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Endothelial dysfunction, thrombophilia, and nailfold capillaroscopic features in livedoid vasculopathy

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ABSTRACT

Background: Livedoid vasculopathy (LV) is a rare, disabling disease characterized by painful ulcers, livedo reticularis and atrophy blanche. Hypercoagulation, endothelial, and microcirculatory dysfunction are believed to be responsible for the pathogenesis of this difficult-to-treat disease.
Objectives: This study sought to investigate the frequency of endothelial dysfunction, hypercoagulability, and nailfold capillaroscopic features in LV patients to shed light on its etiology.
Methods: This case-control study included 16 patients with LV, 24 with systemic sclerosis (SSc), and 23 control subjects. Serum markers of endothelial dysfunction soluble endoglin, endocan, endothelin-1, lipoprotein a, plasminogen activator inhibitor-1 (PAI-1), soluble thrombomodulin, and von Willebrand factor were measured using enzyme-linked immunosorbent assays. Flow-mediated dilation and carotid intima-media thickness were examined as markers of endothelial dysfunction, and microcirculation was assessed with nailfold capillaroscopy. Thrombophilia-related parameters, including gene polymorphisms of factor V Leiden, prothrombin, PAI-1 genes, methylenetetrahydrofolate reductase (MTHFR) and factor XIII mutation and serum levels of protein C, protein S, antithrombin, homocysteine, D-dimer and antiphospholipid antibodies were investigated in LV patients.
Results: Plasminogen activator inhibitor-1 and soluble thrombomodulin levels were significantly higher in LV patients compared to control subjects (2.3 [2.05–2.79] ng/ml vs. 1.89 [1.43–2.33] ng/ml, $p = 0.007$; 1.15 [0.88–1.4] ng/ml vs. 0.76 [0.56–0.9] ng/ml, $p = 0.004$, respectively). Flow-mediated dilation was 25.4 % lower in the LV patients compared to the control group (14.77 % [11.26–18.26] vs. 19.80 % [16.47–24.88], $p = 0.034$). Capillaroscopic features, including ramifications (75 % vs. 8.7 %, $p < 0.001$), avascular areas (25 % vs. 0 %, $p = 0.011$) and dilatations (33.2 % vs. 0 %, $p = 0.016$), were significantly higher in LV patients than in controls. LV patients had multiple biochemical or genetic abnormalities related to thrombophilia, including heterozygous factor V Leiden mutations (6.3 %), MTHFR (C677T) mutations (heterozygous 43.8 %, homozygous 18.8 %), MTHFR (A1298C) mutations (heterozygous 37.5 %, homozygous 12.5 %), factor XIII heterozygous mutation (12.5 %), antithrombin deficiency (31.3 %), protein S deficiency (12.5 %), hyperhomocysteinemia (31.3 %), D-dimer elevation (25 %), anti-β2-glycoprotein I (12.5 %), lupus anticoagulant antibodies (6.3 %), and anti-cardiolipin antibodies (6.3 %).
Conclusions: In conclusion, LV patients were characterized by an increased presence of thrombophilia-related parameters, and also exhibited vascular endothelial and microcirculatory dysfunction, resembling SSc. These findings support the complex interaction of thrombophilia, endothelial dysfunction, and microcirculation dysregulation in the pathogenesis of LV. Thus, the treatment of LV patients should be individualized, based on the identification of the predominant pathological pathways.

1. Introduction

Livedoid vasculopathy (LV) is a rare, chronic, recurrent, thromboembolic disease characterized by painful ulcerated lesions and scarring, which often affects the lower extremities bilaterally. Lesions usually begin as purpuric, telangiectatic papules and progress to painful

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ulcers, which take months to heal and result in white atrophic scars if left untreated (Micieli and Alavi, 2018). The proliferation of endothelium, segmental hyalinization of superficial dermal vessels, along with intraluminal fibrin deposits and intravascular thrombosis are characteristic histopathological findings (Criado et al., 2011).

Although the pathogenesis of the disease is not precisely understood, increased thrombotic activity, decreased fibrinolytic activity, and endothelial damage are thought to cause thrombus formation in the capillary vasculature (Majmundar and Baxi, 2023). In fact, there is a well-established association between various prothrombotic and autoimmune conditions, including factor V Leiden mutation, protein C and S deficiency, prothrombin G202110A gene mutation, plasminogen activator inhibitor (PAI-1) mutation, lipoprotein a (Lpa) elevation, methylenetetrahydrofolate reductase (MTHFR) gene mutation, antithrombin deficiency, activated protein C resistance, hyperhomocysteinemia, cryoglobulinemia, and antiphospholipid syndrome (den Heijer et al., 1998; Shankar et al., 2013; Vasudevan et al., 2016). In addition, coexistence of LV with acquired hypercoagulable states, including paraproteinemia, infections (hepatitis B and C virus) or malignancies has been reported (Espinel et al., 2017).

Although thrombophilia and autoimmune conditions are most commonly associated with LV, some patients are still unresponsive to recommended treatments (anticoagulant, antiplatelet, fibrinolytic, vasodilator, and immunosuppressive agents). This suggests that different mechanisms may play a role in the pathogenesis of LV. Criado et al. (2011) proposed changes in blood flow and endothelial injury as possible pathogenetic factors.

The endothelium regulates blood fluidity, vascular contractility, and permeability by releasing mediators such as nitric oxide (NO), prostacyclin, and endothelin. Several processes, such as shear stress, inflammation, and oxidative stress, disturb endothelial function, which may lead to platelet aggregation and adhesion to the endothelium, leukocyte adhesion, and intimal hyperplasia. This process, which is known as endothelial dysfunction (ED), eventually triggers the release of various molecules that are involved in the coagulation cascade, including serum soluble endoglin (sEng), endocan (endothelial cell-specific molecule-1), endothelin-1 (ET-1), soluble thrombomodulin (sTM), and von Willebrand factor (vWF) (Poredos et al., 2021; Tsai et al., 2009).

