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STUDIES ON HYPERCOAGULATION STATE IN THROMBOANGIITIS OBLITERANS

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Thirteen hypercoagulation parameters related to coagulation, anticoagulation, platelet functions, dynamics of clot formation and hemorrheology were determined in 15–109 patients with thromboangiitis obliterans (TAO). The results showed that VIIIR=Ag, PAdT, PagT, HPF, and whole blood and plasma viscosity were elevated, fibrinogen elevated in the active stage, AT–III declined, RBC electrophoresis time prolonged, and changes suggesting hypercoagulation in thromboelastogram. All these indicated the existence of a hypercoagulation state in TAO patients, which could contribute to the process of thrombosis.

The relationship between immune reactions and blood hypercoagulability could be crucial in the pathogenesis of TAO. Immune reactions causing injury of vascular endothelium play a primary and fundamental role, and blood hypercoagulability could play a secondary but important role in the course of the disease.

Thromboangiitis obliterans (TAO) is an inflammatory disease of blood vessel walls accompanied by thrombosis in the lumen. Although its pathogenesis is not completely clear, special attention has been paid to the role of immune reactions and blood hypercoagulation. This article reports the study on the mechanism of thrombus formation in TAO from the point of view of hypercoagulability of blood. 13 parameters related to coagulation, anticoagulation, platelet functions, dynamics of blood clot formation and hemorrheology were determined.

CLINICAL DATA AND METHODS

Patients. The patients were all males, aged 21 to 54 years. The diagnosis of TAO was based on the following criteria: the onset of disease at the age 20 to 40 years; the symptoms usually accompanied by migratory superficial phlebitis in the limbs; ischemic symptoms and signs in the extremities; other peripheral vascular diseases excluded. In some cases, the diagnosis was confirmed by pathological examinations. The patients were divided into three groups representative of the active, remittent and stabilized stages according to the duration of the disease, distance of walking when claudication sets in, degree of rest pain, and boundary of gangrene in the extremeties.

Parameters of hypercoagulability. The para- menters could be classified into four categories.

Tests related to blood coagulation and anticoagulation. a. Factor VIII related antigen (VIII R: Ag), by Laurell's rocket immunoelectrophoresis;[1] b. Antithrombin III (AT–III), by thrombin–agarose diffusion as-

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say; c. Fibrinogen, by fiuret reaction;[2] d. Heparin precipitable fraction (HPF), by Smith method;[3] e. Euglobulin lysis time (ELT); f. Fibrinogen degradation product (FDP);[2] and g. Kaolin partial thromboplastin time (KPTT) by methods described previously.[2]

Tests for platelet function. a. Platelet adhesion test (PAdT) by Salzman's method[2]. b. Platelet aggregation test (PAgT) performed on a chronolog 335 platelet aggregometer using ADP(0.05 μM, 1.0 μM) and adrenaline (0.4 μg / ml) as inducer.

Thromboelastogram (TEG). Hellige thromboelastography was done using the whole blood recalcifying method.

Parameters related to hemorrheology. a. Vis- cosity of whole blood and plasma. Glass capillary type viscosimeter was used. b. Electrophoresis of red blood cells. RBC was suspended in its plasma, and the speed of RBC migration towards the positive electrode in an electric field was determined.

RESULTS

Changes in various parameters of blood coagulation and anticoagulation (Tables 1,2).

Table 1. The parameter values of coagulation and anticoagulation system in controls and TAO patients

	Ⅷ R:Ag (%)	AT–Ⅲ (mm^2)	Fbg (mg%)	HPF (mg%)	ELT (min)	KPTT (s)
Normal Controls	94 ± 32 (120)	162.20 ± 18.00 (21)	333 ± 61 (30)	103.87 ± 17.68 (24)	> 120 (7 / 20)	38.77 ± 5.69 (59)
TAO Patients	150.99 ± 63.94* (109)	124.45 ± 23.39* (48)	350 ± 109 (48)	152.27 ± 44.41* (61)	> 120 (12 / 19)	42.30 ± 9.72 (15)

Note: ( ) = cases, *P < 0.01

Table 2. Results of measurement of the parameters relared to coagulation and anticoagulation

