Original Article

Tissue plasminogen activator and plasminogen activator inhibitor-1 levels in patients with acute myocardial infarction and unstable angina

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Abstract

Objective: To compare levels of tissue plasminogen activator and plasminogen activator inhibitor-1 levels in patients with acute myocardial infarction and unstable angina in order to understand the use of high sensitivity C-reactive protein (hsCRP), coagulation and fibrinolysis markers for cardiovascular risk assessment.

Methods: The cross-sectional case-control study compared circulating concentrations of high sensitivity C reactive protein (hsCRP), fibrinogen, tissue-type plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) between patients of acute myocardial infarction (AMI) (n = 67), Unstable Angina Pectoris (UA) (n = 35) and healthy control subjects (n = 39) at the King Khalid University Hospital, Riyadh, Saudi Arabia, from June 2006 to August 2007.

Results: The patients had significantly higher hsCRP (1.06 ± 0.11 vs 0.52 ± 0.14, p<0.01), fibrinogen (426.21 ± 24.09 vs 329.32 ± 13.93, p<0.05), PAI-1 (44.02 ± 6.05 vs19.35 ± 3.94, p<0.01) and tPA (12.31 ± 1.16 vs 9.49 ± 0.86, p<0.05) compared to the controls. Fibrinogen (329.32 ± 13.93) and PAI-1 (19.35 ± 3.94) were higher in both angina and infarction groups compared to the healthy subjects (p<0.01). Between the two categories of patients the difference between Fibrinogen (449.60 ± 52.98 vs 419.46 ± 23.42) and PAI-1 (52.00 ± 17.34 vs 43.19 ± 6.10) levels were non-significant. Also, the difference in tPA levels between the controls and angina patients was non-significant (9.49 ± 0.86 vs 9.91 ± 1.24 p>0.05). It was higher in infarction patients (14.79 ± 3.14) compared to angina patients and the controls, (p<0.05). Compared to the controls, hsCRP levels were significantly higher in both the patient groups (0.52 ± 0.14, 1.05 ± 0.28, 1.40 ± 0.20, p<0.01). Moreover, they were significantly higher in infarction patients than those suffering from angina (p<0.05).

Conclusions: CAD patients had a procoagulant state and presented with higher levels of hsCRP compared to the healthy individuals. Moreover, there were significant differences in coagulation markers and hsCRP between angina and infarction patients.

Keywords: Unstable angina, Acute myocardial infarction, hsCRP, Coronary artery disease, tissue-type plasminogen activator, plasminogen activator inhibitor-1. (JPMA 62: 681; 2012)

Introduction

The overall prevalence of coronary artery disease (CAD) in the Kingdom of Saudi Arabia has been reported to be 5.5%.[1] Three pathophysiologic processes are involved in Acute Coronary syndrome, including a dyslipidemic, inflammatory and prothrombotic state.[2] Besides, traditional risk factors such as hypercholesterolaemia, hypertension, and smoking explain only part of the individual risk of cardiovascular events.[3] Plaque rupture and thrombus formation have been identified as the most common mechanistic events in myocardial infarction.[4] In this respect, studies have focused particularly on various components of the haemostatic system[5] and various measures of coagulation and fibrinolytic activity have been proposed as possible predictors of CAD. Elevated concentrations of fibrinogen, the conversion of which leads to the formation of the fibrin meshwork of a thrombus, have been associated with the development of cardiovascular disease (CVD) in healthy individuals.[6]

Initially, several cross-sectional studies of patients with angina pectoris or previous myocardial infarction suggested that such patients had an impaired fibrinolytic system, and subsequently a few prospective cohort studies supported the notion that fibrinolytic factors such as plasminogen activator inhibitor (PAI-1) and tissue-type plasminogen activator (tPA) mass concentrations were predictors of future events in this type of patients.[7]

More than 20 large prospective trials have shown that the inflammatory biomarker high-sensitivity C-

reactive protein (hsCRP) is an independent predictor of future cardiovascular events and that it predicts the risk of hypertension and diabetes.[8] Regarding markers of homeostasis, most prior investigations have compared cases of CAD with healthy controls or compared AMI patients with stable exertional angina.[9-11]

There is scant data on an integrated approach comparing hsCRP and markers of hoemostasis between patients with AMI and unstable angina. The present study was, therefore, carried out to test the hypothesis that variations in the concentrations of CRP, fibrinogen, tPA, and PAI-1 would distinguish patients who developed an AMI from patients who had unstable angina as their initial clinical manifestation of CAD.