ED affects both microcirculation and macrocirculation simultaneously. An imbalance in vascular homeostasis is associated with an impaired peripheral vascular dilation response. Evaluation of the flow-mediated dilation (FMD) response to reactive hyperemia induced by transient ischemia in the brachial artery is an important non-invasive way to measure ED (Poredos et al., 2021; Yang et al., 2012; Moroni et al., 2017). Another method of ED evaluation involves assessing the carotid intima-media thickness (CIMT) (Wang et al., 2019).

ED is considered an etiological factor in various disorders, including atherosclerosis, and current evidence supports its central role in the etiology and disease progression of established SSc (Pacholczak-Madej et al., 2020; Saygin et al., 2019). However, data on the role of ED in the etiology of LV is limited to a study by Yang et al. (2012), who reported reduced brachial FMD in 16 LV patients.

Considering the gaps in existing knowledge regarding LV etiology, this study seeks to provide information about the role of thrombophilia, endothelial dysfunction, and microcirculatory abnormalities in the pathogenesis of LV. The primary aim of the study is to investigate the markers of ED in LV patients. The secondary aims are to determine the frequency of thrombophilia, microcirculatory abnormalities, and the association between nailfold capillaroscopy (NFC) changes and ED-associated parameters. In addition, the study compares the findings of LV patients with those of SSc patients, a disorder characterized by ED and impaired microcirculation.

2. Materials and methods

2.1. Subjects

This case-control study was conducted at the Marmara University Pendik Research and Training Hospital, Istanbul, Turkey. The study was conducted from July to November 2022. Sixteen patients with LV, 24 with SSc, and 23 control subjects were included in the study. The diagnosis of LV was made by an experienced dermatologist (TE) based on typical clinical features (livedo racemosa, typical recurrent ulcers, and atrophy blanche) and histopathological examination if indicated. Histological features of LV included fibrin thrombus in the vessel walls, fibrinoid deposits without significant vasculitis, possibly with erythrocyte extravasation, and dermal sclerosis for advanced lesions. The diagnosis of SSc was made by an experienced rheumatologist (FA) in patients with a total score of $\geq$9 according to the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification (van den Hoogen et al., 2013). Twenty-three control subjects from the same hospital were recruited. Control subjects were physically healthy with no history of comorbidity, and none were taking systemic medications. Since smoking affects endothelial functions, the smoking habits of all participants were recorded. Current smoking was defined as daily smoking (at least 1 cigarette per day, 7 per week, or 1 pack per month) for at least the past 12 months.

All patients who attended their last three visits were included in the sample size. They were classified according to LV activity and severity scores (LVAS) based on four clinical parameters (livedo reticularis, atrophie blanche, ulceration, and pain), and the highest possible score was six points (Monshi et al., 2014). Patients with malignancy, immunodeficiency, pregnancy, lactation, and chronic diseases of the leg, including severe venous insufficiency, were excluded from the study.

Approval for the study was granted by the Institutional Review Board (Marmara University Ethics Committee 09.2021.772), and all procedures were applied in compliance with the Declaration of Helsinki ethical guidelines. All participants signed an informed consent form.

2.2. Clinical features and biochemistry

Demographic features, disease characteristics, and comorbidities were recorded for all the study participants. Ten milliliters of peripheral venous blood were collected from LV patients. Protein C and antithrombin were measured using clotting time-based assay and an enzyme-linked immunosorbent assay (ELISA). Free protein S antigen was screened by ELISA. Homocysteine and D-dimer levels were investigated with liquid chromatography-tandem mass spectrometry and turbidimetric immunoassay, respectively, and vitamin B12 and folate levels were assessed based on chemiluminescence immunoassay.

Anti-β2-glycoprotein I (GPI) IgG/IgM and anticardiolipin (ACA) antibodies were assessed by ELISA, and lupus anticoagulant (LA) was screened by LA sensitive aPTT/ diluted Russell's viper venom time (dRVVT) test (Devreese et al., 2020). Cut-off values are defined as follows: <60 % for protein C and S deficiency, <23 mg/dl for antithrombin deficiency, <60 % and < 70 % for protein C and antithrombin low activity respectively, and > 14 μmol/L for hyperhomocysteinemia, >0.55 mg/L for elevated D-dimer, <150 ng/L for vitamin B12 deficiency, <4 ng/mL for folate deficiency, screening test positivity for LA, >18 IU/mL for anti-β2-GPI I (IgG/IgM) positivity, and > 18 GPL (MPL)/mL for ACA (IgG/IgM) positivity.

In addition, the frequency of genetic thrombophilia parameters, factor V Leiden, prothrombin (G20210A), MTHFR (C677T and A1298C), factor XIII mutations, and PAI-1 gene polymorphisms were investigated with real-time polymerase chain reaction (PCR) in LV patients.

2.3. Serum endothelial dysfunction markers

After 8–12 h of fasting, 5 ml of venous blood was collected from

participants. All samples were centrifuged at 2500 rpm for 20 min and stored at −80 °C until analysis. Levels of sEng, endocan, ET-1, Lp(a), PAI-1, sTM, vWF, and high-sensitivity C-reactive protein (hs-CRP) were assessed by ELISA. KC Junior software was used to run the ELISA at 450 nm (BT-LAB ELISA kits, Opakgen Medical and Chemical Products Co. Ltd., Istanbul, Turkey).

