Thrombophilia in Patients With Hypercholesterolemia

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To investigate a possible interrelationship between hypercholesterolemia and the coagulation and fibrinolytic system, the Cardiovascular Disease Risk Factor Two-Township Study in Taiwan was undertaken as a longitudinal prospective study focusing on the evolution of cardiovascular disease risk factors, with an emphasis on hemostatic factors. Hemostatic parameters measured in this study included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor VIIc, factor VIIIc, antithrombin III, and plasminogen. Subjects of both sexes with hypercholesterolemia (>6.2 mmol/L) also had significant elevations of diastolic blood pressure, plasma glucose, triglycerides, fibrinogen, and factor VIIc and reduced PT and APTT compared with subjects with lower cholesterol. The hypercholesterolemic women additionally had significant elevations of systolic blood pressure and factor VIIIc. Levels of the anticoagulant factors, antithrombin III and plasminogen, were also higher in both hypercholesterolemic men and women. In men, only factor VIIIc had no statistically significant elevation. In women, only PT showed no statistical difference. Established coronary risk factors such as fibrinogen and factor VIIc showed remarkable elevations in patients of both sexes. Using Pearson correlation and multiple regression, the most significant parameter related to cholesterol level was factor VIIc. The present results show that hemostatic abnormalities do exist in patients with hypercholesterolemia, and this thrombophilic phenomenon sheds further light on the study of higher cardiovascular mortality in these subjects.

THE THREE MAIN reversible cardiovascular disease risk factors—cigarette smoking, hypertension, and hypercholesterolemia—were able to predict only about one third of the coronary heart disease (CHD) events in early epidemiological studies.[1] Therefore, in recent years investigators have turned their attention to measurement of other factors that may promote atherosclerosis, thrombosis, or ischemia.[2,3] Such factors include parameters related to vascular endothelial function, platelets, coagulation, and fibrinolysis. Their biological significance is related hemostasis and bleeding disorders on the one hand and thrombosis and atherosclerosis on the other.[2,3]

Hypercholesterolemia has been linked to an increase in coagulation factors[4,5]; an increase in serum total cholesterol—a surrogate measure of serum low-density lipoprotein cholesterol (LDL-C)—is associated with an increased risk of CHD.[6] The risk of CHD can be decreased by treatment that reduces serum cholesterol level.[7] One possibility is that a decrease in lipids has an effect that reduces the risk of coronary thrombosis. Additionally, data concerning hypercholesterolemia and coagulation from the Atherosclerosis Risk in Communities Study show that both factor VIIc and fibrinogen are associated with LDL-C in both univariate and multivariate analysis.[8,9] In the Northwick Park Heart Study,[10] although significant correlations were shown for factor VIIc, factor VIIIc, and fibrinogen in relation to cholesterol components (total cholesterol and LDL-C) in the multiple regression analysis, only fibrinogen was significantly associated with LDL-C in men. The present study was performed to evaluate whether other important hemostatic variables have any relationship to hypercholesterolemia in a relatively large population of middle-aged people.

SUBJECTS AND METHODS

Patient Population

The Cardiovascular Disease Risk Factor Two-Township Study is a longitudinal study ongoing in two suburban communities: Chu-Dung (northwest Taiwan) and Pu-Tzu (southwest Taiwan). The study focuses on the evolution of cardiovascular disease risk factors. The participants have been described in detail in previous reports.[11,12] Briefly, Chu-Dung is a Hakka township in northwest Taiwan,[13] and Pu-Tzu is a Fukienese township in southwest Taiwan. Both the Hakka and Fukienese are groups of Chinese who migrated to Taiwan a few hundred years ago from mainland China. Five villages in each of the two townships were selected randomly from those with greater than 1,000 people or a population density greater than 200/km^2. All residents were invited to participate in the study by a mailed invitation letter that described the study and its purpose. The response rate was approximately 50%. Subjects were divided into those with a higher total cholesterol and a control group with lower total cholesterol, with a cutoff value of 6.2 mmol/L (240 mg/dL). The study included 2,158 people aged 45 to 69 years. There were 939 men in the control group and 179 in the study group, and 901 women in the control group and 139 in the study group.

