
The Hemostatic Changes in Active Pulmonary Tuberculosis

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ABSTRACT

Severe pulmonary tuberculosis (PTB) is sometimes complicated with deep venous thrombosis (DVT). We have examined the role of possible hemostatic disturbance, which are predisposing factors for venous thrombosis in patients with PTB. Coagulation and platelet function tests have been studied in 40 patients with severe PTB and 40 healthy control volunteers before therapy and they were compared with 30th day results and controls. Analysis in patients with active PTB showed anemia, leucocytosis, thrombocytosis, elevation in plasma fibrinogen, factor VIII, plasminogen activator inhibitor 1 (PAI-1) with depressed antithrombin III (ATIII) and protein C (PC) levels. On the 30th day of treatment, anemia, leucocytosis and thrombocytosis were improved. Fibrinogen and factor VIII levels decreased to normal levels, PC and AT III levels increased to normal levels whereas there was no difference in PAI-1 levels. Platelet aggregation studies demonstrated increased platelet activation. Activated protein C resistance was not determined. DVT was not detected in patients during the follow up period. Decreased AT III, PC and elevated plasma fibrinogen levels and increased platelet aggregation appear to induce hypercoagulable state seen in PTB and improves with treatment.

Key Words: Pulmonary tuberculosis, Hypercoagulable state, Protein C, Antithrombin III.

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INTRODUCTION

Deep vein thrombosis (DVT) is clinically observed and can be confirmed with laboratory methods in 3-4% of patients with pulmonary tuberculosis (PTB)^[1]. But the real in-

cidence may be closer to 10%, because most of the patients are thought to be clinically inapparent^[2]. It was reported that the link between inflammation and vascular complications might be found in acute phase reac-

tions. It is possible that large amounts of interleukins synthesized by monocyte-macrophage system during inflammation induce hepatic dysfunction and hemostatic abnormalities^[3]. Hemostatic changes in PTB may favor the development of hypercoagulable states^[4]. Antithrombin III (AT III), protein C (PC), protein S (PS) deficiency were found as a causative factor in about 15% of patients, with primary DVT. Recently, resistance to activated protein C has been reported the most common coagulation defect associated with hypercoagulability^[5,6]. We investigated the possible role of hemostatic disturbances leading to hypercoagulability in pulmonary tuberculosis (PTB).

MATERIALS and METHODS

This study included forty patients with pulmonary tuberculosis. Patients were excluded if there was a history of previous venous thromboembolic disease, neoplasm, recent trauma, significant liver or autoimmune disorders, heavy smoking or medical interventions in the last 6 months that included anticoagulants, immunosuppressive agents. All patients received standard doses of rifampicin (600 mg/day), isoniazid (300 mg/day), pyrazinamide (2 g/day) and streptomycin (1 g/day). Doppler venous flow measurements were carried out by a experienced radiologist. The laboratory tests were done before commencement of anti-tuberculosis drugs and at the end of the first month of therapy. Healthy volunteers were used as control group.

Blood Collection: Venous samples were collected between 08.00 am and 09.00 am without occlusion. Nine mL of venous blood was added to 1 mL of 3.8% sodium citrate. Plasma was separated by centrifugation and analyzed immediately or frozen at -20°C. A biochemical profile was obtained by automated analysis (R-A 1000, RA-XT autoanalyser, Technicon, Tarrytown, New York, USA) in the department of Biochemistry. Coulter MB II was used for whole blood count and ACL 200 for activated partial prothrombin time (aPTT) and Prothrombin time (PT).

Activated Protein C Resistance: Activated protein C resistance (APCR) was studied with commercial kit Coatest APC resistance kit (Chromogenix AB, Taljegardsgatan, Mölnådal, Sweden) with ACL coagulometer. Nine mL of venous blood anticoagulated with 1 mL 0.1 mol/L of sodium citrate, centrifugated at 2000 g for 20 minutes. Plasma samples were stored at -70°C for 1 month or studied in a 4-hour period. 100 microliters of plasma with 100 microliters of aPTT solution incubated for 5 minutes. The anticoagulant effect of APC was estimated as a ratio of recalcification times with or without APC on ACL-200 coagulometer. APC ratio was calculated as follows:

$$\text{APC ratio} = \frac{\text{Clotting time (APC/CaCl)}}{\text{Clotting time (CaCl)}}$$

APC ratios below the "cut-of" values were studied for a second time. An APC ratio less than 1.37 (mean-2 standard deviation of healthy control group) defined as APC resistance. Direct measurement of coagulation factor levels. Factor VIII was studied by and aPTT assay.

(Factor VIII Deficient Plasma for F VIII:C Assay by STA, using severe haemophilia A plasma as substrate and an automatic clot timer). The assay measures the ability of exogenously added F VIII to shorten F VIII deficient plasma; comparison with normal pooled plasma (standart 100% activity) enables quantitation of the percentage of activity. F VIII levels between 60-150% was considered as normal range.