2.4. Non-invasive methods for investigating of ED

FMD and CIMT, which are non-invasive indicators of endothelial dysfunction, were evaluated in all participants. High-resolution ultrasound scanning was performed by an experienced cardiologist (TG) using Epiq 7 (Philips Medical Systems, Andover, MA, USA) and an L18–5 (10 MHz) linear transducer. For FMD measurement, the inner diameter of the brachial artery was measured in three different cardiac cycles, and the average value was calculated. The cuff was inflated to 200 mmHg for 5 min to induce ischemia and then completely deflated, and the brachial artery inner diameter was remeasured in three different cardiac cycles at the 60th second of reactive hyperemia. The mean increase in arterial diameters was compared to the mean of the baseline diameters and expressed as the percentage of FMD (Moroni et al., 2017). For the CIMT measurement, the total thicknesses of the intima and media layers were recorded as the CIMT value in mm (Oflaz et al., 2005).

2.5. Nailfold capillaroscopy

All subjects were advised to avoid traumatic procedures, such as manicures, for four weeks and to avoid smoking or caffeine consumption for at least 4–6 h before the imaging procedure. Eight finger (excluding the thumb) nailfolds were evaluated using a Dino-Lite CapillaryScope 200 Pro capillaroscopy device and DinoXcope 2.0 software. Capillary parameters were analyzed, including quantitative (capillary diameter and capillary density) and qualitative changes (ramification, avascular area, tortuosity, crossing, buschy capillary, microhemorrhage, subpapillary venous plexus, and meandering). As proposed by Ingegnoli et al. (2009), bushy, meandering, branching, and megacapillaries were considered major pathological changes. The minor, non-specific changes included tortuosity, dilatation, and crossing capillaries ($\leq$2). Moreover, avascular areas (when >2 consecutive capillaries are missed), subpapillary venous plexus visibility, and microhemorrhages were evaluated. Capillary density was measured as the number of distal capillaries per 1 mm^2. Capillary diameter measurements were made from the apical loop, and values as >20 μm for capillary dilatation and > 50 μm for megacapillaries were accepted (Ingegnoli et al., 2009; Smith et al., 2020). All images were evaluated by two researchers simultaneously.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS software version 25.0. The conformity of the variables to normal distribution was investigated using a Shapiro–Wilk test. Mean, standard deviation, minimum–maximum, and median (25p–75p) values were used in the descriptive analyses. Categorical variables were expressed as absolute frequencies and percentages. Categorical variables were compared with the Fisher's exact test due to the small sample size. Variables were compared between the groups using the non-parametric Kruskal–Wallis test, and a post hoc Dunn's test was used for multiple comparisons. Relationships between the data were examined using Spearman correlation analysis. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic features and treatments

Sixteen LV patients (age 41.4 ± 17.4 years, 13 [81.2 %] females, and three [18.8 %] males) were evaluated, along with 24 SSc patients (age 42.8 ± 10.5 years, 20 [83.3 %] females, 4 [16.7 %] males), and 23 control subjects (age 41.3 ± 11.3 years, 18 [78.3 %] females, 5 [21.7 %] males) were evaluated. No one was excluded, as all participants attended their targeted review and screening appointments. There were no significant variations in sex, age, body mass index (BMI) or smoking status between the groups ($p = 0.90$; $p = 0.75$, $p = 0.49$, and $p = 0.19$ respectively). In addition, there was no significant difference between the groups in terms of comorbidities, and p values are presented in Table 1.

The current treatments of the LV patients were as follows: acetylsalicylic acid (ASA) (12.5 %), factor Xa inhibitor (6.25 %), hydroxychloroquine (6.25 %), ASA with hydroxychloroquine (12.5 %), ASA with pentoxifylline (12.5 %), factor Xa inhibitor with IVIG (6.25 %), hydroxychloroquine with factor Xa inhibitor (6.25 %), hydroxychloroquine with pentoxifylline (6.25 %), topical corticosteroid (6.25 %), and no treatment (25 %). Meanwhile, the SSC patients were receiving an endothelin receptor antagonist (20.8 %), mycophenolate mofetil (20.8 %), azathioprine (20.8 %), hydroxychloroquine (12.5 %), pentoxifylline (4.2 %), sildenafil (4.2 %), or no treatment (16.6 %).

The mean age at disease onset was 32.4 ± 14.0 years for the LV patients. The mean time from disease onset to diagnosis was 5.3 ± 3.0 years, and the min–max ranged from 1 to 10 years. The mean LVAS was 2.6 ± 1.3, and 75 % of the patients had active disease requiring treatment for ulcers or pain. No seasonal exacerbation was observed in nine patients (56.3 %), while four (25 %) had summer and three (18.8 %) winter exacerbations. One patient with five-year disease remission experienced a flare-up on the tenth day after the second dose of the SARS-CoV-2 mRNA vaccine. Chronic complications of bilateral peripheral polyneuropathy were detected in one patient, and mononeuritis multiplex was also detected in one patient.

3.2. Thrombophilia parameters of LV patients

All LV patients were screened for protein C, protein S, antithrombin, homocysteine, D-dimer, antiphospholipid antibodies, factor V Leiden, prothrombin (G20210A), MTHFR (C677T and A1298C), factor XIII mutation, and PAI-1 gene polymorphism. The genetic thrombophilia panels of the LV patients revealed heterozygous factor V Leiden and heterozygous and homozygous MTHFR (C677T) mutations in 6.3 %, 37.5 %, and 18.8 % of patients, respectively. Heterozygous and homozygous MTHFR (A1298C) mutations were detected in 43.8 % and 12.5 % of patients, respectively, while factor XIII heterozygous mutations were found in two patients (12.5 %). No prothrombin (G20210A) mutations were detected in any LV patients.

The PAI-1 polymorphisms analysis showed that 56.3 % of patients had 4G/5G polymorphisms, 25 % had 4G/4G polymorphisms, and 18.8 % had 5G/5G polymorphisms. Except for one patient (Patient 10), all the LV patients had multiple biochemical or genetic abnormalities related to hypercoagulability. The positive results are shown in Table 2.