Patients and Methods

This cross-sectional study was conducted at the Departments of Physiology, Cardiology and Emergency Medicine of the College of Medicine and King Khalid University Hospital, Riyadh, Saudi Arabia. A total of 145 subjects were initially recruited for the study and 141 patients were included in the final analysis based on the selection criteria. The project was conducted from June 2006 to August 2007 and was funded by the College of Medicine Research Centre (CMRC). The study protocol was approved by the CMRC Research Ethics Committee. The individuals selected were informed about the objectives and procedures of the study and those who agreed to participate signed the consent form, which was typed both in English and Arabic languages. A clinical record of each individual comprising personal data, demographic data, family history and result of the coronary angiography was filled in a predesigned proforma. Patients were divided into two groups based on the standardised criteria into acute myocardial infarction (AMI) (n = 67), and Unstable Angina Pectoris (UA) (n = 35). Inclusion criteria were adult patients of either gender with AMI and unstable angina. The diagnosis of myocardial infarction required the presence of at least 2 of these criteria (1) A history of characteristic prolonged ($\geq$ 30 min) pain or discomfort; (2) Creatine kinase (CK) elevation exceeding twice the upper limit of normal (or CK-MB $\geq$ 50% of total CK); (3) Presence of new Q waves or new abnormal ST-T features.[12] Unstable angina was defined as pain at rest with at least 2 episodes during the previous 48 hours, at least one of which lasted $\geq$ 20 min, or ST-segment deviations that were diagnostic of myocardial ischaemia during angina attack, with no elevation of MB fraction of CK or lactate dehydrogenase on admission to hospital.[13] Furthermore, individuals with concomitant systemic diseases (thyroid disorders, acute infections, stroke, diabetic ketoacidosis, non-ketotic hyperosmolar coma rheumatic diseases, chronic liver diseases, renal disorders, cancer and sepsis) and subjects who were critically ill or with ongoing or recent (< 1 month) infectious diseases as well as patients with surgical procedure in the preceding 3 months were excluded. Control group included age and gender matched healthy (n = 39) individuals who were free of clinical manifestations of coronary, peripheral or cerebral artery disease by history, physical examination and electrocardiographic findings. Blood samples were collected in plain and Na citrate added tubes when the patients came to the emergency room with chest pain with a duration of less than 12 hours between the onset of the symptoms and the arrival at ER. Plasma was separated and stored at - 70°C. For lipid assays fasting venous blood samples were collected on the second occasion after the patients were metabolically stable.

hsCRP was measured using a high-sensitivity latex-enhanced turbidimetric assay (Quantex CRP ultra sensitive kits supplied by BIOKIT, S.A., Barcelona, Spain) and the autoanalyser Hitachi 911, (ROCHE diagnostics, Indianapolis, Indiana, USA). The kit had a working range from 0.10 to 20.0 mg/L. CRP Reagent is a suspension of polystyrene latex particles of uniform size coated with rabbit IgG antihuman CRP. Results were expressed in ug/dl based on international reference material for measurement of 14 human serum proteins (CRM 470). One important attribute of C-reactive protein is its stability over time and the availability of automated assay methods. Besides, the new assays are very sensitive and provide measurement of C-reactive protein at levels substantially below those measured by other traditional methods.

The plasma concentration of fibrinogen, tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) were measured by standard sandwich ELISA technique using commercial kits (Diagnostica STAGO, R.C.S. Nanterre, France).

Total cholesterol (TC), Triglycerides (TG), Low-density Lipoprotein (LDL) and High-density Lipoprotein (HDL) were analysed by standard enzymatic colorimetric method, using the auto-analyser Dimension (Dade-Behring, USA).

The data was analysed by computer software programme Statistical Package for Social Sciences (SPSS version 10, Chicago). Descriptive characteristics and lipid profile of the study patients were calculated as Mean ± SD (Standard Deviation) for continuous variables and as percentages for categorical variables. To assess differences in age, blood pressure, TC, LDL, HDL, TG and BMI the analysis of variance and Student's t test were utilised. Coagulation parameters and hsCRP, because of their

extreme skewness, were analysed by non-parametric Mann-Whitney U test and Wilcoxon (Kruskal-Wallis) test when comparing two or three groups, respectively. A p value of < 0.05 was considered to be statistically significant.

