissue. It is stored and released from platelets on its activation [12,13]. It is the chief inhibitor of tissue plasminogen activator and urokinase plasminogen activator. Thus, PAI-1 stops the conversion of plasminogen into plasmin, the latter being in control of degradation of fibrin clots [13,14]. Increased expression of PAI-1 may hinder normal fibrin clearance and predispose to thrombophilia [15].Multiple single nucleotide polymorphisms in the PAI-1 gene have been investigated. Among these polymorphisms: 4G/5G insertion/deletion polymorphism at −675 bp of the gene promoter, in addition to C > G polymorphism at the 3′untranslated region (UTR) [16]. PAI-1 4G/5G gene polymorphism and the risk of CAD was thoroughly studied with conflicting data; some studies approving its role in the increased risk of CAD [17,18] while others did not [19,20].

This preliminary study aimed at evaluating the possible role of PAI-1 4G/5G and PAI-1C > G polymorphisms in Egyptian patients with CAD compared to a group of non-ischemic patients.

## 2. Subjects and methods

This was a prospective, double blind, case control, pilot, single tertiary center study. The study was conducted on patients admitted or subjected to myocardial perfusion imaging in the Critical Care Center, Faculty of medicine, Cairo University. The ethical committee of the Faculty of Medicine, Cairo University, approved the study. Informed written consents were taken from the patients for their participation in the study. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in human patients. The blood samples were collected, coded and stored in the Critical Care Center and then processed in the Clinical Pathology Department blindly. The laboratory test results were sent directly to the statistician, similarly the clinical and myocardial perfusion data were sent directly to the statistician.

### 2.1. Subjects

#### 2.1.1. Inclusion criteria

All patients with ST segment elevation myocardial infarction (STEMI) admitted to the Critical Care Center and subjected to primary percutaneous coronary intervention (PCI) were included as STEMI patients [21]. Patients scheduled for stress-rest technetium 99 m sestaMIBI (methoxy Isobutyryl Isonitrite) myocardial perfusion studies with abnormal scans were included as stable angina patients [22]. While patients with negative scan results for ischemia upon maximal exercise testing were considered as controls.

#### 2.1.2. Exclusion criteria

Patients above 70 years, patients with cardiogenic shock, in addition to patients who refused to be enrolled in the study were excluded from the study.

### 2.2. Methods

#### 2.2.1. History taking and laboratory investigations

History was taken from patients for possible CAD risk factors. Withdrawal of 2 ml venous blood sample on trisodium citrate tubes was done for determination of plasma PAI-1 activity according to the manufacturer's instructions using enzyme linked immunosorbent assay (ELISA) kit (Zymutest PAI-1 activity, HYPHEN BioMed, France). In addition, withdrawal of 2 ml venous blood sample under complete aseptic conditions in vacuum tubes containing ethylene-diamine-tetra-acetic acid (EDTA) to be stored at −20 °C for later DNA extraction. Subsequent detection of PAI-1 4G/5G and C > G polymorphisms using allele specific polymerase chain reaction and restriction fragment length polymorphism (RFLP) respectively was done.

#### 2.2.2. DNA extraction and genotyping

Genomic DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Germany) according to the manufacturer's instructions. Amplification of DNA for target polymorphisms detection was performed in a 25 μl volume reaction. The reaction consisted of: 7 μl extracted DNA, 1 μl of the corresponding forward primer and 1 μl of the corresponding reverse primer (20 pmol), 12.5 μl master mix (Dreamtaq Green PCR master mix, Thermo Scientific, USA) and 3.5 μl nuclease free water. Amplification was performed in an automated thermal cycler (Applied Biosystems, USA). The primer sequences and amplification thermal PCR conditions are listed in Table 1.