Assay Procedure

Fasting venous blood samples were collected from every subject together with background data such as age, gender, body mass index (kg/m^2), and waist to hip ratio. Blood sampling was performed according to a standard protocol to avoid activation of the coagulation system. A no. 21 butterfly needle (Nissho, Osaka, Japan) was used for puncture to minimize trauma. No hemostasis or repeated punctures were allowed when collecting blood. The first 10 mL blood was collected into a sodium heparin tube (Sherwood Medical, St Louis, MO) for routine biochemical analy-

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sis. After releasing the tourniquet, two precooled 4.5-mL sodium citrate tubes (Becton Dickinson, Rutherford, NJ) were filled. All samples were kept at 4°C until centrifugation. Samples were centrifuged for 15 minutes at 3,000 rpm at −4°C. After separation, plasma samples were stored at −70°C until the analysis was performed.

Hemostatic parameters measured included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor VIIc, factor VIIIc, antithrombin III, and plasminogen. All hemostatic measurements were made within 1 month of blood sampling on an ACL 300 Plus Automat Clotting and Fibrinolyzing Analysis System (Instrumentation Laboratory, Chicago, IL), using reagents provided by the manufacturer. Cholesterol and triglyceride levels were measured by the Monarch 2000 Autoanalyzer (Instrumentation Laboratory).

Statistics

Statistical analyses were performed using SAS Version 6.06 (SAS, Cary, NC). The data are expressed as the mean ± SD. Comparison among the four groups was made by ANOVA (general linear model program). Where only two groups were being compared, the chi-square test or unpaired *t* test were used. A *P* value less than .05 was regarded as significant. Relationships between cholesterol and each of the hemostatic factors were tested using simple Pearson correlations and multiple regression analysis. The effects of age and body mass index were adjusted for in the multiple regression model.

RESULTS

Table 1 summarizes baseline data and hemostatic variables of the study population. The subjects were well-matched for age and body mass index. Of seven variables (PT, APTT, fibrinogen, factor VIIc, factor VIIIc, antithrombin III, and plasminogen) measured, comparing hypercholesterolemic and control subjects, all hemostatic factors showed significant differences except factor VIIIc in men and PT in women. For the well-established risk factors for CHD (fibrinogen and factor VIIc), both hypercholesterolemic groups of both sexes showed statistically higher levels. For the anticoagulant factors (antithrombin III and plasminogen), again, study groups of both sexes showed higher activity.

Hypercholesterolemic patients, regardless of gender, also had statistically higher blood glucose and triglycerides and diastolic blood pressure. In the women, higher systolic blood pressure and fasting insulin were also noted. Body mass index and waist to hip ratio showed no statistical difference, indicating that hypercholesterolemia was independent of obesity in these subjects.

Table 2 summarizes the relationships between hemostatic parameters and plasma total cholesterol by Pearson correlation and multiple regression analysis. Both PT and APTT showed a significant negative correlation with cholesterol concentration, while almost all the other parameters, ie, factor VIIc, factor VIIIc, fibrinogen, plasminogen, and antithrombin III (ordered in decreasing size of the correlation), showed a positive correlation in men and women in univariate and multivariate analyses. Significance was not shown for factor VIIIc in men in the multiple regression model. The most significant parameter related to cholesterol level was factor VIIc for both sexes.

Table 1. Baseline Data and Hematologic Parameters of Control and Study Groups After Adjustment for Other Confounding Variables Such as Smoking, Alcohol Consumption, and Triglyceride Level

