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Original Article

Serum levels of thrombotic markers in patients with acute myocardial infarction

Shafiqul Islam[1], Sobhy M Yakout[2], Nasser M Al Daghri[1,2], Abdullah S Alhomida[1], Haseeb A Khan[1]

[1]Department of Biochemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; [2]Biomarkers Research Lab, Department of Biochemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

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Abstract: Tissue plasminogen activator (TPA) and plasminogen activator inhibitor-1 (PAI-1) have pro- and anti-fibrinolytic activities respectively. The net fibrinolytic activity is mainly determined by the balance between TPA and PAI-1 levels. Considering the important role of these markers in thrombotic pathway, we determined the levels of TPA and PAI-1 in sera of 50 AMI patients, 100 patients with associated risk factors (dyslipidemia and high blood pressure) and 100 healthy controls. The findings showed significantly high levels of TPA and PAI-1 in AMI patients as compared to control subjects. Both these markers were only non-significantly increased in the risk group. There was no correlation between body mass index and these markers however TPA and PAI-1 were significantly correlated with age and systolic blood pressure, respectively. In conclusion, a combination of these markers could provide a useful tool to assess the prognosis of AMI.

Keywords: Acute myocardial infarction, thrombotic markers, TPA, PAI-1

Introduction

Although cardiac troponin-T is a highly sensitive and specific marker of myocardial injury there is a need of developing technologies for novel biomarkers or signatures discovery towards point-of-care testing for future management of acute myocardial infarction (AMI) [1]. The use of different troponin-T assays and cut-off values may result in a discordant frequency of AMI diagnoses [2]. Recent findings on significant increases in carnitine and acylcarnitine levels in the dried blood spots of AMI patients suggested that a dual-marker strategy using carnitine (longer plasma half-life) in combination with troponin (shorter plasma half-life) could be a more promising biomarker strategy in risk stratification of patients [3]. Elevation of blood carnitine in AMI patients has been attributed to the poor uptake or increased leakage of carnitine through the ischemic myocardium [4]. Role of carnitine homeostasis in AMI was also supported by variations in blood carnitine levels due to the genetic polymorphism in carnitine palmitoyltransferase gene [5].

Recently, it has been found that a reduction in serum triglycerides does not prevent the risk of AMI, whereas a decrease in serum high density lipoproteins (HDL) and increase in C-reactive protein (CRP) strongly predispose the risky individuals to an AMI event suggesting the importance of HDL and CRP measurements for the assessment of a combined lipid-inflammation risk factor that could be a useful predictor of high risk individuals, as well as a prognostic marker in AMI patients [6]. Khan et al [7] observed a significant increase in total and differential leukocyte counts that was significantly correlated with CRP levels indicating a pro-inflammatory cascade in AMI patients and the importance of leukocyte counts for quick prediction of both myocardial necrosis and inflammation in AMI patients. Interestingly, monocytes were found to be significantly increased in AMI patients but not in infected controls however serum creatine kinase (CK) was significantly increased in AMI patients and decreased in infected controls suggesting that differential trends of monocytes and CK in AMI and infective controls could be utilized for the prognosis of AMI patients [8].

Table 1. Characteristics of different study groups

Characteristics	Control (N=100)	Risk group (N=100)	AMI (N=50)	*P* value
Gender ratio (M/F)	2.03	2.22	2.33	NS
Age (years)	39.9 ± 17.1	57.9 ± 9.9*	55.7 ± 12.3*	<0.001
Body mass index (kg/m^2)	25.6 ± 6.8	29.7 ± 5.9*	29.5 ± 5.7*	<0.001
Systolic blood pressure (mmHg)	113.5 ± 8.5	121.3 ± 19.7*	131.3 ± 25.3*	<0.001
Diastolic blood pressure (mmHg)	74.3 ± 6.4	77.7 ± 12.4*	80.3 ± 15.7*	<0.01

Values are mean ± standard deviation; NS, not significant; *significantly different from the control group.

The markers of the extrinsic and intrinsic pathways of coagulation including prothrombin time (PT) and activated partial thromboplastin time (aPTT) were found to be significantly increased in AMI patients [9]. The elevations in PT values were more than 2.5-fold greater than aPTT suggesting a high potential of PT for predicting blood clotting tendency in patients receiving anticoagulation therapy [9]. The plasminogen activator system is an important defense mechanism against intravascular thrombosis. Tissue plasminogen activator (TPA) is a protein involved in the breakdown of blood clots by catalyzing the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown. In a clotting situation, TPA is released from the storage pools in the endothelium and initiates fibrin breakdown. Plasminogen activator inhibitor-1 (PAI-1) functions as a principal inhibitor of TPA and hence counteracts fibrinolysis, the physiological process that degrades blood clots. The overall fibrinolytic activity is mainly determined by the balance between TPA and PAI-1 levels [10]. To further improve the efficacy of thrombolytic therapy, a PAI-1 resistant variant of TPA has been developed for the treatment of AMI [11]. In this study, we compared the serum levels of TPA and PAI-1 in AMI patients versus healthy controls as well as a group of patients (risk group) with dyslipidemia and high blood pressure.