Results

Clinical characteristics, fibrinogen, PAI-1, tPA and hsCRP levels between the controls and CAD patients were noted (Table-1). The CAD group had significantly higher BMI, SBP and DBP compared to the healthy subjects (p<0.01, p<0.05, p<0.05 respectively). Fibrinogen (p<0.05), PAI-1 (p<0.01), tPA (p<0.05) and hsCRP (p<0.01) levels were also significantly higher in CAD patients.

The CAD patients were then divided into UA and AMI groups and their characteristics were compared with the controls (Table-2). Both UA and AMI groups had significantly higher BMI compared to the controls (28.98 ± 1.39, 28.10 ± 0.85, 25.68 ± 0.92, p<0.05 respectively). However, difference was non-significant among UA and AMI patients for blood pressure values (p>0.05). Fibrinogen and PAI-1 levels were higher in both the groups compared to the healthy subjects (p<0.01). Comparison of fibrinogen and PAI-1 between AMI and UA patients showed non-significant difference. The difference in tPA levels between the controls and UA group was non-significant (9.49 ± 0.86 vs 9.91 ± 1.24 p>0.05). However, it was significantly higher in the AMI group (14.79 ± 3.14) compared to the UA and control subjects, p<0.05). hsCRP levels were significantly higher in both CAD groups (0.52 ± 0.0.14, 1.05 ±0.28, 1.40 ± 0.20, p<0.01) compared to the controls. Comparison of hsCRP levels between UA and AMI group was also significant and values were higher in the AMI group (p<0.05).

In lipid profile, TC, TG and LDL were significantly higher and HDL was significantly lower in both CAD groups than the controls (p<0.01). The difference was insignificant for TC, TG, LDL and HDL between UA and AMI groups (Table-3).

Table-1: Clinical and biological data of control and CAD patients.

	Control	Patients with CAD
M/F	39 (23/16)	102 (69/33)
Age	51.09 ± 2.49	54.83 ± 1.21
BMI	25.68 ± 1.26	28.27± 0.68**
SBP mmHg	125.27 ± 1.72	131.97 ± 1.91*
DBP mmHg	71.35 ± 1.31	76.87 ± 1.34*
Fibrinogen	329.32 ± 13.93	426.21 ± 24.09*
PAI-1	19.35 ± 3.94	44.02 ± 6.05**
tPA	9.49 ± 0.86	12.31 ± 1.16*
hsCRP	0.52 ± 0.14	1.06 ± 0.11**

Data is expressed as Mean ± SD
* P < 0.05 vs control
** P < 0.01 vs control
SBP: Systolic blood pressure; DBP: Diastolic Blood pressure; PAI-1: Plasminegen Activator Inhibitor-1; tPA: Tissue type Plasmiuagen Activator; hsCRP: High-senstivity C-Reactive Protein; BMI: Body Mass Index.

Table-2: Clinical and biological data of the three groups.

	Control	Unstable Angina	AMI
M/F	39 (23/16)	35 (21/14)	67 (38/29)
Age	51.09 ± 1.54	51.68 ± 1.90	55.80 ± 1.70
BMI	25.68 ± 0.92	28.98 ± 1.39	28.10 ± 0.85
SBP mmHg	125.27 ± 3.25	132.97 ± 3.57	131.44 ± 2.56
DBP mmHg	71.35 ± 2.13	77.70 ± 2.41	76.97 ± 1.81
Fibrinogen	329.32 ± 13.93	449.60 ± 52.98*	419.46 ± 23.42*
PAI-1	19.35 ± 3.94	52.00 ± 17.34**	43.19 ± 6.10**
tPA	9.49 ± 0.86	9.91 ± 1.24	14.79 ± 3.14#
hsCRP	0.52 ± 0.14	1.05 ± 0.28**	1.40 ± 0.20**#

Data is expressed as Mean ± SD
* P < 0.05 vs control
# P < 0.05 vs unstable angina
** P < 0.01 vs control
SBP: Systolic blood pressure; DBP: Diastolic Blood pressure; PAI-1: Plasminegen Activator Inhibitor-1; tPA: Tissue type Plasmiuagen Activator; hsCRP: High-senstivity C-Reactive Protein; BMI: Body Mass Index. AMI: Acute Myocardial Infarction.

Table-3: Lipid profile of the study participants.

	Control	Unstable Angina	AMI
M/F	39 (23/16)	35 (21/14)	67 (38/29)
TC	4.13 ± 0.28	4.50 ± 0.31*	4.42 ± 0.18*
TG	1.08 ± 0.20	1.78 ± 0.18*	1.98 ± 0.15*
LDL	2.32 ± 0.16	2.73 ± 0.27*	2.79 ± 0.18*
HDL	1.13 ± 0.11	0.65 ± 0.07*	0.76 ± 0.03*

Data is expressed as Mean ± SD. AMI: Acute Myocardial Infarction.
* P < 0.01 vs control
TC: Total cholesterol; TG: Triglycerides' LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein.