Groups	Ⅷ R:Ag (%)	AT–Ⅲ (mm^2)	Fbg (mg%)	HPF (mg%)
Normal Controls	94 ± 32 (120)	162.20 ± 18 (21)	333 ± 61 (30)	103.87 ± 17.68 (24)
Active	167.10 ± 37.75* (11)	123.26 + 18.66* (10)	407 ± 64* (15)	238.17 ± 69.15* (18)
Remittent	137.39 ± 63.52* (20)	123.01 ± 25.85* (21)	335 ± 123 (24)	139.85 ± 35.94* (20)
Stabilized	128.66 ± 55.10* (16)	126.95 ± 23.80* (17)	306 ± 97 (9)	95.85 ± 17.68 (23)

*P < 0.01

It was shown that VIII R:Ag was increased significantly in all groups as compared with the normal control (P < 0.01), and it had a tendency to increase with the severity of the disease. AT–III declined significantly in all

groups of TAO ($P<0.01$). Fibrinogen was elevated but without statistical significance. However, elevation was marked in the active group ($P<0.01$) and declined in accordance with the severity of the disease in the remittent and stabilized groups. HPF increased significantly in the active and remittent groups ($P<0.01$). KPTT and FDP were within the normal range.

Changes of platelet functions (Table 3). The value of PAdT was elevated ($P<0.05$), especially in the active group ($P<0.01$). The values of PAgT differed with different inducers and dosages. A marked increase in PAgT was observed when induced by a lower dose of ADP (0.5μM) as compared with those of the normal controls ($P<0.05$–0.01), but it showed no difference ($P>0.05$) whether induced by those of higher doses of ADP (1 μm) or Adr (0.4 μg / ml).

Changes in parameters related to dynamics of clot formation. The r and k values were shorter than normal ($P<0.05$, $P<0.01$). The m value showed a tendency to be shorter ($P>0.05$). The values of m_ε and m_α were within normal ranges.

Changes in parameters related to hemorrheology (Table 4). The viscosity of whole blood and plasma increased ($P<0.01$).

Table 3. Parameter values of platelet functions in normal controls and TAO patients

	PAdT	PAgT (%)								
		ADP (0.5μm)			ADP (1μm)			Adr (0.4μg / ml)		
		2 min	4 min	Peak	2 min	4 min	Peak	2 min	4 min	Peak
Normal Controls	68.06 ± 7.13 (30)	28.17 ± 9.73 (51)	28.20 ± 11.96 (51)	31.85 ± 10.79 (51)	49.32 ± 15.87 (52)	56.53 ± 19.54 (52)	58.68 ± 16.84 (52)	41.76 ± 14.37 (50)	65.91 ± 7.75 (50)	72.79 ± 15.03 (50)
TAO Patients	72.58 ± 9.17** (33)	38.40 ± 15.48** (15)	42.67 ± 21.81** (15)	46.30 ± 19.04* (15)	53.93 ± 9.23 (19)	57.71 ± 25.26 (19)	61.28 ± 22.27 (19)	48.39 ± 21.01 (19)	62.66 ± 21.31 (19)	65.32 ± 20.53 (19)

*P<0.01, **<0.05

Table. 4. Values of TEG and hemorrheology parameters in normal controls and TAO patients

	TEC					Hemonrrheology		
	r (min)	K (min)	Mε	mα (mm)	m (min)	blood viscosity	Plasma viscosity	RBC Electrophoresis(s)
Normal controls	7.20 ± 1.54 (120)	4.40 ± 1.39 (120)	113.79 ± 24.29 (120)	52.75 ± 5.33 (120)	31.91 ± 7.03 (120)	4.92 ± 0.62 (62)	1.59 ± 0.14 (62)	23.50 ± 1.74 (62)
TAO patients	6.25 ± 1.61** (16)	3.98 ± 1.04** (16)	103.44 ± 22.75 (16)	50.31 ± 5.29 (16)	29 ± 5.93 (16)	4.89 ± 0.51* (19)	1.76 ± 0.23* (19)	25.89 ± 1.68* (19)

*P<0.01, **P<0.05

DISCUSSION

Thirteen laboratory parameters were adopted for studying hypercoagulability in TAO patients. The results showed that nine were abnormal, i.e.; increased VIII R:Ag, PAdT. PAgT, HPF, viscosity of whold blood and viscosity of plasma and decreased AT–III. Fibrinogen was raised in the active group and mobility of RBC in electrophoresis was reduced and some abnormal findings were observed in TEG. These results indicate that hypercoagulability existed in the TAO patients.