Regulatory Proteins of Coagulation and Fibrinolysis: Antithrombin III (AT III) and, protein C (PC) were studied using ACL coagulometer with Accuclot Protein C kit number 106H6123 Sigma diagnostic, 14508, St. Louis, MO 63178, USA and Accucolor AT III Chromogenic kit number 055H6136. Activity of PC and AT III in normal plasma is 70-140% and 80-120% respectively. Plasminogen activator inhibitor-1 (PAI-1) was studied with a commercial solid-state double anti-

body ELISA (Biopool AB, Umea, SWEDEN kit number 1222036) and C-reactive protein (CRP) quantitatively by immunodiffusion.

Platelet Function Tests: Using platelet-rich plasma and a Chrono-Log dual-channel aggregometer (Whole Blood Lumi-Ionized Calcium Aggregometers Chrono-Log 560-Ca Dual Sample, Havertown, PA, USA) patients were compared to controls following the addition of adenosine diphosphate at concentration of 10 μ M/L, collagen 2 μ g/L and ristocetin 1.25 mg/mL. Percent of aggregation from baseline was calculated on each occasion.

Analysis: Results are reported as mean and standard deviation (SD). Laboratory data was compared by Student's-t test.

RESULTS

40 patients (20 was male and 20 was female) with active tuberculosis (TB) and 40 healthy volunteers (20 was male and 20 was female) were included in this study. Median age was 32 (range, 20-49 years) and 32 (range 21-47 years) respectively.

Anemia (Hb 10.2 ± 0.3 g/dL), leucocytosis (mean WBC $14400 \pm 1100/\text{mm}^3$), thrombocytosis (platelet $465000 \pm 85000/\text{mm}^3$), hypoalbuminemia, increased erythrocyte sedimentation rate (ESR 78 ± 22 mm/hour), LDH (543 ± 61 U/L) and CRP (44.4 ± 26.09 mg/dL) and decreased AT III ($64.05 \pm 8.88\%$) and PC ($65.0 \pm 10.38\%$) were found before

treatment. Fibrinogen (803.4 ± 62.77 mg/dL), Factor VIII ($210 \pm 58.42\%$) and PAI-1 (42 ± 1.55 ng/mL) were also higher than the normal levels before treatment. After 4 weeks of therapy Hb and albumine levels increased from 10.2 ± 0.3 to 12.1 ± 0.2 and from 2.8 ± 0.4 to 3.7 ± 0.2 respectively. Leukocyte and platelet counts decreased from 14400 ± 1100 to 8700 ± 1000 and 465000 ± 85000 to 347000 ± 53000 respectively (Table 1).

AT III increased from $64.05 \pm 8.88\%$ to $89 \pm 3.29\%$ and PC from $65 \pm 10.38\%$ to $85 \pm 10.38\%$. Significant elevation in PAI-1 persisted for 4 weeks. Fibrinogen and F VIII levels decreased to normal levels after treatment (Table 2).

Pretreatment fibrinogen, factor VIII and PAI-1 levels of patients were statistically higher than control, all of them, except PAI-1 return back to normal levels with treatment. Whereas PC, AT III levels of patients were statistically lower than control before treatment and return to the normal levels after treatment.

Increased platelet reactivity was seen in patients prior to treatment and improved with treatment (Table 3). There was a significant difference between pretreatment values of % platelet aggregation and first month values. Platelet function tests revealed, $162.25 \pm 29.67\%$ aggregation with ADP, $172 \pm 53.14\%$ aggregation with collagen and $147 \pm 52.59\%$ with ristocetin prior to treatment.

Table 1. Comparative results of several parameters.

Parameter (NR)	Before therapy	4 th week of therapy
WBC ($3.6-9.6 \times 10^3/\text{mm}^3$)	14.4 ± 1.1	8.7 ± 1.0
Hb (13-16 g/dL)	10.2 ± 0.3	12.1 ± 0.2
PLT ($150-400 \times 10^3/\text{mm}^3$)	465 ± 85	347 ± 53
ESR (0-20 mm/h)	78 ± 22	48 ± 22
LDH (0-475 U/L)	543 ± 61	387 ± 63
CRP (0-5 mg/dL)	44.4 ± 26.09	26.45 ± 25.51
Albumin (NR 3.5-5.0 g/dL)	2.8 ± 0.4	3.7 ± 0.2

Results expressed as mean \pm SD, NR= normal range.