For the PAI-1 4G/5G polymorphism: two reactions were performed; each reaction contains a sense control primer, a common antisense primer and an allele specific primer (4G or 5G). An amplified 257 bp control band formed by sense and antisense primers was present in both reactions. In addition, a 136 bp band was observed for each 4G and 5G reactions if their alleles were present. 4G/4G homozygotes yielded 257-bp and 136-bp bands for the 4G allele-specific reaction and a 257-bp band for the 5G allele-specific reaction. 4G/5G heterozygotes showed 257- and 136-bp bands for both reactions. Whereas 5G/5G homozygotes showed a

**Table 1**
Primer sequences for PAI-1 polymorphisms.

| Genetic polymorphism | Primer sequence | Thermal cycler conditions |
|---|---|---|
| PAI-1C/G | F: 5[sps backslash] GCCTCCAGCTACCGTTATTGTACA3 [sps backslash] R:5[sps backslash] CAGCCTAAACAACAGAGACCCC-3 [sps backslash] | Initial denaturation: 94° C for 3 min 30 cycles of: Denaturation 94° C for 30 s, Annealing 60° C for 30 s, Extension 72° C for 30 s, Final extension: 72° C for 1 min |
| PAI-1 4G/5G | | |
| Sense control primer | 5[sps backslash] AGCTTTTACCATGGTAACCCCTGG 3 [sps backslash] | Initial denaturation: 94° C for 3 min, 32 cycles* of: Denaturation 94° C for 35 s, Annealing 65° C for 35 s, Extension 72° C for 70 s, Final extension: 72 °C for 1 min |
| 4G allele-specific primer | 5[sps backslash] AGAGTCTGGACACGTGGGGA 3[sps backslash] | |
| 5G allele-specific primer | 5[sps backslash]-AGAGTCTGGACACGTGGGGG-3 [sps backslash] | |
| Common antisense primer | 5[sps backslash] GCAGCCAGCCACGTGATTGTCTAG3 [sps backslash] | |

* PCR thermal conditions were the same for the 5G and 4G allele reactions, except for the final 22 cycles in 5G allele reactions, in which the annealing temperature was 58 °C.

257-bp band for the 4G allele-specific reaction in addition to 257- and 136-bp bands for the 5G allele-specific reactions [23]. As for the PAI C > G polymorphism: amplification yielded a 755 bp band. Amplified DNA was digested with 5 μl of HindIII restriction enzyme (New England Biolabs, USA) at 37° C for 1 h. A 567 and 188 bp fragments represented the C/C wild-type genotype for the PAI-1C > G polymorphism, whereas 755 bp band corresponded to the G/G polymorphic genotype. All fragments were analyzed by electrophoresis on 2% agarose gel and stained with ethidium bromide [24].

#### 2.2.3. STEMI patients

Patients with STEMI were subjected to clinical examination, 12 lead electrocardiogram (ECG) and Primary PCI by experienced operators within 90 min of admission. In addition, withdrawal of blood samples on admission for routine baseline laboratory investigations, before administration of any anticoagulant or antiplatelet therapy. Creatine kinase (CK), and CK-MB every 6 h for 24 h to detect enzyme peak level, were assayed as per center protocol.

#### 2.2.4. Myocardial perfusion imaging

Patients scheduled for technetium ($Tc^{99m}$) sestaMIBI myocardial perfusion imaging were subjected to ischemia provocation stress testing. This was done using Treadmill exercise stress test according to Bruce protocol to reach 100% of the target predicted heart rate or termination of exercise upon development of chest pain, ECG changes or serious arrhythmias [25]. Patients with exercise terminated due to physical fatigue or who didn't reach the target heart rate were excluded. A dose of 15–20 mCi of technetium sestaMIBI was injected during the peak of exercise to acquire the first set of images (stress images). Another similar dose of technetium sestaMIBI was injected on a separate day at rest to acquire the rest images. Single photon emission computed tomography (SPECT) Image acquisition was done using a double head Siemens Symbia gamma camera (USA). Frames were acquired 30 s each over 120° arc to get 2 studies (stress-rest) demonstrated as a comparison display showing the classical short axis, vertical long axis and horizontal long axis slices. The images were processed using Cedars Sinai software, (2007, USA). Myocardial perfusion scans were interpreted and revised by 2 experienced investigators.