Materials and methods

This study was conducted on 50 AMI patients, 100 patients with associated risk factors (dyslipidemia and high blood pressure) and 100 healthy controls. The participating subjects were recruited and enrolled from King Khalid University Hospital and National Guard Hospital, Riyadh, Saudi Arabia. The characteristics of subjects are given in **Table 1**.

The diagnosis of AMI was made by the European Society of Cardiology/American College of Cardiology (ESC/ACC) diagnostic criteria for AMI. Briefly, the diagnosis required the presence at least two of the following criteria: (i) history of characteristic prolonged (≥30 min) pain or discomfort, (ii) CK levels exceeding twice the upper limit of normal (or CK-MB ≥50% of total CK) and (iii) presence of new Q waves or new abnormal ST-T features [12]. The patient exclusion criteria included recent surgery, active infection, chronic inflammatory diseases, significant hepatic or renal dysfunction and malignancy. The protocol of this study was approved by our Institutional Review Board (IRB) for human studies and all the patients signed informed consent.

Venous blood samples were collected in serum separator tubes. Sera were separated and stored at -20°C until analyzed. The levels of TPA and PAI-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits from Innovative Research, Inc., USA. Prior to analysis, sera were diluted 1:10 and 1:5 for TPA and PAI-1 measurements.

The data were analyzed by analysis of variance (ANOVA) followed by Bonferroni test using SPSS statistical package. Pearson's test was used for correlation analysis. *P* values <0.05 were considered as statistically significant.

Results

There was no significant difference in the gender ratio among the three groups. However, age, body mass index (BMI) and blood pressure were significantly higher in AMI patients as well as in the risk group as compared with healthy controls (**Table 1**).

The levels of serum thrombotic markers including TPA and PAI-1 were slightly increased in the risk group and these changes did not reach the level of significance (**Table 2**). In AMI group, both TPA (ANOVA F=2.28, P=0.016) and PAI-1

Table 2. Serum coagulation markers in different groups

Biomarker	Control	Risk group	AMI	*P* value
Tissue plasminogen activator (ng/ml)				
Model 0 (un-adjusted)	2.5 ± 0.13	4.1 ± 0.11	5.2 ± 0.22*	<0.05
Model 1 (age-adjusted)	3.2 ± 0.20	3.7 ± 0.13	5.6 ± 0.20	NS
Model 2 (BMI-adjusted)	2.3 ± 0.18	4.2 ± 0.13	5.9 ± 0.22*	<0.01
Plasminogen activator inhibitor-1 (ng/ml)				
Model 0 (un-adjusted)	10.1 ± 0.17	12.7 ± 0.15	16.3 ± 0.24*	<0.01
Model 1 (age-adjusted)	10.8 ± 0.18	12.1 ± 0.17	15.8 ± 0.22*	<0.05
Model 2 (BMI-adjusted)	10.3 ± 0.17	12.4 ± 0.17	17.8 ± 0.28*	<0.01

Values are mean ± standard error; NS, not significant; *significantly different from the control group.

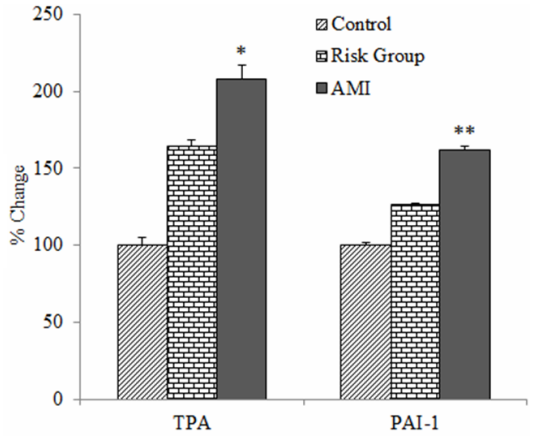

Figure 1. Percent change in serum TPA and PAI-1 levels as compared to controls. *P<0.05 and **P<0.01 versus control group.