Discussion

Our study showed higher levels of hsCRP, fibrinogen, tPA and PAI-1 in CAD patients compared to the healthy controls. There were non-significant differences between unstable angina patients and AMI patients when we compared fibrinogen and PAI-1 levels. However, tPA levels were significantly higher in the AMI group compared to UA group. While hsCRP levels were significantly higher in both CAD groups compared to the controls, suggesting an ongoing pro- inflammatory state in these patients. Additionally, hsCRP levels were significantly higher in AMI compared to UA.

Plasminogen activator inhibitor (PAI-1) is a serine protease inhibitor that regulates fibrinolysis by rapid inhibition of tissue-type and urokinase-type plasminogen activators. PAI-1 elevations have been significantly associated with CAD or CVD in numerous studies and

were an independent risk factor for re-infarction in individuals whose first AMI occurred before age of 45.[14,15]

Tissue-type plasminogen activator (tPA) acts upon plasminogen to form plasmin, an enzyme that lyses fibrin in thrombi. Although both elevation and depression of circulating tPA levels have been associated with CAD, the prevailing observation has been that increasing tPA levels are an independent risk factor for CAD.[16,17]

Fibrinogen, tPA, and PAI-1 have been reported to be strong independent risk factors for both initial and recurrent CVD, with each SD increase associated with a 20% to 30% independent increment in risk.[18] A recent meta-analysis of 31 prospective studies reported that every 1 g/L increase in fibrinogen increased the hazard of developing CVD by a factor of 1.8.[19]

The mean PAI activity in CAD patients was significantly higher than in non-CAD patients. Moreover, tPA antigen was also elevated in CAD patients compared to the non-CAD group.[9] However, in our study although PAI-1 level was higher in both UA and AMI groups, but tPA levels were higher only in the AMI group but not in the UA group compared to the controls. The probable reason is that we studied separately the two CAD groups, while they were combined in the above mentioned study.

At a median of 15 weeks after presentation, patients with AMI had slightly higher d-dimer concentrations than patients with stable angina ($p = 0.057$), but were not significantly different in other markers. By contrast, fibrinogen, d-dimer, and tissue-type plasminogen activator were significantly higher ($p < 0.001$) and PAI-1 lower in patients with CAD than in the controls. After statistical adjustment for clinical covariates, cardiac risk factors, medications, and other confounders, fibrinogen, d-dimer, and PAI-1 remained significantly associated with CAD. Selected plasma markers of coagulation and fibrinolysis did not distinguish patients presenting with AMI from those with stable exertional angina.[10] Our results are in line with these reports except for tPA levels which were significantly higher in the AMI group compared to the UA. The difference in our study is in the grouping of patients. We compared AMI with UA patients.

PAI-1 levels were significantly increased in diabetic CAD patients (5.26 ± 1.96 ng/ml), but not in the stable angina patients without diabetes (2.97 ± 1.44 ng/ml). Immunologically-reactive tPA released after exercise was higher in the 16 CAD patients without diabetes than in the controls. Our data could indicate that in stable angina without diabetes there is no chronic latent activation of the clotting system, with no impairment of fibrinolytic activity. On the other hand, the presence of diabetes mellitus seems to influence the fibrinolytic capacity in CAD, particularly increasing PAI levels.[11]

However, the question whether increased tPA antigen levels are the result of prevalent endothelial dysfunction or represent a net activation of endogenous fibrinolysis in response to underlying atherosclerosis, increased inhibition of fibrinolysis, or delayed turnover needs direct experimental testing and would not be answered in an epidemioloigally designed study. At present it seems difficult to say whether these changes in the haemostaic parameters is the cause of atherosclerotic mechanism or an effect of this complicated process. It is prudent to have further studies done in this regard. We observed that hsCRP levels were significantly higher in both CAD groups who had a prothrombotic state. This is important because impaired endogenous fibrinolytic capacity might be an important contributor to the increased coronary event rate associated with elevated CRP levels.[20,21]

A long-term predictive value of elevated hsCRP levels has been found in patients with documented CAD and angina[22] and in individuals with multiple risk factors.[23] Our study also showed that hsCRP levels were significantly higher in CAD patients compared to the healthy individuals. The difference was also significant when we compared UA patients with AMI. This is an interesting observation because high levels of hsCRP not only predict first myocardial infarction, but also recurrent events.[24,25] The limitation of our study is its small sample size and cross-sectional design. Large-scale prospective studies are required to determine the exact predictive value of these markers.