#### 2.2.5. Statistical analysis

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as median and range or mean and standard deviation when appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using either Student *t*-test or Mann-Whitney test as appropriate. Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then multiple comparisons was done based on Kruskal-Wallis test. Odds ratio (OR) with 95% confidence interval (CI) was used for risk estimation. Spearman-rho method was used to test correlation between numerical variables. A p-value < 0.05 was considered significant.

## 3. Results

### 3.1. Patients' characteristics

One hundred and forty-four patients were included in the study: 42 STEMI patients, 63 stable angina patients, and 39 as a control group.

Mean age of the studied subjects was 61 ± 10.3 years. Compared to controls, all risk factors for CAD (except dyslipidemia) were significantly more prevalent in the STEMI and Stable Angina patients (Table 2). The CK and CK-MB on admission and peak levels are shown in Fig. 1. The mean number of perfusion defects in Stable Angina patients was 1.6 ± 0.9.

### 3.2. PAI polymorphism

PAI-1 4G/5G gene polymorphism genotypes and allele frequencies did not differ significantly between the three studied groups (Tables 3 and 4). The 4G4G mutant genotype was detected in only 2 patients with stable angina, so we considered patients with 4G/4G or 4G/5G as one group.

PAI-1C > G polymorphism genotypes and allele frequencies did not differ significantly between the three studied groups (Tables 3, and 4). The GG mutant genotype was detected in 10 STEMI patients, 4 patients with stable angina, and in only 2 of the control group so we considered patients with GG and CG genotypes as one group.

In addition, CK and CK-MB and their peak levels did not differ significantly between mutant and non-mutant genotypes in both polymorphisms (p value > 0.05).

Most of the heterozygous STEMI patients (89.2%) for 4G5G polymorphism were found to have simultaneously either mutant GG genotype (35.7%) or one mutant G allele (53.6%) for PAI C > G polymorphism (p value 0.016).

### 3.3. PAI-1. Activity

The expected normal level for PAI-1 activity is < 5 ng/ml according to manufacturer's instruction as most of the PAI-1 is in the latent or in the inactive form. Comparison of the level of PAI-1 activity between the 3 studied groups did not yield any significant difference (Table 5). In addition, PAI activity did not differ significantly between different genotypes of PAI polymorphisms among the different groups (Table 6). No correlations between PAI activity and different parameters including age, lipid profile, CK and CK-MB levels, fibrinogen or number of defects among STEMI patients were detected (p value > 0.05).

### 3.4. CAD risk factors

Diabetes, smoking and hypertension were more prevalent in CAD patients compared to the controls while the prevalence of dyslipidemia, family history, genotypic and allelleic frequencies of the studied PAI 4G/5G, PAI C/G were similar among CAD and controls.

(Table 4).

## 4. Discussion

Discovering new CAD risk factors at the level of genetic regulation of the coagulation and fibrinolysis cascades was subjected to intense research investigations. These research studies aimed at a better prediction and consequently prevention of this critical condition. Many polymorphisms have been implicated in the pathophysiology of CAD [26]. Among these are: polymorphisms of PAI-1 at different sites of the gene, which are important in determining PAI-1 activity thus controlling the balance between coagulation and fibrinolysis [27].

PAI-1 gene polymorphisms could change its generation level. Moreover it has been linked to worsening of some thrombotic diseases; as coronary heart disease, atherosclerosis and stroke [28,29].