(ANOVA F=4.77, P=0.009) levels were significantly higher as compared to control subjects (**Table 2**). Statistical models adjusting for age and BMI resulted similar trends (**Table 2**). On percent scale, the increases in serum TPA were 64% and 108% while in PAI-1 were 25.74% and 61.38% in risk group and AMI patients, respectively (**Figure 1**).

There was a significant correlation between serum TPA and age (R=0.16, P<0.05) whereas TPA was not correlated with BMI and blood pressure (**Table 3**). Serum PAI-1 showed a significant correlation with systolic blood pressure (R=0.14, P<0.05) however it was not correlated with age and BMI (**Table 3**).

Discussion

We observed a significant increase in serum TPA levels in AMI patients (**Table 2**). Elevated TPA antigen at initial presentation in patients with AMI has been associated with higher short-term risk of death, suggesting that TPA may be a useful prognostic biomarker for the early risk stratification of these patients [13]. Mannucci et al [14] have reported that although higher levels of von Willebrand factor (VWF), fibrinogen, CRP and TPA were associated with AMI, only TPA maintained an independent association with AMI after adjustment for both classical and hemostatic risk factors. Ehlers et al [15] have observed elevated TPA levels in patients with initial ECG changes suggesting that association of myocardial damage and a disturbed hemostatic system might stratify patients who are at high risk of suffering further coronary events. Although TPA related to the risk of death and to nonfatal acute coronary syndromes, adjusting for significant covariates reduced the strength of the associations [16]. It is important to note that intravenous TPA infusion is one of the effective therapeutic approaches for AMI [17, 18]. Complications of intravenous thrombolysis with TPA are commonly related to hemorrhage, anaphylaxis, or arterial occlusion; however, the disruption of intracardiac thrombus and subsequent embolization to coronary arteries may be an important mechanism in the occurrence of AMI after administration of TPA for acute ischemic stroke [19].

The levels of serum PAI-1 were significantly higher (1.61-fold) in AMI patients, which is in agreement with earlier reports [20, 21]. Ilić et al [21] have observed significant increase in PAI-1 values in re-infarction patients (2.32-fold) than in myocardial infarction patients (1.54-fold), whereas both these values were significantly higher as compared with healthy individuals. In another study, PAI-1 concentrations have been found to be 2.5- and 4.6-fold higher in non-diabetic and diabetic AMI patients as compared to the control group on the first day from the myocardial infarction onset [22]. Increased PAI-1 levels have been associated with the occurrence of cardiogenic shock after AMI, with prognostic value for subsequent complications in these patients [23]. The acute release of PAI-1

Table 3. Correlation between the different parameters and blood coagulation markers

Parameter	TPA		PAI-1	
	R	P	R	P
Age	0.16	0.04*	0.09	0.17
Body mass index	0.04	0.65	0.09	0.18
Systolic blood pressure	-0.007	0.93	0.14	0.04*
Diastolic blood pressure	0.06	0.45	0.07	0.34

*Statistically significant.

over the first 24 hours of myocardial infarction has been associated with death and heart failure [24]. Sinkovic and Pogacar [25] measured the PAI-1 and troponin T levels within the first 48 h and then based on 30-day mortality and/or reinfarction concluded that simultaneous assessment of troponin T and PAI-1 would provide complementary prognostic information and enable clinicians to stratify risk more effectively among patients with AMI and unstable angina.

High plasma PAI-1 plays a considerable role in the higher incidence of unsuccessful reperfusion and impaired left ventricular function after thrombolytic therapy [26]. Genser et al [27] have suggested that a marked PAI-1 increase after thrombolytic therapy for AMI could be a common, drug-independent antifibrinolytic rebound phenomenon in response to thrombolytic treatment. However, elevated pre-treatment PAI-1 activity in patients with AMI is the most significant independent risk factor of failed fibrinolysis with streptokinase therapy [28]. The level of PAI-1 is mainly influenced by obesity and insulin resistance whereas changes in life style such as weight reduction and physical activity can reduce plasma PAI-1 levels [29].

In conclusion, both TPA and PAI-1 levels are significantly higher in AMI patients whereas the risk group (dyslipidemia and hypertension) showed only non-significant increases. Both these markers were not correlated with BMI however TPA showed significant correlation with age and PAI-1 with systolic blood pressure (**Table 3**). The combination of these markers could provide a useful tool to assess the prognosis of AMI.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Haseeb A Khan, Department of Biochemistry, College of Science, King Saud University, Bld 5, Rm 1B20, P.O. Box 2455, Riyadh 11451, Saudi Arabia. E-mail: khan_haseeb@yahoo.com; haseeb@ksu.edu.sa

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