3.3. Serum ED markers

Significantly higher levels of serum sTM and PAI-1 were found in the LV and SSc patient groups compared to the controls (sTM: LV: 1.15 [0.88–1.4] ng/ml, SSc: 0.98 [0.84–2.59] ng/ml, control group: 0.76 [0.56–0.9] ng/ml; PAI-1: LV: 2.3 [2.05–2.79] ng/ml, SSc: 2.53 [1.9–5.89] ng/ml, control group: 1.89 [1.43–2.33] ng/ml) (see Table 3). Graphical comparisons of serum markers between groups are presented in Fig. 1.

PAI-1 polymorphisms were classified into subgroups (4G/5G, 5G/5G, 4G/4G) in the LV patients, and the highest serum PAI-1 value was found in the 4G/4G polymorphism group, with no statistically significant difference found between the groups ($p = 0.13$).

Table 1
Demographic data and comorbidities of the groups.

		LV ($N = 16$)		SSc ($N = 24$)		Control ($N = 23$)		p^1
		n/M ± SD	%/Mdn (25p-75p)	n/M ± SD	%/Mdn (25p-75p)	n/M ± SD	%/Mdn (25p-75p)	
Age (years)		41.4 ± 17.4	36.5 (27–52)	42.8 ± 10.5	41.5 (34–51)	41.3 ± 11.3	43 (29–48)	0.76[2]
Sex	F	13	(81.3)	20	(83.3)	18	(78.4)	0.92
	M	3	(18.7)	4	(16.7)	5	(21.6)	
Smoking	No	10	(62.5)	19	(79.2)	20	(87)	0.22
	Yes*	6	(37.5)	5	(20.8)	3	(13)	
BMI		26.1 ± 5.0	24.7 (22.5–31.7)	26.4 ± 5.8	25.6 (23.8–29.7)	24.5 ± 2.9	25 (21.8–26.2)	0.5[2]
Comorbidites								p^1
Arterial Hypertension		1	(6.3)	2	(8.3)	0	(0)	0.46
Type 2 diabetes		1	(6.3)	1	(4.2)	0	(0)	0.71
Hyperlipidemia		0	(0)	1	(4.2)	0	(0)	1.0

M: Mean; Mdn: Median; LV: Livedoid vasculopathy; SSc: Systemic sclerosis; SD:Standart deviation; (25p-75p): 25th–75th percentile.
[1] Fisher's exact test [2]Kruskal–Wallis test.
* current smokers: daily smoking (at least 1 cigarette per day, 7 per week, or 1 pack per month) for at least the past 12 months.

Table 2
Overview of genetic and biochemical coagulation risk factors in LV patients.

Pt No	Sex	Age at onset	LVAS	PAI-1 polymorphism	MTHFR		FXIII mutation	Factor V Leiden	Positive biochemical parameters
					C677T	A129C			
1	f	22	2	4G/5G	het	nd	het	nd	Hyperhomocysteinemia
2	f	18	1	4G/5G	nd	homo	nd	nd	nd
3	f	30	4	4G/4G	homo	nd	nd	nd	Protein S deficiency
4	f	19	1	4G/5G	nd	het	nd	nd	Antithrombin deficiency, LA (positive)
5	f	53	1	5G/5G	het	het	nd	nd	Hyperhomocysteinemia, folate deficiency, anti- β2-GPI (IgG/M), D-Dimer (positive)
6	m	41	5	4G/5G	nd	het	nd	nd	Hyperhomocysteinemia, Vitamin B12 and Protein S deficiency
7	f	40	2	4G/4G	homo	nd	nd	nd	Hyperhomocysteinemia, Antithrombin deficiency, D-Dimer (positive)
8	m	33	2	4G/5G	het	het	nd	nd	Hyperhomocysteinemia, folate deficiency
9	m	13	3	4G/5G	nd	het	nd	nd	Antithrombin deficiency, anti-β2-GPI (IgG/M) and ACA (positive)
10	f	38	4	4G/5G	nd	nd	nd	nd	nd
11	f	35	2	4G/4G	homo	nd	het	nd	Antithrombin deficiency
12	f	66	3	4G/5G	nd	het	nd	het	D-Dimer (positive)
13	f	48	2	5G/5G	nd	homo	nd	nd	nd
14	f	17	2	5G/5G	het	nd	nd	nd	D-Dimer (positive)
15	f	18	4	4G/4G	het	nd	nd	nd	Antithrombin deficiency
16	f	26	4	4G/5G	het	het	nd	nd	nd

M: Male; f: Female; het: Heterozygous; homo: Homozygous; nd: Not detected; LA: Lupus anticoagulant; anti-β2-GPI: Anti-β2-glycoprotein I antibodies; ACA: Anti-cardiolipin antibodies; LVAS: LV activity and severity score (Monshi et al., 2014).

3.4. CIMT and brachial artery FMD values

ED-related ultrasonography parameters were evaluated in the LV, SSc, and control groups.

The CIMT values measured via high-resolution ultrasonography were comparable among groups ($p = 0.142$). FMD (%) responses were significantly lower in both the LV (14.77 %, 11.26–18.26) and SSc groups (10.06 %, 6.12–13.85) compared to the controls (19.8 %, 16.47–24.88; $p < 0.001$) (Table 3). A graph comparing FMD between groups is presented in Fig. 2.

In the correlation analysis, while CIMT was not correlated with any marker, an inverse correlation was found between FMD and ET-1 ($r = -0.32$; $p = 0.019$). No association was observed between FMD and CIMT values and coagulation parameters. CIMT was weakly but positively correlated with D-dimer and homocysteine, but the association was not statistically significant ($r = 0.29$, $p = 0.06$ and $r = 0.27$, $p = 0.09$, respectively).

