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A Study of Coagulation and Anti-endothelial Antibodies in Idiopathic Livedo Reticularis

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Livedo reticularis is associated with collagen vascular diseases and other vaso-occlusive disorders in a substantial number of cases. In the remaining cases the cause of livedo reticularis is still unknown (i.e. idiopathic). We sought to determine a possible causal relationship between idiopathic livedo reticularis and autoimmune factors associated with the coagulation system, including antiendothelial cell antibodies.

Nine patients with idiopathic livedo reticularis were studied. All patients were found to have normal platelet count, fibrinogen levels, and prothrombin and activated partial thromboplastin times, as well as negative results for Venereal Disease Research Laboratory and D-timer tests. Anticoagulant activity was detected in 2 patients: one had positive results of thromboplastin titration index and Russell's viper venom test, as well as increased levels of anticardiolipin antibodies and anti-endothelial cell antibodies; the other has positive thromboplastin titration index, mildly increased levels of anti-endothelial cell antibodies, and markedly increased levels of antinuclear antibodies. A third patient had mildly increased levels of anti-endothelial cell antibodies alone, and a fourth patient had mildly increased levels of antinuclear antibodies only. The clinical outcome was uneventful in all of the patients during an 18-month follow-up period.

These findings suggest involvement of autoimmune factors associated with the coagulation system in some patients with idiopathic livedo reticularis, whose clinical significance remains to be determined. ***Key words: cardiolipin; lupus; anticoagulant.***

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Livedo reticularis is often associated with collagen vascular diseases and other vaso-occlusive disorders (1, 2). In the remaining cases the cause of livedo reticularis is still unknown (i.e. idiopathic) (1, 3, 4). Histologically, livedo reticularis sometimes shows vascular intimal hypertrophy and/or intraluminal thrombosis (5–11). Livedo reticularis is seen more frequently in a subset of systemic lupus erythematosus (SLE) patients with anticardiolipin antibodies (1, 4, 8, 10, 12–14) and in primary antiphospholipid syndrome (15–18). Several investigators have also found increased frequency of anticardiolipin antibodies in patients with idiopathic livedo reticularis (4, 15). Anticardiolipin antibodies have been associated with increased diathesis for intravascular thrombosis for a still unknown mechanism (11, 15, 16, 18–24).

A substantial association between lupus-like anticoagulant activity, anticardiolipin antibodies, and the presence of anti-endothelial cell antibodies in patients with SLE has been described (25, 26). It has been suggested that anti-endothelial cell antibodies represent a primary event rather than a secondary immune response against determinants exposed as a consequence of vascular inflammatory processes (26). Recently, anti-endothelial cell antibodies were found in one third of patients with widespread livedo reticularis and cerebrovascular accidents, i.e. Sneddon's syndrome (27).

The present study was performed in order to explore a possible causal relationship between idiopathic livedo reticularis and autoimmune factors associated with the coagulation system, including anti-endothelial cell antibodies.

MATERIALS AND METHODS

The study group included 9 patients (1 male and 8 females, mean age 24.8 years, range 10–64) with livedo reticularis lasting 1–6 years (mean 2.4). The patients were diagnosed as having idiopathic livedo reticularis because none of them had any history or clinical findings suggestive of collagen vascular disease or primary antiphospholipid syndrome, nor did they demonstrate cutaneous vasculitis. In all patients the livedo reticularis was limited to the lower and, occasionally, upper extremities. None of the patients were taking any medication, except for one patient (No. 3, Table I), who had been on carbamazepine therapy for convulsive disorder for several years. All patients were followed up for 18 months after blood sampling.

Blood samples were tested for the following: complete blood count, Venereal Disease Research Laboratory standard slide flocculation test, fibrinogen levels, prothrombin time and activated partial thromboplastin time, using the routine methods. The blood samples were also tested for antinuclear and anticardiolipin antibodies, using enzyme immunoassay kits (Eldan Tech Ltd., Jerusalem, Israel). Antinuclear and anticardiolipin antibodies were marked as increased when above 200 U/ml and 15 IgG phospholipid units/ml, respectively. Determination of fibrin degradation products in plasma containing D-dimers domain was performed using a D-dimer latex assay kit (Baxter Diagnostics, Deerfield, IL, U.S.A.)

In the following tests, a normal control was included in each assay:

Thromboplastin titration index

Antithromboplastin in plasma was determined by measuring the prothrombin time of the patient and control with commercial thromboplastin (Difco Laboratories, Detroit, MI, U.S.A.), diluted to 1:50, 1:200, and 1:500. The thromboplastin titration index was calculated by the average patient/control ratio (28). Thromboplastin titration index was considered positive when the ratio was greater than 1.3.

Dilute Russell's viper venom test

Plasma was incubated with Russell's viper venom (Wellcome, Beckenham, U.K.), diluted to 1:200 and actin (FSL Dade, Miami, FL, U.S.A.) diluted to 1:16. Clotting time was measured after calcification (29). The dilute Russell's viper venom test was calculated by the patient/control ratio and was considered positive when this ratio was greater than 1.1.