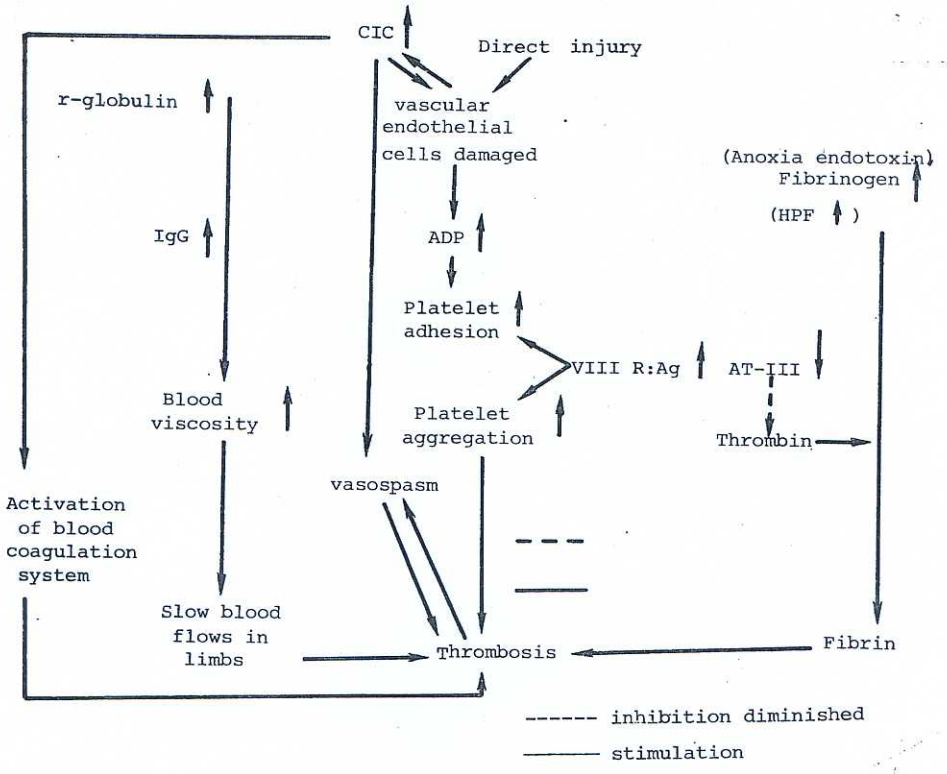

Fig 1. Preliminary of working hypothesis of thrombosis in TAO

HPF appears to be very similar to fibrinogen and is a polymer formed during the conversion of fibrinogen to fibrin. Its increase facilitates the aggregation of fibrinogen and affects the stability of blood cell suspension, favoring the formation and development of thrombus.[3] Smith reported that HPF could be elevated under the action of gram–negative bacillus endotoxin, thus changing the stability of fibrinogen.

VIII R:Ag is synthesized within vascular endothelial cells; this has been proved by immunofluorescent technique.[5,6] When the vascular endothelial cells are damaged, its production and release increased. VIII R:Ag is an important factor in platelet adhesion and combines with platelet glucoprotein I_b receptor. Therefore, elevated VIII R:Ag reflects damaged vascular endothelial cells and is beneficial to the adhesion of platelet. Based on immunological studies in TAO,[7,8] the vascular endothelial cells are damaged by and immunocomplex deposited in vascular

walls. This reveals the close relationship between the production of hyper- coagulability and the vascular endothelial cells damaged by immune reaction.

AT–III is one of the most important anti- coagulation factors in plasma. It can inhibit thrombin and other coagulation factors containing serine as the active centre. Whether its decline is due to an increase of consumption or decrease of production, leading to a predisposition of thrombosis needs further investigation.

These results suggest that the pathogenesis of thrombosis in TAO is probably due to the following mechanism.

The increase in coagulability and hyperviscosity of blood is probably the result of vascular endothelial damage caused by an immune reaction. Thus, anticoagulation factors decline (AT–III, etc.), leading to enhancement of blood coagulation and decrease of blood fluidity (changes of hemorrheology, TEG, etc.), facilitating the formation of thrombus at the site of damaged endothelium of the limb vessels (Fig 1).

The process of thrombosis can activate the kinin system,[9] which releases vasoactive substances damaging the vessel wall. Therefore, the immune reaction and blood hypercoagulation are the two factors which interact with each other in the pathogenesis of TAO. The inflammation in the vessel wall caused by the immune reaction probably play primary and fundamental role, while the blood hypercoagulability state play a secondary but important role in the course of the disease.

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