3.5. Nailfold capillascopic parameters

The patients with LV had several capillaroscopic features resembling the SSc pattern. Fig. 3 shows the leg lesions and nailfold capillaroscopic patterns of an LV patient. The median capillary density was significantly lower in both the LV 7/mm^2 (6–8) and SSc 6/mm^2 (4.5–6.5) groups compared with control subjects (10/mm^2, 9–11) ($p = 0.014$, $p < 0.001$, respectively). In parallel, the frequency of avascular areas increased statistically significantly in LV patients, similar to SSc. Moreover, ramification, meandering, and dilated capillaries (>20 μm), which are characteristic of neoangiogenesis, are also significantly increased in the LV patients, similar to SSc. However, importantly, megacapillary (>50 μm) structures, which are frequently observed in the active period in SSc (in 62.5 % of SSc patients in the current study), were not seen in any LV patients. A comparison of capillary parameters among groups is presented in Table 4.

There was a statistically significant negative and weak correlation between capillary density and sTM and PAI-1 ($r = -0.49$, $p < 0.001$; $r = -0.4$, $p = 0.002$, respectively). There was a statistically significant positive weak correlation between capillary density and FMD ($r = 0.43$, $p = 0.001$).

Additionally, there was a statistically significant negative and weak correlation between capillary diameter and FMD ($r = -0.27$, $p = 0.039$).

4. Discussion

Hypercoagulability, endothelial, and microcirculatory dysfunction parameters were investigated in patients with LV to illuminate the major

Table 3
Comparison of serum markers and USG parameters between groups.

	LV	SSc	Control	*p*
vWF (ng/ml)	22.36 (19.66–24.24)	25.21 (19.33–57.04)	18.92 (17.41–24.95)	0.098
sTM (ng/ml)	1.15 (0.88–1.4)	0.98 (0.84–2.59)	0.76 (0.56–0.9)	**0.002**[a]
PAI-1 (ng/ml)	2.3 (2.05–2.79)	2.53 (1.9–5.89)	1.89 (1.43–2.33)	**0.004**[b]
Lpa (ng/ml)	23 (21.1–31.14)	28.26 (20.84–70.24)	23.56 (19.2–28.55)	0.275
ET1 (ng/ml)	60.68 (57.62–81.45)	63.92 (56.32–188.57)	44.21 (38.53–69.52)	**0.008**[c]
sEng (ng/L)	8 (6.53–8.93)	8.76 (5.72–23.16)	6.48 (5.02–10.31)	0.267
Endocan (ng/L)	192.14 (175.76–266.51)	217.2 (176.11–650.56)	178.86 (150.15–350.68)	0.175
hS-CRP (ng/ml)	4.85 (3.99–5.81)	4.43 (3.89–9.51)	3.65 (3.24–5.4)	**0.038**[d]
CIMT (mm)	0.59 (0.48–0.64)	0.51 (0.44–0.57)	0.48 (0.4–0.55)	0.142
FMD (%)	14.77 (11.26–18.26)	10.06 (6.12–13.85)	**19.8 (16.47–24.88)**	**<0.001**[*]

Values are shown in median and 25th–75th percentile distribution.
[a,b,c,d]Dunn's post-hoc test; *p* value[a]: LV-Control: 0.014, SSc-Control:0.005, LV-SSc:1; *p* value[b]: LV-Control: 0.023,SSc-Control:0.007, LV-SSc:1.0; *p* value[c]: LV-Control: 0.16, SSc-Control:0.007, LV-SSc:1.0; *p* value[d]: LV-Control: 0.10, SSc-Control:0.074, LV-SSc:1.0.
LV: Livedoid vasculopathy; SSc: Systemic sclerosis.
Bold numbers indicate significant *p*-values (<0.05) (Kruskal–Wallis test).
[*] Dunn's post-hoc test (SSc-Control: $p < 0.001$; LV-Control: $p = 0.034$; LV-SSc: $p = 0.21$).

pathogenetic mechanisms. The results of this study showed multiple biochemical and genetic hypercoagulability-associated abnormalities in almost all LV patients. In addition, LV patients had significantly higher sTM and PAI levels and lower FMD, indicating vascular ED. Microcirculatory disturbances resembling an SSc pattern, such as decreased capillary density and neovascularization, were also detected at a significantly higher rate in the LV group than in the control group.

The association of LV with various hereditary or acquired coagulopathies has been demonstrated conclusively (Schiffmann et al., 2021; Weishaupt et al., 2019; Vasudevan et al., 2016). Consistent with the literature, the current study results showed at least one hypercoagulability parameter in 15 of 16 (94 %) LV patients. The frequency of these prothrombotic markers is higher than the rates of 44 % (11/25) (Weishaupt et al., 2016) and 67 % (48/75) (Criado et al., 2021) previously reported in LV patients. This may be due to the additional parameters screened in the current study, such as MTHFR, factor XIII mutations, D-dimer, and antithrombin. In addition, ethnic and regional variations in some of these parameters, such as MTHFR, may play a role in this difference (Sazci et al., 2005). Among the hypercoagulability parameters, antithrombin deficiency, hyperhomocysteinemia, factor V Leiden, MTHFR mutations, and PAI-1 polymorphisms were the most prevalent in the current study.

In line with these findings, an increase in MTHFR polymorphisms, which impair homocysteine metabolism, and in PAI-1, which decreases fibrinolysis, have been shown previously (Lee and Cho, 2021). Likewise, the antiphospholipid antibody positivity detected in 18 % of the LV patients in the current study is similar to the rate reported by Di Giacomo et al. (2010) of 17.64 %. Consequently, hypercoagulopathy is a common finding, and all LV patients should be investigated in detail for acquired or inherited coagulation disorders. Those LV patients exhibiting multiple thrombophilia markers may be treated with rivaroxaban or low-molecular weight-heparin, foregoing less effective options like ASA and pentoxifylline.