	Men		Women	
Parameter	Normal (n = 839)	Chol ↑ (n = 179)	Normal (n = 901)	Chol ↑ (n = 139)
Age (yr)	57.6 ± 5.8	57.3 ± 5.4	56.2 ± 5.6	57.9 ± 5.4
Weight (kg)	65.8 ± 9.7	67.9 ± 11.2	58.7 ± 9.2	58.6 ± 8.0
BMI (kg/m²)	24.3 ± 3.2	24.8 ± 3.5	25.0 ± 3.7	24.8 ± 5.0
COI	0.89 ± 0.09	0.89 ± 0.06	0.81 ± 0.06	0.82 ± 0.07
SBP	122.3 ± 18.3	125.3 ± 16.3	122.1 ± 18.7	125.1 ± 20.3‡
DBP	79.0 ± 11.2	82.4 ± 10.0†	76.1 ± 10.4	77.1 ± 10.2‡
Glucose (mmol/L)	5.81 ± 1.96	6.27 ± 2.29‡	5.70 ± 1.68	6.27 ± 2.41*
Insulin (pmol/L)	59.6 ± 11.6	61.2 ± 13.8	63.4 ± 45.0	74.7 ± 59.8‡
TG (mmol/L)	1.32 ± 0.60	1.71 ± 0.67*	1.18 ± 0.61	1.52 ± 0.75*
Fibrinogen (g/L)	2.71 ± 0.74	2.97 ± 0.70§	2.87 ± 0.76	3.03 ± 0.83‡
APTT (s)	27.5 ± 3.7	26.5 ± 3.3‡	26.5 ± 4.1	25.8 ± 3.4§
PT (s)	13.3 ± 1.4	13.0 ± 1.0‡	13.0 ± 1.81	12.9 ± 1.6
Factor VIIc (%)	126.9 ± 39.8	139.8 ± 30.5†	133.6 ± 37.6	151.4 ± 43.2*
Factor VIIIc (%)	134.6 ± 55.0	140.3 ± 57.1	141.0 ± 62.2	157.2 ± 77.5†
AT-III (%)	105.8 ± 22.2	112.5 ± 21.1‡	105.6 ± 21.2	122.6 ± 22.2*
Plasminogen (%)	122.0 ± 32.9	133.0 ± 37.6†	126.1 ± 37.2	141.4 ± 43.2*

NOTE. Values are the mean ± SD. Cutoff value for cholesterol, 6.21 mmol/L.

Abbreviations: COI, central obesity index (waist to hip ratio); BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; Chol, cholesterol; Chol ↑, hypercholesterolemic; AT-III, antithrombin III.

*$P < .0001$, †$P < .001$, ‡$P < .01$, §$P < .05$: *v* normal group of same sex.

DISCUSSION

The parameters that have been documented in the long-term prospective Framingham Heart Study as independent risk factors for CHD are (1) increased age, (2) male gender, (3) decreased high-density lipoprotein cholesterol, (4) increased total cholesterol or LDL-CL, (5) elevated blood pressure, (6) cigarette smoking, (7) diabetes, and (8) left ventricular hypertrophy determined by electrocardiogram.[14] Additional cardiovascular risk factors such as elevation of fibrinogen and factor VIIc have also been shown by other epidemiological studies.[15-17]

This study shows that men with hypercholesterolemia have a significant elevation of fibrinogen and factor VIIc along with shortened PT and APTT. This indicates a thrombogenic tendency in these patients. The elevation of antithrombin III and plasminogen was also significant. Factor VIIIc also increased in the men, but showed no statistical significance. Until recently, opinions concerning hemostatic factors in CHD were based primarily on cross-

Table 2. Relationship Between Hemostatic Parameters and Cholesterol by Pearson Correlation and Multiple Regression Analysis

Variable	Pearson Correlation Coefficient	P	Multiple Regression Coefficient	P
APTT (s)				
Men	−.185	.0001	−.0109	.0001
Women	−.237	.0001	−.0133	.0001
PT (s)				
Men	−.215	.0001	−.0003	.0001
Women	−.198	.0001	−.0003	.0001
Fibrinogen (g/L)				
Men	.135	.0001	.1661	.0075
Women	.144	.0001	.1161	.0303
Factor VIIc (%)				
Men	.304	.0001	.1871	.0001
Women	.334	.0001	.1769	.0001
Factor VIIIc (%)				
Men	.115	.0009	.0005	NS
Women	.244	.0002	.0013	.0001
Plasminogen (%)				
Men	.131	.0002	.0821	.0032
Women	.195	.0001	.1200	.0001
AT-III (%)				
Men	.099	.0043	.0626	.0027
Women	.090	.0015	.0471	.0021

NOTE. Adjusted for age and body mass index.
Abbreviations: NS, not significant; AT-III, antithrombin III.

sectional studies, epidemiological characteristics, and cross-cultural comparisons.[18] However, the information derived from prospective epidemiological and longitudinal cohort studies has expanded considerably in the past couple of years. Nevertheless, prospective studies cannot resolve the cause-and-effect issue, because altered levels of certain hemostatic factors relating to CHD may be a mere consequence of the risk state itself or a result of other processes causally related to or associated with CHD.