**Table 2**
Comparison of demographic data and risk factors among STEMI, stable angina and the control groups.

| | STEMI* N = 42 | Stable angina N = 63 | Control N = 39 | P value |
|---|---|---|---|---|
| Age (years): mean ± SD | 55.4 ± 9.9 | 45.2 ± 8.5 | 42 ± 7.8 | <0.001** |
| Gender: Men (N/%) | 38 (90.5%) | 41 (65.1%) | 16(41%) | <0.001** |
| Women (N/%) | 4 (9.5%) | 22(34.9%) | 23(59%) | |
| Diabetic | 9 (21.4%) | 13 (20.6%) | 1 (2.6%) | 0.028** |
| Yes (N/%) | 33(78.6%) | 50(79.4%) | 38(97.4%) | |
| No (N/%) | | | | |
| Smokers | 28 (66.7%) | 16 (25.4%) | 6 (15.4%) | 0.001** |
| Yes (N/%) | 14(33.3%) | 47(74.6%) | 33(84.6%) | |
| No (N/%) | | | | |
| Hypertensive | 12 (28.6%) | 30 (47.6%) | 8 (20.5%) | 0.012** |
| Yes (N/%) | 30(71.4%) | 33(52.4%) | 31(79.5%) | |
| No (N/%) | | | | |
| Dyslipidemia | 2 (4.8%) | 5 (7.9%) | 7 (17.7%) | 0.133 |
| Present (N/%) | 40(95.2%) | 58(92.1%) | 32(82.1%) | |
| Absent (N/%) | | | | |
| FH*** for CAD**** | 15 (35.7%) | 11 (17.5%) | 6 (15.4%) | 0.043 |
| Present (N/%) | 27(64.3%) | 52 (82.5%) | 33(84.6%) | |
| Absent (N/%) | | | | |

* STEMI; ST elevation myocardial infarction.
** P value < 0.05 is significant.
*** Family history.
**** Coronary artery disease.

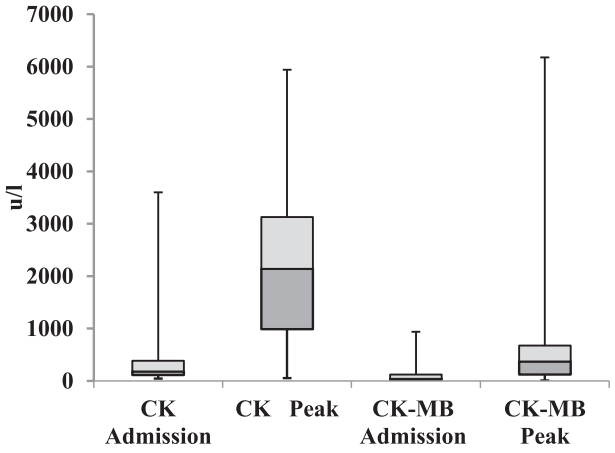


**Fig. 1.** CK and CK-MB levels among STEMI patients.

In this study we investigated the association between PAI-1 4G/5G, PAI-1C/G polymorphisms and the risk of coronary artery disease and their relation to PAI-1 activity.

The clinical practice guidelines (CPG) published by the European Society of Cardiology (ESC) emphasized the high prevalence of STEMI in women and elderly and commented on the diagnostic difficulties encountered in these populations [30]. The present study showed a significantly higher age among the STEMI patients compared to the other 2 groups. Acute coronary syndrome (ACS) exists three to four times more common in men than in women below the age of 60 years; however above the age of 75 years, women have a higher incidence [31]. In this study; male prevalence was significantly higher between the STEMI and the stable angina patients but less among the control.

In this study, regarding conventional risk factors of CADs, diabetes mellitus, smoking and hypertension were associated with increased risk of CADs. On the other hand, dyslipidemia and positive family history of CADs did not have a significant effect on hazard rate among our patients.

The genotype and allele frequencies for the PAI-1 4G/5G polymorphism in the control group were (5G/5G) 21.6%, (4G/4G + 4G/5G) 78.4%, (5G) 60.8% and (4G) 39.2% respectively. The genotypic distribution of 4G/5G polymorphism differed significantly in several studies by race. Panahloo et al., detected frequencies of 21% for 5G/5G, 50% for 4G/5G and 29% for 4G/4G genotype among white control participants and of 67%, 33% and 0% among African–Americans respectively [32]. Festa et al. studied the same polymorphism among 3 ethnic groups (non-Hispanic whites, Hispanics and black) and detected significant differences regarding the genotypic distribution (p = 0.001) [33].