Although LV seems to be primarily an occlusive condition rather than an inflammatory condition, not all LV patients exhibit prothrombosis. In addition, a subgroup of patients is unresponsive to antithrombotic therapy, indicating the presence of additional pathogenetic pathways. A few recent studies have suggested a possible role of endothelial and microcirculatory dysfunction in the pathogenesis of LV (Kawabe et al., 2022; Yang et al., 2012). Considering the sparsity of data on this pathway, the current study investigated ED in LV patients using a battery of surrogate markers. The novel biomarkers of Lp(a), vWF, sTM, endocan, sEng, and ET-1 were used, all of which have been studied in different vasculopathic diseases and shown to be associated with ED (Hickey et al., 2018; Kostner et al., 2013; Leite et al., 2020; Vieceli Dalla Sega et al., 2021).

The results showed an increase in sTM and PAI-1. Indeed, sTM; has been found to be remarkably elevated in various diseases characterized by impaired endothelium, such as SSc, and it has been suggested as a biomarker of ED (Budzyń et al., 2019; Miwa et al., 2015; Ziętek, 2021). Additionally, Agirbasli et al. (2011) reported increased PAI-1 antigen levels in the plasma and tissue of 20 LV patients and approximately 100 times increased functional stability of PAI-1. Homozygosity for 4G in the 4G/5G polymorphism of PAI-1 has been associated with high PAI-1 levels (Deng et al., 2006; Eriksson et al., 1995). In the current study, in 4G/4G homozygous LV patients, PAI-1 was high but not statistically significant, which may have been due to the small sample size. When all these findings are considered together, although high PAI-1 levels have been shown in multiple studies, its role in pathogenesis remains unclear.

Recently, a link between another thrombophilic factor, Lp(a), and LV has been reported (Criado et al., 2021). However, no significant elevation was found in our LV patients, which may be due to the shorter disease duration in this group.

One of the leading causes of ED is increased oxidative stress, which reduces NO bioavailability, leading to the development of ED and microcirculatory dysfunction. Moreover, the damaged endothelium cannot maintain its antithrombotic effect and shifts the balance in homeostasis toward coagulation. It has been reported that microvascular damage due to ischemia-reperfusion events in SSc causes a pro-oxidant imbalance (Dziedzic et al., 2023). In the same study, oxidative stress and inflammatory markers were found to be correlated in SSc patients. Evaluation of new biomarkers associated with oxidative stress in LV and potentially endothelial and microcirculatory dysfunction may contribute to elucidating the pathogenesis of LV.

In addition to serum ED markers, impaired FMD was observed in the LV and SSc groups in the current study. Similarly, Yang et al. (2012) found reduced FMD in LV patients. FMD is largely caused by shear stress-induced NO release, and poor FMD values indicate impaired NO production by endothelial cells (Takahashi et al., 2014). Although the current findings revealed ED in the LV patients, the consequences of this finding remain are not known. Thus, the impact of impaired endothelial function on microcirculation was investigated by NFC examination.

Capillary density was significantly lower in patients with LV and SSc than in the control group. In addition, the incidence of several major changes, such as ramification, meandering, and avascular areas, which indicate dysfunctional microcirculation, was higher in LV patients than in healthy control subjects. Overall, it can be hypothesized that ED facilitates the prothrombotic process in individuals with genetic or acquired hypercoagulopathy, leading to thrombus formation and impairment of the microcirculation in the pathogenesis of LV.

An interesting finding of the current study was the remarkable overlap between ED and NFC findings in the LV and SSc groups. The role of ED in SSc has been well documented, and abnormal nailfold capillaries have been included in the ACR-EULAR criteria for the classification of SSc (van den Hoogen et al., 2013). Although the major capillaroscopic changes detected in this study are indicative of microcirculation dysregulation in the LV, the absence of megacapillaries, and capillary densities above the cut-off value for SSc (<7/mm^2) (Smith et al., 2020), do not match a sclerodermoid pattern, which may partly

