

Alu-repeat Polymorphism in the Tissue Plasminogen Activator Gene and Risks of Myocardial Infarction in Tunisian Population

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Abstract

This study aimed to determine whether *I/D* polymorphism predispose to the development of MI in Tunisia. 50 patients with coronary artery disease complicated by MI and 70 healthy controls were analyzed. Quantitative analyses lipids are founded. DNA was isolated from blood samples. Polymorphisms were assessed by polymerase chain reaction method. Statistical differences between groups were determined by Chi-square test. Compared to controls, cases have significantly higher levels of total cholesterol, triglycerides and LDL-cholesterol and lower level of HDL-cholesterol. There are no significant differences between patients and controls in terms of allelic and genotypic frequencies. However, the frequency of the "I" allele is higher in cases which have 2 MI ($p=0.0354$). Additionally, a statistically significant association between diabetes, arterial high blood pressure, family history and cigarette smoking with MI was founded ($p<0.01$). *I/D* polymorphism of *t-PA* gene is not associated with MI in this sample of Tunisian population.

Keywords: Myocardial infarction; t-PA gene; *I/D* polymorphism

Introduction

Myocardial infarction (MI) is a coronary artery disease (CAD) caused by the degeneration of heart tissues as a result of blood clot formation in coronary arteries [1]. It represents one of the most important health problems in many nations worldwide [2].

Although the major risk factors of MI are environmental factors such as high blood pressure, hypercholesterolemia, diabetes and cigarette smoking, evidence is accumulating which suggest that genetic polymorphisms have been implicated to the disease progression [3,4]. Furthermore, MI is directly related to the thrombosis-fibrinolysis system which is regulated by factors involved either in clot formation and lysis [5]. Several gene polymorphisms of the fibrinolytic proteins and their cross regulators have been studied [6] and some studies founded that polymorphisms in genes encoding the components of thrombosis-fibrinolytic cascade may be a contributing factor in disease etiology by creating an imbalance between the two processes [7].

Tissue plasminogen activator (*t-PA*) produced by endothelial cells is involved in the breakdown of fibrin clot by converting plasminogen into plasmin, which then leads to fibrinolysis [8]. The *t-PA* protein encoded by *t-PA* gene (*PLAT*) is the inducer of fibrinolysis. The increased enzyme activity causes hyper-fibrinolysis which might leads to excessive bleeding whereas it's decreased activity result in thrombosis. *t-PA*-antigen might therefore either play an important role in the development and progression of vascular disease, culminating in thromboembolic complications or death, or *t-PA*-antigen might just indicate the co-existence of confounders such as hypertension, insulin resistance, heart failure or ischemic heart disease, all of which can cause endothelial dysfunction and vascular injury [9]. Several studies founded that a common insertion of an *Alu-repeat* in the intron 8 of the gene is considered to be involved in plasma levels of *t-PA* [8,10,11]. In fact, the presence of one *I/D* allele was associated with a 50% increase in risk of MI, whereas homozygous carriers had a two fold adjusted increase in risk, suggesting an association between the number of *Alu-repeats* and arterial thrombosis [5,12].

The aim of this study is to determine whether *I/D* polymorphism on the *PLAT* gene predispose to the development of MI in a sample of Tunisian population.

Materials and Methods

Study population

We conducted a case-control study in 50 patients, age 49 ± 6.25 (years), with CAD complicated by MI (confirmed by coronary angiography) ascertained from the Department of Cardiology in Fattouma Bourguiba University Hospital Monastir, Tunisia. The control group included 70 healthy volunteers, age 57 ± 8.37 (years). All subjects participated in the study on a voluntary basis, were interviewed by the same interviewer. The study, in accordance with Helsinki Declaration, was reviewed and approved by the local ethics committee and included only individuals that agreed to participate after reading and signing a free and informed consent form.

