

ACTIVATED PROTEIN C RATIO, PLASMA TISSUE FACTOR ACTIVITY AND ACTIVATED FACTOR VII IN CHINESE PATIENTS WITH CORONARY HEART DISEASE

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Abstract

Objective: There is close relationship between abnormal coagulation system and progression of coronary heart disease (CHD), our purpose is to evaluate the contribution of hematologic factors and some other risk factors to the development of coronary heart disease (CHD) in Chinese population.

Methods: 56 patients with CHD at admission and 54 controls were enrolled. Plasma levels of protein C, free protein S, total protein S, thrombomodulin, activated factor VII (FVIIa), factor VII:Ag, P-selectin, tissue-type plasminogen activator, plasminogen activator inhibitor-1 were measured by enzyme linked immunosorbent assay, activity of tissue factor (aTF) by chromogenic activity assay, and activated protein C (APC) ratio, prothrombin time, aPTT, fibrinogen, D-dimer and thrombin time by full-automated coagulation analyzer.

Results: Compared with controls, plasma level of thrombomodulin, FVIIa, factor VII:Ag and aTF were raised in CHD group ($p < 0.05$, 0.001, 0.05, and 0.05, respectively). The average APC ratio in CHD group was lower than that in controls ($p < 0.001$). The result of binary logistic regression analysis showed that activated factor VII (OR2.680, 95%CI1.539-4.665) and tissue factor activity (OR1.019, 95%CI1.004-1.035) were risk factors and high density lipoprotein (OR0.008, 95%CI0-0.478) and activated protein C ratio (OR0.001, 95%CI0-0.011) were protective factors for CHD.

Conclusions: Low activated protein C ratio, elevated tissue factor activity and increased activated factor VII in plasma may contribute to development of coronary heart disease.

Key words: Coagulation; Factor VII; Fibrinolysis; Coronary Heart Disease; Tissue Factor; Thrombosis

Abbreviations: TC, total cholesterol; TG, triglycerides; HDL, High density Lipoprotein; LDL, Low density Lipoprotein; FBG, Free blood glucose; PC, protein C; FPS, free protein S; TPS, total protein S; TM, thrombomodulin; FVIIa, activated factor VII; tPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; APC ratio, activated protein C ratio;

APC resistance, activated protein C resistance; aTF, tissue factor activity; Fbg, fibrinogen; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time.

INTRODUCTION

Coronary atherosclerosis results from multifactorial progressive vascular alterations that lead to the development of plaques within the coronary arteries (Jawed Fareed et al., 1998). There is close relationship between abnormal coagulation fibrinolytic system and development as well as progression of coronary heart disease. Thrombogenesis is the final process whereby the exposed tissue factor (TF) triggers the activation of coagulation and the freshly formed clot fills the coronary artery lumen. During pre-infarction phases, a hypercoagulable state caused by vascular distress, fibrinolytic deficit, procoagulant behavior of various mediators, cytokines, and adhesion molecules may be detectable (Jawed Fareed et al., 1998). Use of laboratory assays of hemostatic activation may represent an approach to identify high-risk patients in whom several of these indicators can be used to guide therapy (Amiral J and Fareed J et al., 1996). During the past few years, several methods to evaluate various tests for thrombogenesis have been described (Broze G and Miletich JP, 1987; Broze G et al., 1988; Bauer KA and Rosenberg RD, 1987; Messmore HL, Walenga JM and Fareed J, 1984; Aurousseau MH, Amiral J and Boffa MC, 1991; Stephens CJM, 1991; Donner MG et al., 1998; Kohler HP et al., 1998). Various analytes that are found in increased circulating amounts during the activation or inhibition of the coagulation process include tissue factor, tissue factor pathway inhibitor, TAT complex, fibrinogen degradation products, D-dimer, platelet factor 4, thromboxane B2, endothelin, vWF, anti-phospholipid antibody and so on.

The aim of the present study is to test the plasma levels of anticoagulation factors, including protein C, free protein S, total protein S and activated protein C ratio, coagulation factors, including activated FVII, FVII: Ag and tissue factor activity, fibrinolytic factors, including tPA and PAI-1, P-selectin and thrombomodulin, and to compare the differences of the factors between Chi-

nese CHD patients and controls so as to find out significant hematologic factors, which may promote or delay the development and progression of CHD.