The present study did not detect a significant difference in the genotype and allele frequencies of the PAI-1 4G/5G polymorphism when CAD patients were compared to the control group (p 0.25, p 0.55 respectively). Association of the PAI-1 4G/5G promoter polymorphism and CAD is still a controversial issue with some studies linking its association [27,34,35] while others not [19,36–42]. Senol et al., did not find any statistical significant differences when preoperative coronary artery bypass grafting (CABG) patients with STEMI were compared to a control group [43]. However, Gong

**Table 3**
Genotypic frequencies of PAI-1 4G/5G, and PAI-1C > G polymorphisms in the studied groups.

| | STEMI* N (%) | Stable angina N (%) | Control group N (%) | P value** |
|---|---|---|---|---|
| *PAI 4G/5G polymorphism* | | | | |
| 5G/5G | 11(26.8%) | 22(34.9%) | 8 (21.6%) | 0.343 |
| 4G/4G + 4G/5G | 30 (73.2%) | 41(65.1%) | 29 (78.4%) | |
| *PAI C > G polymorphism* | | | | |
| CC | 7(20.6%) | 13(32.5%) | 12 (35.3%) | 0.360 |
| CG + GG | 27(79.4%) | 27(67.5%) | 22 (64.7%) | |

* STEMI; ST elevation myocardial infarction.
** P value < 0.05 is significant.

**Table 4**
Association between *PAI-1* 4G/5G, and PAI C/G polymorphisms and different parameters and risk of CADs.

| | STEMI* & stable angina patients | Control group | P value | Odds ratio (95% CI) |
|---|---|---|---|---|
| Gender | | | | |
| Men (N/%) | 79(75%) | 16(41%) | <0.001** | 4.37 (2.01–9.50) |
| Women (N/%) | 26(25%) | 23(59%) | | |
| Diabetic | | | | |
| Yes (N/%) | 22(21%) | 1 (2.6%) | <0.001** | 10.07 (1.31–77.50) |
| No (N/%) | 83(79%) | 38(97.4%) | | |
| Smokers | | | | |
| Yes (N/%) | 44(42%) | 6 (15.4%) | 0.001** | 3.97 (1.53–10.29) |
| No (N/%) | 61(58%) | 33(84.6%) | | |
| Hypertensive | | | | |
| Yes (N/%) | 42(40%) | 8 (20.5%) | 0.024** | 2.58 (1.08–6.18) |
| No (N/%) | 63(60%) | 31(79.5%) | | |
| Dyslipidemia | | | | |
| Present (N/%) | 7(7%) | 7 (17.7%) | 0.054 | 0.32 (0.11–1.00) |
| Absent (N/%) | 98(93%) | 32(82.1%) | | |
| FH*** for CAD | | | | |
| Present (N/%) | 26(25%) | 6 (15.4%) | 0.216 | 1.81 (0.68–4.80) |
| Absent (N/%) | 79(75%) | 33(84.6%) | | |
| PAI 4G/5G | | | | |
| *Genotypes* | | | | |
| 5G/5G | 33 (31.7%) | 8 (21.6%) | 0.25 | 0.59 (0.25–1.43) |
| 4G/4G + 4G/5G | 71 (68.3%) | 29 (78.4%) | | |
| *Allele frequency* | | | | |
| 5G | 64.7% | 60.8% | 0.55 | 0.85 (0.49–1.46) |
| 4G | 35.3% | 39.2% | | |
| PAI C/G | | | | |
| *Genotypes* | | | | |
| CC | 20 (27%) | 12 (35.3%) | 0.38 | 1.43 (0.62–3.51) |
| CG + GG | 54 (73%) | 22 (64.7%) | | |
| *Allele frequency* | | | | |
| C | 54.1% | 64.7% | 0.14 | 1.56 (0.86–2.82) |
| G | 45.9% | 35.3% | | |

* STEMI; ST elevation myocardial infarction.
** P value < 0.05 is significant.
*** Family history.

**Table 5**
Comparison of PAI-1 activity in STEMI, stable angina and control groups.

| | STEMI* N = 41 | Stable angina N = 56 | Control N = 31 | P value** |
|---|---|---|---|---|
| PAI-1 activity ng/ml Median (range) | 2(0–13.6) | 2(0–13.6) | 2(0–12) | 0.933 |

* STEMI; ST elevation myocardial infarction.
** P value < 0.05 is significant.