Laboratory measurements

Quantitative analyses lipids are founded in controls and patients within 24 h from collection. Plasma levels of total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) were measured by an enzymatic colorimetric assay (using a clinical system, Beckman instruments). LDL-cholesterol (LDL-C) concentrations were calculated by using the Friedewald formula [13].

Leukocyte DNA was extracted from blood samples by routine phenol-chloroform method and amplified by polymerase chain reaction (PCR) using oligonucleotide primers:

5' primer: 5'GTAAGAGTTCGGTAACAGGACAGCT3'

3' primer: 5'CCCCACCTAGGAGAAGTCTCTTT3'

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Amplification was performed in an automated thermal cycler (Master cycler). The PCR conditions were 10 min for initial denaturation at 94°C, 30 cycles at 94°C for 1 min for denaturation, 1 min at 60°C for annealing and 2 min at 73°C for extension, followed by 5 min at 72°C for final extension. The amplified product was digested with restriction enzyme. The gel visualizing system was used (Vilber Lourmat, France). The fragments were separated on a 2% agarose gel stained with ethidium bromide.

Statistical analysis

Allelic and genotypic frequencies were calculated by direct gene counting method. Lipid and plasma parameters measures were compared through the student's t-test. Chi-square test was used to determine if there are any significant differences in polymorphism frequencies in cases compared to control population.

Results

The distribution of the selected characteristics of MI cases and controls are presented in Table 1. The statistical comparison between them showed a significant difference in terms of frequency of diabetes, arterial hypertension and family myocardial infarction history ($p < 0.01$). Compared to controls, cases had significant higher levels of TC, TG, LDL-C and lower levels of HDL-C (Table 1).

Different genotypes of *PLAT* gene in cases and controls were identified in electrophoresis patterns of PCR fragments (Figure 1). The two groups showed no significant difference in terms of allele frequency ($p = 0.143$) and genotype frequency ($p = 0.154$) (Table 2). Therefore, the genotype frequency of myocardial infarction cases was 0.24% homozygotes *I/I*, 0.32% homozygotes *D/D* and 0.44% heterozygotes *I/D*, controls showed 0.27% *I/I*, 0.21% *D/D* and 0.51% *I/D*.

The distribution of allele frequency of *PLAT* gene according to MI number for each patient is given in Table 3. The frequency of the allele "I" is higher in cases which have 2 MI ($p = 0.0354$).

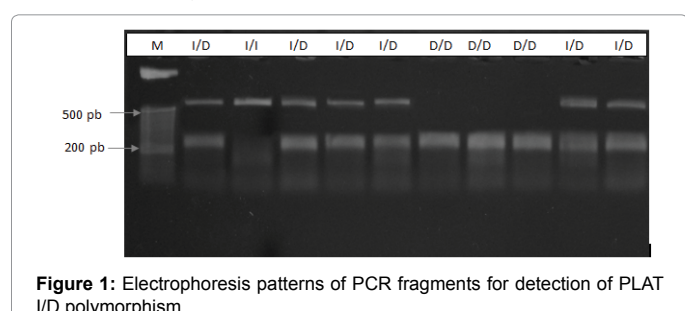
Discussion

This work focused on the relation between the polymorphism of

	Cases (n=50)	Controls (n=70)	P
Age (Mean ± SD)	49 ± 6.25	57 ± 8.37	0.115
Smoking (n)	39	25	0.0124
Diabetes (n)	25	5	<0.01
Arterial hypertension (n)	23	8	<0.01
Family history (n)	20	6	<0.01
TC (mmol/L)	4.62 ± 0.91	4.01 ± 0.46	<0.001
TG (mmol/L)	1.76 ± 0.77	0.78 ± 0.18	<0.001
HDL-C (mmol/L)	0.92 ± 0.26	1.07 ± 0.12	<0.001
LDL-C (mmol/L)	2.95 ± 0.86	2.57 ± 0.44	<0.01

SD: Standard deviation

Table 1: Selected characteristics of myocardial infarction cases and controls and lipid levels of the study population.