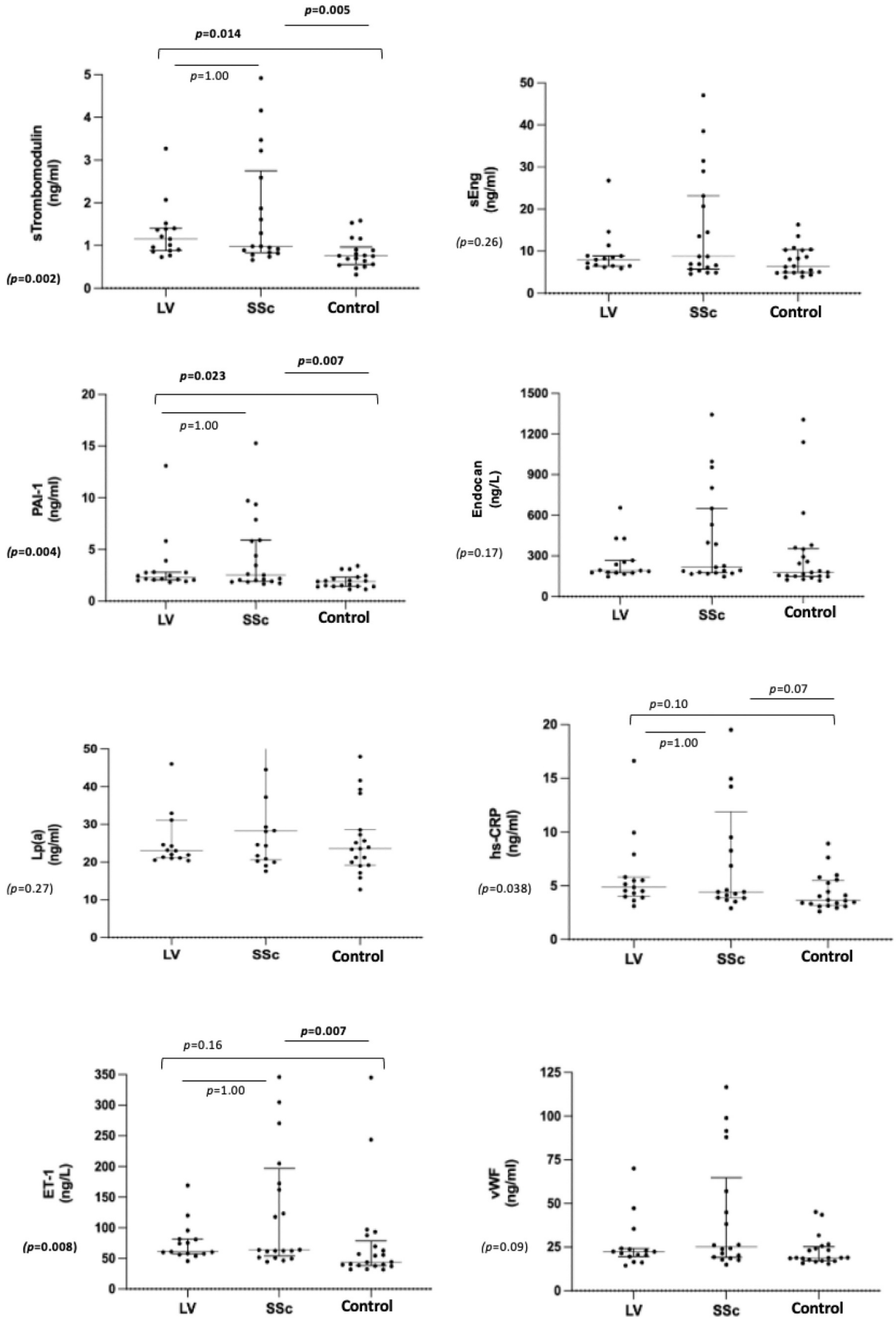

Fig. 1. Serum markers in LV and SSc patients and control subjects.
Datapoints indicate individual measurements, while horizontal lines show median values (IQR).
LV: Livedoid vasculopathy; SSc: Systemic sclerosis; sEng: Soluble endoglin; PAI-1: Plasminogen activator inhibitor; Lp(a): Lipoprotein a; hs-CRP: High-sensitivity C-reactive protein; ET-1: Endothelin-1; vWF: von Willebrand factor.

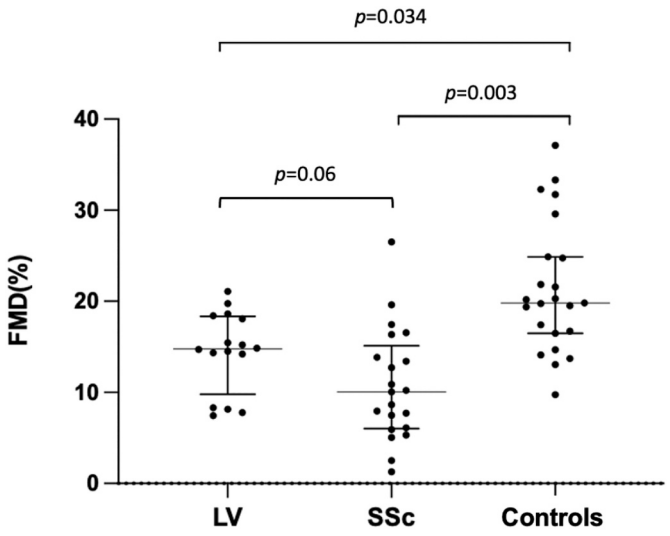

Fig. 2. Comparisons of FMD (%) values in LV, SSc, and controls.
Datapoints indicate individual measurements, while horizontal lines show median values (IQR).
LV: Livedoid vasculopathy; SSc: Systemic sclerosis.

explain the differences in the clinical features of these two disorders.

There is no consensus on the optimal treatment for LV (Coromilas and Micheletti, 2023), and an individualized approach is not possible due to the inability to stratify the patients based on predominant pathological pathways. Hence, the results of the current study may have practical implications. The evaluation of patients with LV may be optimized by screening for hypercoagulopathy and NFC. Mild patients who do not exhibit abnormalities in the screening can be treated with first-line modalities, such as pentoxifilline, ASA, and hydroxychloroquine. For patients with high PAI-1 levels and 4G/4G homozygosity, t-PA treatment can be given priority (Deng et al., 2006). Patients having multiple pathologies related to a prothrombotic state and nonspecific changes in NFC, can be treated with heparin or rivaroxoban. Meanwhile, patients exhibiting a sclerodermoid pattern in NFC can be treated with calcium channel blockers and hydroxychloroquine. Severe patients with signs of thrombophilia and ED can be treated using a combined approach, including rivaroxoban/ low-molecular-weight heparin and intravenous immunoglobulin/ baricitinib/ tumor necrosis factor inhibitors (Kofler et al., 2021; Song and Tu, 2022; Gao and Jin, 2022).

Moreover, there is a need for a valid scoring system to improve LV follow-up. Consequently, the correlation of LVAS with serum ED markers and capillaroscopic findings was investigated. However, no significant correlation was found (all $p > 0.05$), implying that clinical parameters may not fully reflect pathophysiological processes.

The most important limitation of the current study was its small sample size. Although the study groups were matched in terms of comorbid diseases, possible confounding effects of comorbidities cannot be ruled out. In addition, a statistical limitation of the study is that a multiple regression model could not be established due to the sample size and inadequate normality distribution for the number of confounding factors.

In conclusion, LV is associated with thrombophilia, impaired vascular endothelial function, and microcirculation, and sTM and PAI-1 may be indicators of ED in patients with LV. In individuals with hypercoagulability, ED may be associated with a prothrombotic process, impaired microcirculation, tissue ischemia, and necrosis, as seen in LV. It is crucial to shed light on the pathogenesis of LV to inform the development of new treatment modalities. There is a need for further studies with larger sample sizes, to examine different pathophysiological mechanisms of LV while excluding confounding factors.