MATERIALS AND METHODS

SUBJECTS

56 Chinese patients with coronary heart disease (CHD) admitted to department of cardiology in Wuhan Union Hospital from December 2005 to April 2006 were proved by percutaneous coronary arteriography according to the diagnostic criteria of World Health Organization for coronary heart disease. There were 26 patients with stable angina (SA) and 30 patients with acute coronary syndromes (ACS). 54 controls excluded thromboembolic disease, diabetes mellitus, hypertension, malignancy, acute or chronic liver and kidney disease, connective tissue disease, surgery or trauma within 4 weeks before admitted were drawn from attenders in the same hospital during the same period. Both groups were matched for age, sex and sampling time. All of them had not been treated with any anticoagulants or fibrinolytics before blood was collected. Clinical and demographic details of the subjects were listed in Table 1. All patients gave written informed consent for the investigation.

DATA COLLECTION AND POTENTIAL RISK FACTOR DEFINITION

Information about demographic characteristics and risk factors was collected in a case report form (CRF). Hypertension was defined as a history of receiving antihypertensive agents or systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg for at least 2 times in different day. Diabetes was considered when random blood glucose ≥ 11.1 mmol/L with relevant symptoms, FPG. ≥ 7.0 mmol/L, or 2HPG (2-hour plasma glucose) in OGTT ≥ 11.1 mmol/L. Status of smoking was coded as none or current users. APC resistance was defined when APC ratio was less than 2.0 in this study.

SAMPLE COLLECTION AND LABORATORY PROCEDURES

Blood sample for hemostatic assays were drawn into evacuated glass tubes containing 1/9 volume of 3.2% sodium citrate anticoagulant within 24 hours after admission. Within 30 minutes after the blood was collected, plasma was obtained by centrifugation at 2500g for 6 minutes at room temperature, placed in aliquots, and stored frozen at -80°C until analysis. In some instance, the samples were temporarily stored at -20°C for up to 1 weeks but then transferred to -80°C .

Table 1. Clinical and Demographic Details of the Subjects with CHD and Controls.

Variables	Controls (n=54)	Patients with CHD (n=56)	p value
Age (years)	62 \pm 9	59 \pm 12	0.158
Sex (%male)	61.1	69.6	>0.05
TC (mmol/L)	4.4 \pm 0.8	4.3 \pm 1.2	0.643
TG (mmol/L)	1.4 \pm 1.1	1.5 \pm 0.5	0.665
HDL (mmol/L)	1.3 \pm 0.3	1.1 \pm 0.3	0.001
LDL (mmol/L)	2.5 \pm 0.6	2.7 \pm 1.0	0.433
Hypertension (%)	0	53.6	< 0.001
FBG (mmol/L)	5.1 \pm 1.2	5.3 \pm 1.6	0.309
Smokers (% verbally admitting)	27.8	46.4	<0.05

Note: Data presented as mean and standard deviation or as a percentage.

Table 2. Details of the Methods.

Factors	Name of kits	Manufacturer
Protein C	ZYMUTEST PROTEIN C	HYPHEN BioMed, France
Free protein S	ZYMUTEST FREE PROTEIN S	HYPHEN BioMed, France
Total protein S	ZYMUTEST TOTAL PROTEIN S	HYPHEN BioMed, France
TM	IMUBIND [®] Thrombomodulin ELISA Kit	American Diagnostica inc., USA
FVIIa	IMUBIND [®] Factor VIIa ELISA Kit	American Diagnostica inc., USA
FVII	AssayMax Human Factor VII ELISA Kit	AssayPro LCC, USA
P-selectin	Human sP-selectin ELISA	GeneMay Inc., USA
tPA	AssayMax Human Tissue-Type Plasminogen Activator ELISA Kit	AssayPro LCC, USA
PAI-1	AssayMax Human Plasminogen Activator Inhibitor-1 ELISA Kit	AssayPro LCC, USA
aTF	AssaySense Human Tissue Factor Chromogenic Activity Assay Kit	AssayPro LCC, USA

The level of protein C (PC), free protein S (FPS), total protein S (TPS), thrombomodulin (TM), activated factor VII (FVIIa), factor VII: Ag (FVII), P-selectin, tissue-type plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) in plasma were measured by enzyme linked immunosorbent assay (ELISA). Tissue factor activity (aTF) was measured by chromogenic activity assay. The details of the methods were listed in Table 2. At the same time, we also measured activated protein C (APC) ratio and levels of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (Fbg), D-dimer (DD) and thrombin time (TT) by full-automated coagulation analyzer (Sysmex CA-7000, Japan). Levels of blood lipids were assayed by Department of Laboratory of Union Hospital.