Table 2. Sequential measurements of parameters of coagulation in PTB patients

Parameter (NR)	Before therapy	4 th week of therapy	p value
Fibrinogen (200-400 mg/dL)	803.40 ± 62.77	517 ± 65.20	p< 0.05
PT (10.7-13 sec)	10.7 ± 0.4	10.7 ± 0.5	p> 0.05
APTT (27-35 sec)	32.54 ± 1.08	32.11 ± 1.38	p> 0.05
Factor VIII (50-150%)	210.0 ± 58.42	169.33 ± 40.74	p< 0.05
AT III (80-120%)	64.05 ± 8.88	89.0 ± 3.29	p< 0.05
PC (70-140%)	65.0 ± 10.38	85.0 ± 10.38	p< 0.05
PAI-1 (< 5 ng/mL)	42.0 ± 1.55	43.85 ± 1.13	p> 0.05

Results expressed as mean ± SD, NR= Normal range, PC= Protein C, AT III= Antithrombin III, PAI-1= Plasminogen activator inhibitor-1

Table 3. Platelet function studies of patients

Aggregation agent	Pretreatment (% aggregation)	4 th week of therapy (% aggregation)	p value
ADP (10 µ/L)	162.25 ± 29.67	68.40 ± 11.72	p< 0.05
Collagen (2 µg/L)	172.00 ± 53.14	87.34 ± 28.56	p< 0.05
Ristocetin (1.25 mg/mL)	147.00 ± 52.59	67.20 ± 28.60	p< 0.05

With treatment aggregation with ADP, collagen and ristocetin were $68.4 \pm 11.72\%$, $87.34 \pm 28.56\%$ and $67.2 \pm 28.6\%$ respectively.

Activated protein C resistance was not found in patients. Venous thrombosis was not detected. There was no significant changes in hepatic function tests (AST, ALT, ALP, bilirubin) with treatment.

DISCUSSION

Severe pulmonary tuberculosis is characterized by an acute phase response and hypercoagulable state. Experimental studies have shown that peripheral blood mononuclear cells in PTB can be readily induced to produce IL-1, IL-6 and TNF-alpha^[7]. It is likely that the vascular endothelium could be primed as a result of the interaction between mycobacterial products and the host-monocyte-macrophage system which then synthesize large amounts of TNF-alpha and IL-6^[7,8]. These cytokines induce hepatic acute-phase responses that alter levels of coagulation proteins such as fibrinogen and

FVIII^[9,10]. These widespread disturbances in hemostasis could focus on the vascular intima that is activated and rendered more thrombogenic by proinflammatory cytokines^[11].

Fibrinogen is an acute-phase reactant, and its production rate may increase greatly as a result of various essentially nonspecific stimuli. It was shown that the risk of DVT is significantly (4 times) higher in patients whose first period of DVT occur before age of 70 and has a fibrinogen level over 5 g/L. Factor VIII and CRP is also an acute-phase reactant. In our study the levels of fibrinogen, factor VIII and CRP were increased. It is possible that large amount of interleukins such as IL-6 synthesized by monocyte-macrophage system induce hepatic acute-phase reactants like fibrinogen and CRP.

Reduced AT III, PC levels are commonly found hemostatic abnormalities in secondary hypercoagulable states such as malignancy, hepatic disease^[12-14]. Protein C levels decre-

ase in patients with hepatic disease and using anticoagulant drugs because it is a vitamin K dependent protein. It was found that the levels of AT III and protein C were decreased before treatment and came to normal levels with antituberculosis treatment. This is probably due to normalization of hepatic dysfunction with treatment. Robson et al, reported same results^[15].

Fibrinolytic system has a minor role in the development of thrombosis if coagulation inhibitors act normally. It was reported that some temporary changes such as increase in PAI-1 levels, might be seen in DVT and pulmonary embolism^[16]. In our study PAI-1 levels were high and remained unchanged with antituberculosis treatment. It may be a contributing factor for hypercoagulability in TBC.

The mechanism of platelet hyperaggregability seen in most cases such as myocardial infarction or stroke is unclear. Increased platelet activity may be a response to vascular changes and organ infarction and it may also result from inherent differences in platelet reactivity among the population or from hormonal and autocrine factors such as plasma catecholamine^[17-20]. Plasma fibrinogen which is the functionally important ligand for GPIIb/IIIa and produce platelet aggregates, has been shown by several epidemiological studies to be an independent risk factor. A large epidemiological study by Meade et al showed that the plasma fibrinogen concentration is an important determinant of platelet aggregation^[17]. Platelet hyperaggregability which was found in our study and others is probably due to hyperfibrinogenemia.

Although resistance to activated protein C is the most recently described and the commonest coagulation defect associated with DVT, we did not find APC resistance in the patients^[5,6,20,21]. DVT was not detected in this study. This is probably due to small number of patients who were included in the study. We could not find any study investigating APC resistance in patients with TBC in the literature.

In conclusion, hypercoagulable state which is seen in TBC develops secondary to inflammation and return back to normal with antituberculosis treatment.

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