Conclusion

CAD patients have a prothrombotic state and also present with higher levels of hsCRP compared to the healthy individuals. Moreover, there are also significant differences in haemostatic markers and hsCRP between UA and AMI patients, with levels of tPA and CRP being significantly higher in AMI than UA. The difference in PAI-1 levels was non-significant. Long-term prospective studies are needed to determine the predictive values of these markers for first and recurrent cardiovascular events.

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References

1. Al-Nozha MM, Arafah MR, Al-Mazrou YY, Al-Maatouq MA, Khan NB, Khalil MZ, et al. Coronary artery disease in Saudia Arabia. Saudi Med J 2004; 25: 1165-71.
2. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 2001; 104: 365-72.

3. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364: 937-52.
4. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995; 92: 657-71.
5. Hamsten A. The hemostatic system and coronary heart disease. Thromb Res 1993; 70: 1-38.
6. Francis RB, Jr., Kawanishi D, Baruch T, Mahrer P, Rahimtoola S, Feinstein DI. Impaired fibrinolysis in coronary artery disease. Am Heart J 1988; 115: 776-80.
7. Lijnen HR, Collen D. Impaired Fibrinolysis and the Risk for Coronary Heart Disease. Circulation 1996; 94: 2052-4.
8. Ridker PM. C-Reactive Protein and the prediction of cardiovascular events among those at intermediate risk: Moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol 2007; 49: 2129-38.
9. Huber K, Resch I, Stefenelli T, Lang I, Probst P, Kaindl F, et al. Plasminogen activator inhibitor-1 levels in patients with chronic angina pectoris with or without angiographic evidence of coronary sclerosis. Thromb Haemost 1990; 63: 336-9.
10. Itakura H, Sobel BE, Boothroyd D, Leung LL, Iribarren C, Go AS, et al. Do plasma biomarkers of coagulation and fibrinolysis differ between patients who have experienced an acute myocardial infarction versus stable exertional angina? Am Heart J 2007; 154: 1059-64.
11. Davi G, Violi F, Catalano I, Giammarresi C, Putignano E, Nicolosi G, et al. Increased plasminogen activator inhibitor antigen levels in diabetic patients with stable angina. Blood Coagul Fibrinolysis 1991; 2: 41.
12. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000; 36: 959-69.
13. Braunwald E. Unstable angina. A classification. Circulation 1989; 80: 410-4.
14. Kohler HP, Grant PJ. Plasminogen-Activator Inhibitor Type 1 and Coronary Artery Disease. N Engl J Med 2000; 342: 1792-801.
15. Hamsten A, Walldius G, Szamosi A, Blombäck M, Faire U, Dahlén G, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 1987; 330: 3-9.
16. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998; 98: 2241-7.
17. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 2004; 109 (25 Suppl 1): IV6-19.
18. Kannel WB. Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. Lipids 2005; 40: 1215-20.
19. Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005; 294: 1799-809.
20. Devaraj S, Singh U, Jialal I. The evolving role of C-reactive protein in atherothrombosis. Clin Chem 2009; 55: 229-38.
21. Singh U, Devaraj S, Jialal I. C-reactive protein decreases tissue plasminogen activator activity in human aortic endothelial cells: evidence that C-reactive protein is a procoagulant. Arterioscler Thromb Vasc Biol 2005; 25: 2216-21.
22. Zebrack JS, Anderson JL, Maycock CA, Horne BD, Bair TL, Muhlestein JB. Usefulness of high-sensitivity C-reactive protein in predicting long-term risk of death or acute myocardial infarction in patients with unstable or stable angina pectoris or acute myocardial infarction. Am J Cardiol 2002; 89: 145-9.
23. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996; 144: 537-47.
24. Biasucci LM, Liuzzo G, Grillo RL, Caligiuri G, Rebuzzi AG, Buffon A, et al. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation 1999; 99: 855-60.
25. Correia LC, Lima JC, Rocha MS, D'Oliveira Junior A, Pericles Esteves J. Does high-sensitivity C-reactive protein add prognostic value to the TIMI-Risk Score in individuals with non -ST elevation acute coronary syndromes? Clinica Chimica Acta 2007; 375: 124-8.