**Table 6**
Comparison of PAI-1 activity between mutant and non-mutant PAI-1 4G/5G and C/G genotypes in STEMI patients.

| | PAI-1 activity (ng/ml) | | P value** |
|---|---|---|---|
| PAI-4G/5G polymorphism | Wild (5G/5G) | Mutant (4G/4G + 5G/4G) | |
| Control group | 1(1–6) | 4.6 (0–11) | 0.262 |
| Stable angina | 1.25(0–13.6) | 2 (0–13.6) | 0.373 |
| STEMI* patients | 1.8 (0–13) | 2 (0–13.6) | 0.379 |
| PAI-1C/G polymorphism | Wild (CC) | Mutant (CG + GG) | |
| Control group | 3.3 (1–6) | 4.6 (0–11) | 0.495 |
| Stable angina | 3.3(0–13.6) | 2(0–13) | 0.756 |
| STEMI patients | 2.0(0–13.6) | 2(0–13) | 0.944 |

* STEMI; ST elevation myocardial infarction.
** P value < 0.05 is significant.

et al., associated the 4 G allele to the risk of MI [44], while Hindorff et al. study, associated it to the decreased risk of MI in young women [42].

Our study assessed the active PAI-1 levels. The active PAI-1 levels in the 3 studied groups did not differ significantly (p = 0.933) and no significant differences were detected between wild and mutant genotypes regarding the level of active PAI-1 levels in the different studied groups. A former study investigated the PAI-1 levels in different races: its level was the lowest in blacks followed by non-Hispanic whites then Hispanics (p 0.0001) [33].

It has been postulated that PAI-1 4G/5G polymorphism is associated with different levels of PAI -1 protein as the presence of 4G/4G genotype is associated with increased levels of PAI-1 in plasma and increased myocardial infarction risk [27,34,35]. Isordia-Salas et al. [45] and Eriksson et al. [27] detected a higher plasma level in patients who were homozygous for the 4G allele. Similarly, Festa et al., detected the highest PAI-1 plasma level in subjects with a 4G/4G genotype, intermediate level for those with a 5G/4G genotype and the lowest for the 4G/4G genotype [33].

Among our studied groups the genotype 4G/4G was detected only in 2 cases of the stable angina patients and was not detected in any of the control or STEMI patients and this hampered reaching

a conclusion on the association between 4G allele and active PAI-1 levels. Extended studies are needed to elucidate the effect of 4G/4G genotype on PAI-1 activity and levels among the Egyptian population.

The genotype distribution and allele frequencies for the PAI-1C/G polymorphism in the control group were: (C/C) 35.3%, (C/G) 62.7% (G/G) 2%, (C) 64.7% and (G) 35.3% respectively. Among normal subjects, Barlik et al., detected frequencies of (C/C) 35.6%, (C/G) 44.4%, (GG) 20%, (C) 57.8% and (G) 42.2% [46], while Torres-Carillio et al. in 132 Mexicans, detected frequencies of 49.2%, 44.7% and 6.1% [24] in genotypes respectively.

To the best of our knowledge, the PAI-1C/G polymorphism has not been extensively investigated in CAD patients. The present study did not detect a significant difference in the distribution of genotypes and allele frequencies of the PAI-1C/G polymorphism when CAD patients were compared to the control group (p 0.38, p 0.14 respectively).

On studying the effect of the presence of combined mutant genotypes or alleles in CAD patients, STEMI patients showed significantly higher frequencies for the presence of combined mutant alleles of PAI-1 4G/5G and PAI-1C/G polymorphisms (p 0.016) raising the possibility of multifactorial genetic alteration predisposition to acute coronary syndrome.

In conclusion, no significant association between PAI-1 4G/5G and C > G polymorphisms and the risk of coronary artery disease or the activity level of PAI-1 were detected among the studied population. However, STEMI patients showed presence of combined mutant allele of both genes more frequently. Further multicenter studies on a larger sample are recommended to study the effect of combined PAI-1 genetic alteration on the risk of coronary artery disease.

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## Conflict of interest

The authors declare no conflict of interest.

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