STATISTICAL ANALYSIS

SPSS 13.0 was used for data statistical analysis. Differences between 2 groups were analyzed by the unpaired-samples t test for continuous variables with normal distribution, nonparametric test for continuous variables with skewness distribution, and χ^2 test for non-continuous variables. Binary logistic regression analysis was used to estimate risk factors of CHD. A p value of <0.01 or <0.05 was regarded as statistically significant.

RESULTS

Compared with controls, plasma level of TM, FVIIa, FVII: Ag and aTF were significantly raised ($p<0.05$,

Table 3. Levels of Hematologic Factors in Plasma between CHD and Controls.

Variable	Control group (n=54)	CHD group (n=56)
PC (%)	108.7±18.1	115.2±18.2
FPS (%)	88.5±17.8	86.6±21.0
TPS (%)	113.4±17.0	113.0±18.1
TM (ng/ml)*	3.66±0.68	4.02±1.03
FVIIa (ng/ml) ***	4.77±2.06	8.01±2.74
FVII Ag (g/ml)*	0.54±0.04	0.58±0.10
p-selectin (ng/ml)	164.1±58.9	178.6±161.0
tPA (ng/ml)	1.19±0.84	1.26±1.17
PAI-1 (ng/ml)	9.5 (5.1)	8.9 (6.4)
APC ratio ***	2.68±0.15	2.44±0.18
APC resistance (%)	0	1.79
aTF (pg/ml)*	163.1±38.3	205.0±58.2
Fbg (g/L)	3.829±0.900	3.921±0.608
PT (s)	11.2±1.08	12.3±1.21
APTT (s)	27.7±4.8	30.4±19.3
TT (s)	10.4±2.8	13.6±2.7
D-dimer (mg/L)	0.151 (0.195)	0.124 (0.108)

Note: Data presented as mean and standard deviation, Median (Inter-quartile range, IQR) or a percentage.

* $p<0.05$, *** $p<0.001$.

Table 4. Results of risk parameter estimates.

Parameter	OR	95%Confidence interval	p Value
aTF	1.019	1.004-1.035	0.016
FVIIa	2.680	1.539-4.665	<0.001
HDL	0.008	0-0.478	0.021
APC ratio	0.001	0-0.011	0.001

$p<0.001$, $p<0.05$ and $p<0.05$, respectively) in CHD group. The average APC ratio in CHD group was significantly lower than that in controls ($p<0.001$). Incidence rate of APC resistance in CHD patients was higher than that in controls, while there was no statistical significance. At the same time, we also found HDL concentrations in plasma were significantly higher in controls than those in CHD patients ($p<0.001$), number of smokers in CHD patients was significantly higher than that in controls ($p<0.05$). However, there were no significant differences in PC, FPS, P-selectin, tPA, PAI-1, PT, APTT, DD, TT and fibrinogen as well as LDL, TG and TC in plasma between CHD group and control group. See Table 1 and Table 3 for details of relevant information.

In addition, we also found that plasma FVIIa and aTF were risk factors and HDL and elevated APC ratio were protective factors for coronary heart disease by binary logistic regression analysis. Details see Table 4.

DISCUSSION

TF is a 30 kDa integral membrane glycoprotein (Jawed Fareed et al., 1998) and is the key initiator of the coagulation cascade. Previous study has proved that an aberrant expression of TF within the vasculature occurs in a variety of diseases, including atherosclerosis. TF expression in these diseases is associated with thrombotic events (Tilley R and Mackman N, 2006). In present study, we found that tissue factor activity in plasma was significantly higher in CHD patients than in controls, which may be caused by release of TF in plaque or other unclear reasons, suggesting that TF might contribute to development of hypercoagulable state and acute thrombogenesis is easy to occur in CHD population. The result of this study also showed that TF activity was a risk factor for CHD, however, we are not sure that it is elevated TF activity that contributes to progress of CHD, perhaps CHD affects activity of TF inversely, therefore, we can not conclude that TF activity is a risk factor for CHD, nevertheless, we have to think highly of the role of TF activity in CHD.