	Cases (n=50)	Controls (n=70)	P
Allele frequency			
I	0.46	0.52	0.143
D	0.54	0.47	
Genotype frequency			
I/I, n (%)	12 (0.24%)	19 (0.27%)	0.154
I/D, n (%)	22 (0.44%)	36 (0.51%)	
D/D, n (%)	16 (0.32%)	15 (0.21%)	

Table 2: Allele and genotype frequencies of cases and controls.

Allele	Myocardial infarction number (N)		P
	N=1 (19 Cases)	N=2 (31 Cases)	
I/I	4 (0.210)	8 (0.258)	0.0354
I/D	6 (0.315)	16 (0.516)	
D/D	9 (0.473)	7 (0.220)	

Table 3: Allele frequency according to myocardial infarction number.

PLAT gene and MI disease in Tunisian population. Several studies have investigated the regulating role of *t-PA* in the degradation of the thrombus and founded that the protein appears to activate the transformation of plasminogen to plasmin, which digests the fibrin clot [14,15]. The *I/D* polymorphism in the gene coding for *t-PA* was associated with an increased or decreased levels in serum *t-PA*. Further, the homozygous genotype *I/I* of the *t-PA* gene was associated with a higher risk of MI [16]. In this study, we found no significant differences between the two groups in terms of allelic and genotypic frequencies. Our results are contradictory to findings reported by van der Bom et al. [13] in which the authors founded that *I/I* genotype was increased of twice in cases group, but they are consistent with results from others studies. In fact, Steed et al. [17] have founded that cases and controls was relatively similar in term of frequency of "I" allele (0.57% vs 0.58%, respectively) and no association between *I/D* polymorphism and MI have been noted ($p > 0.05$). Furthermore, Hooper et al. [18] founded that the frequencies of "I" allele was 0.37% in cases and 0.44% in controls. Moreover, in a recent meta-analysis included seven studies which investigated the relationship between *t-PA* *I/D* polymorphism and MI, the authors suggests that the polymorphism showed no consistent association with MI [19].

However, in our study we found that *I/I* and *I/D* genotype frequencies have a significant increase in patients with two MI compared to patients with one MI ($p = 0.0354$), which may be explained by the impact of the two genotypes on *t-PA* enzymatic activity.

In terms of lipid levels, plasma TC, LDL-C and TG were raised in the disease group whereas HDL-C was reduced. Interestingly total plasma cholesterol did not discriminate between cases and controls as in other European countries [20]. Multiple regression analysis confirmed the independent predictive role for all of the above mentioned lipid parameters [21,22].

In addition, we have noted a statistically significant association between the environmental factors, including diabetes, arterial high blood pressure, family history and cigarette smoking with MI. This latter result confirms previous association studies where *t-PA* protein expression was found to be influenced by environmental factors [7,18,23-25].

A discrepant findings support the idea that MI is a multifactorial disease which may be the result of "gene-environment" interaction.

In interpreting the results from our current study, several potential limitations need to be considered. A potential concern is the study sample size that is relatively small. Another potential limitation of our

study concerns use of hospital-based cases and controls. While every effort was made to include all cases admitted to the study hospitals, we did not attempt to ascertain patients who may not have come to this hospital. Therefore, the generalizability of our results may be limited because of the hospital-based study design. Additionally, controls have a higher average age than the MI patients, a study sample with a quite comparative age need to be considered.

Despite these limitations, the study contributes to our understanding of MI in this population and provides some of the first data on relationship between t-PA polymorphism and MI in Tunisia.

Conclusions

Our results demonstrated that the risk allele "T" was not found to be associated with MI. However, *I/I* and *I/D* genotype frequencies have a significant increase in patients with two MI compared to patients with one MI. Additionally, we noted a statistically significant association between the environmental factors and MI.

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