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Regular Article

Prothrombin fragment 1 + 2 is associated with intima media thickness of the carotid artery in patients with myocardial infarction

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ABSTRACT

Background and aim: Previous studies have shown that a decreased fibrinolytic activity or a hyperactivated coagulation system increase the risk of myocardial infarction. The aim of this study was to investigate the relationship between the presence of atherosclerotic lesions and coagulation and fibrinolysis factors and high sensitivity CRP (hs-CRP) in patients with myocardial infarction.
Methods: In a cross-sectional study, 123 patients, aged 31-80 years, with a history of previous myocardial infarction were examined with B-mode ultrasound of the common carotid artery. Blood samples were collected for measurements of fibrinogen, plasminogen activator inhibitor-1 activity (PAI-1), von Willebrand factor (vWF), prothrombin fragment 1 + 2 and hs-CRP.
Results: Prothrombin fragment 1 + 2 and hs-CRP were significantly ($p<0.05$) and positively associated with common carotid artery intima media thickness (IMT). PAI-1 was significantly ($p<0.05$) and negatively associated with IMT. IMT was also significantly associated with systolic blood pressure and age. When IMT was used as an dependent variable and systolic blood pressure, age, PAI-1 and prothrombin fragment 1 + 2 were used as independent variables in the multiple stepwise regression analysis a significant and independent relationship was observed between IMT and systolic blood pressure and age ($p<0.05$).
Conclusion: The levels of prothrombin fragment 1 + 2 and hs-CRP are associated with intima media thickness in the common carotid artery in patients with previous history of myocardial infarction.

Introduction

Thrombosis provoked by a rupture of an atherosclerotic plaque is an important risk factor for ischemic cardiovascular event [1,2]. Prospective studies have reported increased risk of cardiovascular events in patients with coagulation activation or impaired fibrinolytic function [3–6]. Cross sectional studies have shown that levels of prothrombin fragment 1 + 2 and thrombin-antithrombin complex are associated with cardiovascular disease [7–9]. Plasminogen activator inhibitor-1 (PAI-1), a fast acting inhibitor of plasminogen activation, is produced by the vascular endothelium, but is also present in platelets and is considered to be an important regulator of fibrinolysis [10]. High levels of PAI-1 have been reported in patients with acute coronary syndromes [11–13]. Common carotid artery intima-media thickness (IMT) is an indicator of a subclinical atherosclerosis which, in previous studies, has been shown to predict the incidence of cardiovascular disease [14–16]. A few studies have reported conflicting results concerning PAI-1 and failed to show any association to cardiovascular disease [4,17,18].

The aim of this study was to investigate the relationship between IMT with haemostatic factors and hs-CRP in patients with myocardial infarction.

Methods

Subjects

One hundred and twenty-three patients, men (76%) and women (24%) aged between 32-73 years with a history of acute myocardial infarction were included. The patients were recruited from the department of Cardiology at Karolinska University Hospital Huddinge, Sweden. A majority of the patients were recruited 2-3 days after the myocardial infarction. The examinations of this study were performed 1-12 months after the index event. Ninety percent of the patients in this study were examined within 3 months after the myocardial infarction. This time interval was chosen to be sure that patients were examined when they were clinically in stable condition. The exclusion criteria were previously well-known and established diabetes mellitus and oral anticoagulation therapy. All examinations including ultrasound and blood sampling were done at the same time. All subjects gave informed consent after written and oral information. The ethics

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committee of the Karolinska Institute at Karolinska University Hospital Huddinge approved the study.

Acute myocardial infarction was defined using the criteria of the European Society of Cardiology and the American College of Cardiology [19]. Thus, patients were diagnosed as having an acute myocardial infarction if they had two values of serum troponin T greater than 0.05 g/L together with either typical symptoms or new Q-waves in at least two of the twelve standard electrocardiographic leads, or electrocardiogram changes indicating acute ischemia (ST-elevation, ST-depression, or T-wave inversion).

Measurements

Venous blood was drawn after an overnight fast and 5 min of supine rest for determination of serum levels of cholesterol and triglycerides using established methods. Plasma glucose concentrations at 0, 30, 60, 90 and 120 min following ingestion of 75 g glucose were analysed, using glucose oxidase technique on a Hitachi 917 system. An immunophelometric assay, N High Sensitivity CRP (DadeBehring), was used to estimate CRP levels [20].Venous blood was also drawn with minimal cuff pressure for analysing the hemostatic markers including fibrinogen, von Willebrand factor, Plasminogen activator inhibitor 1 (PAI-1) and prothrombin fragment 1 + 2. The citrated blood samples were centrifuged within 30 minutes, and plasma was immediately frozen in aliquots and stored at -70 °C until analysis. PAI- 1 activity was determined by using the Spectrolyze PAI-1 kit (Biopool AB) on the citrated plasma samples that had been stored at -70 °C. Von Willebrand factor antigen was measured by a commercially available ELISA method (Liatest® vWF kit, Stago provided by Triolab AB). Fibrinogen levels in plasma were determined by conventional techniques (Sysmex CA-1500). Level of prothrombin fragment 1 + 2 was assessed by using enzyme-linked immunosorbent assay kits, Enzygnost F1 + 2 (Behring). Patients were asked to bring 2 overnight (12-hour) urine samples for determination of urinary albumin excretion using an immunochemistry nephelometry method. The mean of 2 measurements was used in the statistical analysis. Microalbuminuria was defined as excretion of 20 to 200 μg albumin/min. Resting blood pressure was measured in the right arm after about 10 min supine rest. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Smoking status was assessed by a questionnaire.