FVII is a liver coagulation protein which plays an important role in vivo clotting initiation and thrombus formation (Porreca E et al., 2002). The Prospective Cardiovascular Munster Study (PROCAM) (Heinrich J et al., 1994) showed that elevated FVII coagulant activity was a risk factor for ischemic heart disease, and the Northwick Park Heart Study found that VIIc was significantly related to CHD mortality (B. L. De Stavola and T. W. Meade, 2007). However, not all stud-

ies have confirmed FVII as an independent risk factor and the significance of FVII in coronary heart disease remains unclear (Junker R et al., 1997; Folsom AR et al., 1997; Tracy RP et al., 1999; Cooper JA et al., 2000). The study of Woodhouse PR et al. (Woodhouse PR et al. 1994) suggested that there was a possible link between an inflammatory state and hemostatic factors including FVII. Moreover, a growing body of evidence suggests that inflammatory processes play a role in the initiation and in the progression of atherosclerosis (Ridker PM, 1997). Elevated FVII correlated with inflammation may promote development of CHD. The results of our study showed that FVIIa and FVII:Ag plasma levels were significantly raised in CHD patients compared with controls and FVIIa was a risk factor for CHD. We presume it is impaired endothelium that promotes FVII protein synthesis and activation of FVII. Considering elevated aTF which was also an important factor of CHD in our study, we conclude that extrinsic coagulation pathway plays an important role in progression of CHD or atherothrombosis.

APC has an important role in regulating coagulation because it inactivates FVIIIa and FVa by proteolytic cleavage. Addition of APC to an in vitro plasma clotting test causes a prolongation of the time to clot formation as a result of the accelerated degradation of FVa and FVIIIa (Laffan MA, 1998). The ratio of time after and before addition of APC is represented as APC ratio. When the ratio is lower than a certain value, activated protein C resistance may exist, which means low response to APC in this type of test. In our study, APC ratio is significantly lower in CHD than that in controls, however, only 1.79% CHD patients (1 case) had activated protein C resistance, and there was no statistical difference in incidence of APC resistance. Sakata T et al. (Sakata T et al., 1996) found that venous and arterial patients showed significantly lower normalized APC-sensitivity ratio as compared with healthy control. Avellone G et al. (Avellone G et al., 2001) and Makris TK et al. (Makris TK et al., 2000) also arrived at similar results. However, Hayashi K et al. (Hayashi K et al., 1997) and Prohaska W et al. (Prohaska W et al., 1995) achieved opposite conclusion, i.e. there is no increased risk of developing coronary atheroma or consecutive myocardial infarction resulting from APC resistance. What causes the differences of these results? We guess the differences may owe to race of people, sample size and methods. Some studies have found that APC resistance plays little role in progress of arterial or venous diseases in Asians. Therefore, we conclude that we could regard low activated protein C ratio as a marker of CHD though there is no difference in APC resistance between patients and controls.

Thrombomodulin is a transmembrane protein expressed constitutively in endothelial cell. Its extracellular region binds to thrombin and resultantly acts on protein C. It is digested by proteases into diverse-sized fragments collectively called soluble thrombomodulin (sTM), which is considered a marker of vascular injury. Atherosclerosis Risk In Communities study showed a positive association between sTM and carotid atherosclerosis in Caucasians, but there was no

significant association between sTM and carotid atherosclerosis in African American (Salomaa V et al., 1999). Our study found that sTM was higher in CHD patients compared with controls, however, it was not a risk factor for CHD. The reason for these inconsistent conclusions is not entirely clear. Some authors indicated one possible explanation. The plasma sTM level in healthy subjects may be contributed primarily by the constitutive cleavage of endothelial TM, and thus may be correlated with the level of TM expression in endothelial cells, a high sTM level may therefore imply a high vasoprotective action and is therefore associated with a low CHD risk. A fraction of sTM detected in plasma of unhealthy subjects may be contributed by pathological cleavage induced by pro-inflammatory mediators. As pro-inflammatory mediators downregulate endothelial surface TM, an excessive cleavage further reduces the endothelial TM, thereby increasing the risk of atherosclerosis (Wu KK, 2003). The study of Olivot JM et al. (Olivot JM et al., 2004) confirmed the explanation.

Decreased HDL level is regarded as a risk factor of CHD. In this study, HDL concentrations in plasma were significantly higher in controls than those in CHD patients ($p < 0.001$), and elevated HDL concentrations was protective factor of coronary heart disease by binary logistic regression analysis. This finding implied that HDL played a key role against progression of CHD, and may be regarded as a good indicator for patients with CHD.

Number of smokers in CHD patients was significantly higher than that in controls in present study. Some studies have confirmed that incidence and case fatality of CHD in smokers is 2-6 times of those in non-smokers, which is in accordance with our conclusion.

In conclusion, results of present study showed that low APC ratio, elevated plasma aTF and FVIIa were indicators for CHD patients and they may contribute to development of coronary heart disease. Larger sample case-control study and prospective study should be done for disputed or other risk factors of CHD in the future.

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