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Table I. *Results of several tests in the study*

No.	TTI	dRVVT	ACA (GPL U/ml)	AECA (−/+/++)	ANA (U/ml)
Normal values	<1.30	<1.1	<10		<150
1	N	N	N	−	N
2	1.41	N	N	+	554
3	N	N	N	−	N
4	N	N	N	−	N
5	1.40	1.33	23	++	N
6	N	N	N	−	200
7	N	N	N	−	N
8	N	N	N	+	N
9	N	N	N	−	N

TTI = thromboplastin titration index; dRVVT = dilute Russell's viper venom test; ACA = anticardiolipin antibodies; GPL = IgG phospholipid; AECA = anti-endothelial cell antibodies; ANA = antinuclear antibodies; N = normal; −/+/++ = negative/mildly increased/increased.

Binding of antibodies to endothelial cells

This assay was performed using a cellular enzyme immunoassay (30), which was modified in our laboratory. Briefly, human umbilical vein endothelial cells were obtained according to the method of Jaffe et al. (31) and grown in the presence of M-199 medium enriched with 10% fetal calf serum, 8% human serum, and 50 μg/ml endothelial cell growth factor (Sigma, St. Louis, MO, U.S.A.). Confluent monolayers cultured on a microtiter plate (Nunc, Roskilde, Denmark) were washed with phosphate-buffered saline (PBS), and the cells were fixed with 27% formaldehyde in PBS. Following washing, cells were incubated with patient or control sera at 1:100 dilution for 60 min and then washed again and further incubated with peroxidase-conjugated anti-human IgG (Sigma), diluted to 1:1,000 for 1 h. After the substrate had been added, the absorbance was measured at 492 nm. Samples were assayed in triplicates, and the average of at least three different assays was calculated. Results were considered "increased" when the calculated optical density of patients/control was ≥2.0. Results close to 2.0 were considered "mildly increased".

All patients were followed up clinically for 18 months after blood collection.

RESULTS

All patients were found to have normal platelet counts, fibrinogen levels, prothrombin time and activated partial thromboplastin time and negative results for Venereal Disease Research Laboratory and D-dimer tests. The other results are presented in Table I. Anticoagulant activity was detected in 2 patients (22%): one had positive results of thromboplastin titration index and dilute Russell's viper venom test, as well as increased anticardiolipin antibodies and anti-endothelial cell antibodies levels, and the other had positive thromboplastin titration index, mildly increased anti-endothelial cell antibodies level and markedly increased antinuclear antibody level. A third patient had mildly increased anti-endothelial cell antibodies level alone, and a fourth patient had mildly increased antinuclear antibody level alone. The patient on carbamazepine therapy (No. 3, Table I) did not show any laboratory abnormalities. Further laboratory work-up of the patient with markedly elevated level of antinuclear antibodies (No. 2, Table I) did not reveal any abnormalities consistent with SLE. During the 18-month follow-up period none of the patients showed any thrombotic tendencies or clinical signs of collagen-vascular disease.

DISCUSSION

The demonstration of anticoagulant activity using the more sensitive tests, such as dilute Russell's viper venom test and thromboplastin titration index in 2 of our 9 patients, of whom 1 also had increased anticardiolipin antibody level and the other markedly elevated antinuclear antibody level, and both of whom had increased anti-endothelial cell antibody levels, suggests the involvement of anticoagulant activity. In addition, the demonstration of a mildly increased anti-endothelial cell antibody level in a third patient and a mildly increased antinuclear antibody level in a fourth patient increases the likelihood of an association of idiopathic livedo reticularis with autoimmune features, although during the 18-month follow-up period none developed a collagen-vascular disease or any thrombotic event.

There is no direct evidence that antiphospholipid antibodies are pathogenic *in vivo*, even though the circumstantial evidence is strong (16). Possible mechanisms are that these antibodies react directly with activated platelet membrane lipids so as to promote platelet aggregation, or that they might fix to endothelial cells and inhibit their antithrombotic properties, or have an effect on the fibrinolytic system (16, 18, 21, 22, 24).

Anti-endothelial cell antibodies have been demonstrated in sera from patients affected by SLE, and their presence has been related to vascular injury (25). In addition, a significant association between IgG anti-endothelial cell antibodies and lupus-like anticoagulant activity has been shown by some authors (25). Other authors have not found such a relationship but have demonstrated that anticardiolipin antibodies can be a part of anti-endothelial cell antibodies in certain lupus sera (16). Meroni et al. (26) claim that anti-endothelial cell antibodies seem to be characteristic of patients with small-vessel vasculitis. The frequent presence of anti-endothelial cell antibodies in diseases with primary small-vessel vasculitides, such as Wegener's granulomatosis, the lower prevalence of anti-endothelial cell antibodies in vasculitides secondary to SLE and the virtual absence of anti-endothelial cell antibodies in essential cryoglobulinemia and vasculitis associated with HIV infection suggest that anti-endothelial cell antibodies might represent a primary event in vascular injury rather than an immune response against determinants exposed as a consequence of vascular inflammatory processes (26). Interestingly, endothelial cytotoxic activity was reported in the majority of sera of 11 patients with livedo racemosa generalisata Ehrmann (32). This type of livedo, mostly found in women between 20 and 40 years of age, is frequently associated with cerebrovascular disorders in Sneddon's syndrome (27). Francès et al. (27) recently demonstrated anti-endothelial cell antibodies in 35% of patients with Sneddon's syndrome. Therefore, the presence of anti-endothelial cell antibodies in 3 (33%) of our patients, although only mildly elevated in 2 of them, could suggest primary vascular injury mediated by these antibodies in the pathogenesis of some cases of idiopathic livedo reticularis.

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