Table 1
Baseline characteristics of the patients (n = 123).

Age (yrs)	61 ± 11
Male n (%)	94 (76)
Female n (%)	29 (24)
SBP (mm Hg)	138 ± 21
DBP (mm Hg)	80 ± 9
Total-Cholesterol (mmol/l)	4.7 ± 0.9
Triglycerides (mmol/l)	1.6 ± 1.0
BMI (kg/m^2)	26.5 ± 3.5
FPG (mmol/l)	5.4 ± 1.3
2-h PG (mmol/l)	7.7 ± 3.0
UAE (mg/l)	17.9 ± 70.4
vWF (IU/ml)	1.4 ± 0.4
PAI-1 (IU/ml)	19.5 ± 19.3
Fibrinogen (g/l)	3.3 ± 0.7
Prothrombin (nmol/l)	0.9 ± 0.7
Hs-CRP, mg/l	3.4 ± 5.6
Prevalence of DM n (%)	13 (11)
Prevalence of IGT n (%)	32 (26)
Prevalence of NPG n (%)	78 (63)
Smoking habits	
Current, n (%)	20 (16)
Previous, n (%)	71 (58)
Never, n (%)	28 (23)
Snuff, n (%)	4 (3)
Current treatment	
Aspirin	99 (99)
ß-blockers	111 (90)
Statins	111 (90)
ACE-inhibitors	31 (25)

SBP, systolic blood pressure. DBP, diastolic blood pressure. FPG, fasting plasma glucose. 2-h PG, 2-h plasma glucose. UAE, urinary albumin excretion. PAI-1, Plasminogen activator inhibitor 1 activity, vWF, von Willebrand factor. Hs-CRP, high sensitivity C-reactive protein. IGT, impaired glucose tolerance. NPG, normal plasma glucose. ACE, angiotensin- convering enzyme.

Table 2
Spearmans rank order correlation between intima media thickness of common carotid artery and study variables in focus.

Variable	CCA IM Thickness, Mean of both side
Age (year)	0,54*
BMI (kg/m^2)	-0,17
SBP (mm Hg)	0,41*
DBP (mm Hg)	0,01
Total-Cholesterol (mmol/l)	0,06
Triglycerides (mmol/l)	0,04
FPG (mmol/l)	0,13
2-h PG (mmol/l)	0,21*
UAE (mg/l)	0,15
vWF (IU/ml)	0,16
PAI-1 (IU/ml)	-0,21*
Fibrinogen (g/l)	0,16
Prothrombin (nmol/l)	0,37*
Hs-CRP, mg/l	0,22*

* Marked correlations are significant at $P<0.05$. UAE, urinary albumin excretion. PAI-1, Plasminogen activator inhibitor 1 activity, vWF, von Willebrand factor. FPG, fasting plasma glucose. 2-h PG, 2-h plasma glucose. SBP, systolic blood pressure. DBP, diastolic blood pressure.

Carotid B-mode ultrasonography

The right and left carotid arteries were examined with a duplex scanner (Aspen, Acuson, Mountain View, Ca, USA) by using a 7 MHz linear array transducer. The same trained sonographer performed all scannings. The far wall of the common carotid artery (CCA), 0.5 to 1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of the IMT and lumen diameter. The intima-media thickness was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The examinations were video taped for subsequent analyses by a computer system [21] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 10 mm long section of CCA in late diastole, defined by a simultaneous electrocardiographic recording. The mean values of the IMT and lumen diameter within the 10 mm long section were calculated. When a plaque was observed in the region of the CCA measurements, the intima-media thickness was not measured. Carotid plaque was defined as a localised intima-media thickening of greater than 1 mm and at least a 100% increase in thickness compared with adjacent wall segments. The differences between repeated measurements of IMT and lumen diameter, by using the automated analysing system, were 3.2% and 0.6% (coefficient of variation), respectively (with an IMT of 0.48 to 1.04 mm and a lumen diameter of 4.34 to 7.91 mm). The ultrasonographic methods used have been described in detail previously [22,23].

Statistical analysis

Results are reported as mean ± SD except where indicated otherwise. All data analyses were done using Statistica for Windows software version 7.0. Mann-Whitney U test, X^2 test, Spearman's correlation of coefficient and forward stepwise regression analysis were performed. Since coagulations factors were not normally distributed a log