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IRB approval status

Reviewed and approved by IRB (Marmara University Ethics Committee); approval #09.2021.772.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

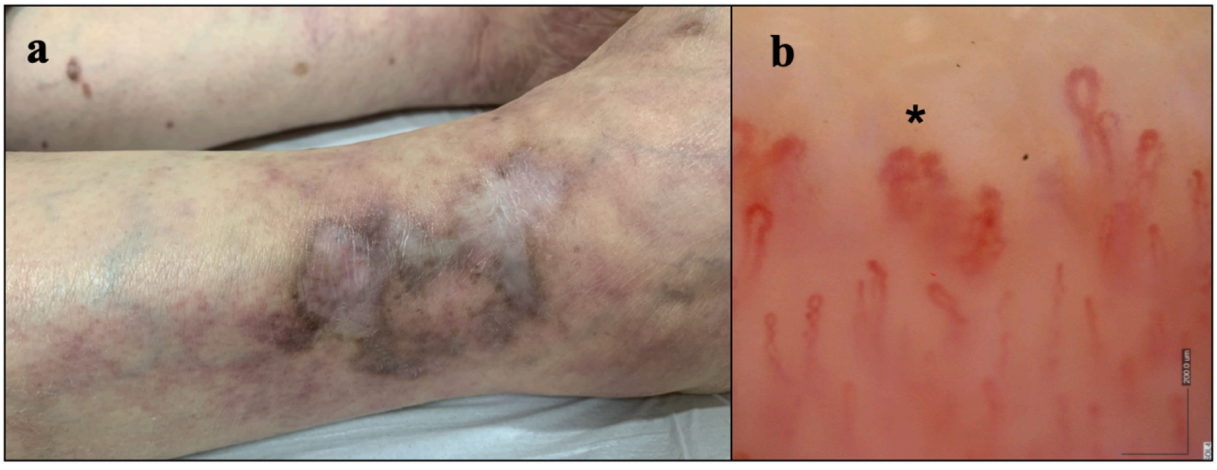

Fig. 3. Clinical and NFC images of a patient with LV
Livedo racemosa and atrophie blanche lesions on the leg of a 47-year-old female patient with LV.
(b) Capillary irregularity and buschy capillary (*) on capillaroscopy (x220 magnification).
LV: Livedoid vasculopathy; NFC: Nailfold capillaroscopy.

Table 4
Comparison of groups in terms of capillary parameters.

Index		LV	SSc	Control	*p*
		%/ Median (25p-75p)	%/ Median (25p-75p)	%/ Median (25p-75p)	
Capillary density		7 (6–8)	6 (4.5–6.5)	10 (9–11)	**<0.001**[a]
Capillary diameter		14.78 (13.38–17.5)	24.12 (18.72–40.85)	15.09 (13.2–16.18)	**<0.001**[b]
Avascularity	Absent	12 (75)	5 (20.8)	23 (100)	<0.001
	Present	**4 (25)**	19 (79.2)	**0 (0.00)**	
Tortuosity	None	0 (0.00)	5 (20.8)	7 (30.4)	0.001
	<%33	4 (25)	2 (8.3)	10 (43.5)	
	%33–66	6 (37.5)	4 (16.7)	5 (21.7)	
	> %66	**6 (37.5)**	13 (54.2)	**1 (4.4)**	
Crossing	None	0 (0.00)	1 (4.2)	9 (39.1)	<0.001
	<2	10 (62.5)	8 (33.3)	13 (56.5)	
	>2	**6 (37.5)**	15 (62.5)	**1 (4.4)**	
Dilatation	None	11 (68.75)	5 (20.8)	23 (100)	<0.001
	<%33	**4 (25)**	12 (50)	**0 (0.00)**	
	%33–66	1 (6.25)	0 (0.00)	0 (0.00)	
	>%66	0 (0.00)	7 (29.2)	0 (0.00)	
Buschy capillary	None	15 (93.75)	18 (75)	23 (100)	0.013
	%33–66	1 (6.25)	6 (25)	0 (0.00)	
Megacapillary	None	16 (100)	15 (62.5)	23(100)	<0.001
	%33–66	0 (0.00)	9 (37.5)	0 (0.00)	
Meandering	None	9 (56.25)	14 (58.3)	22 (95.65)	0.01
	<%33	3 (18.75)	5 (20.8)	1 (4.35)	
	%33–66	0 (0.00)	1 (4.2)	0 (0.00)	
	>%66	**4 (25)**	4 (16.7)	**0 (0.00)**	
Ramification	None	**4 (25)**	0 (0.00)	**21 (91.3)**	<0.001
	<%33	**7 (43.75)**	16 (66.7)	**2 (8.7)**	
	%33–66	5 (31.25)	0 (0.00)	0 (0.00)	
	>%66	0 (0.00)	8 (33.3)	0 (0.00)	
Microhemorrhages	None	13 (81.25)	9 (37.5)	21 (91.3)	<0.001
	<%33	3 (18.75)	15 (62.5)	2 (8.7)	
Subpapillary venous plexus	NV	12 (75)	18 (75)	15 (65.2)	0.77
	Visible	4 (25)	6 (25)	8 (34.8)	

Bold *p* values indicate significance with Kruskal–Wallis test.
[a,b]Dunn's post-hoc test; *p* value[a]: LV-Control: 0.014, SSc-Control < 0.001, LV-SSc: 0.09; *p* value[b:] LV-Control: 1.0, SSc-Control:<0.001, LV-SSc:<0.001.
The frequencies of categorical variables that were statistically significant in the pairwise comparison between the LV and control groups are indicated with bold (Fisher's exact test).
LV: Livedoid vasculopathy; SSc: Systemic sclerosis; NV: Non-visible;; (25p-75p): 25th–75th percentile.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mvr.2023.104591.

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