Recent prospective studies have provided new information on the relationship between fibrinolytic function and CHD. With the accumulation of further CHD episodes in the Northwick Park Heart Study,[19] a strong independent relationship has emerged between low fibrinolytic activity, as measured by dilute clot lysis time, and increased risk of future CHD in men between 40 and 54 years of age at the time of entry into the survey. Previous data from the same study group showed that increased levels of plasma fibrinogen, factor VIIc, and serum cholesterol are independently associated with an increase in acute ischemic heart disease events in middle-aged men.[15,20] Although an increased level of factor VIIIc has also been demonstrated to be associated with an increased risk of CHD, it did not attain the usually accepted level of significance.[15]

In hypercholesterolemic women in our study, the phenomena were similar to those observed in male subjects. However, there are still some differences worth mentioning. Factor VIIIc showed a prominent elevation, and determining whether this phenomenon implies that women sustained a higher risk for CHD needs further study. A similar phenomenon has been observed previously in diabetic women.[12] Additionally, elevations of fibrinogen, factor VIIc, antithrombin III, and plasminogen were also more significant in women than in men, leading to a presumption that in hypercholesterolemic women, the risk of CHD is probably higher than for men. However, considering the Pearson correlation and multiple regression analysis (Table 2), the strongest associations found between cholesterol and hemostatic factors were those for factor VIIc in both sexes.

The elevation of antithrombin III and plasminogen in hypercholesterolemic patients may be a protective mechanism, since antithrombin III is one plasma inhibitor for which a decrease in quantity or activity has been associated with a thrombotic tendency in humans.[21] Previous studies have shown that an elevation of antithrombin III in diabetic patients is considered a protective mechanism against other changes favoring the onset of vascular disease.[22,23]

Plasminogen becomes absorbed and concentrated into fibrin, thereby allowing and facilitating the formation of plasmin directly on its substrate,[24] and fibrinolysis, which results in the dissolution of fibrin clots, is ultimately mediated by the proteolytic enzyme, plasmin. Therefore, the elevation of antithrombin III and plasminogen in these hypercholesterolemic patients may be a protective mechanism against an excessive thrombotic tendency associated with elevation of coagulation factors. However, determining whether this represents an increase in quantity but defective activity needs further study.

In these patients with hypercholesterolemia, the procoagulation activity of increased levels of fibrinogen, factor VIIc, and factor VIIIc with shortened PT and APTT was perhaps balanced by an increased anticoagulant activity of antithrombin III and plasminogen, probably indicating that such activity was a natural defense mechanism against thrombotic events in these patients. There are few previous studies showing a link between hypercholesterolemia and increased coagulability.[4,5] Besides fibrinogen, this study investigated other coagulation and anticoagulation factors that also showed a parallel increase with fibrinogen.

The role of insulin resistance in the development of CHD has generated considerable interest in recent years. Subjects with CHD tend to have a higher insulin response to an oral glucose load than subjects without the disease.[25,26] Hyperinsulinemia is associated with a worsened profile of cardiovascular risk factors, including obesity, elevated triglyceride levels, low levels of high-density lipoprotein cholesterol, and elevated blood pressure.[25] From the present data, hypercholesterolemic women showed significantly increased levels of fasting insulin compared with the other three groups. However, further study is needed to determine whether this phenomenon shows additional coronary risk in female hypercholesterolemic patients.

In conclusion, this study is the first to demonstrate the elevation of various coagulation and anticoagulation factors in hypercholesterolemia in a relatively large Chinese population, implying that these phenomena may partially contribute to the cardiovascular risk of hypercholesterolemia.

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