Structural and functional changes in the microcirculation of lepromatous leprosy patients - Observation using orthogonal polarization spectral imaging and laser Doppler flowmetry iontophoresis

Curt Treu[1], Maria das Graças Coelho de Souza[1] *, Omar Lupi[2], Fernando Lencastre Sicuro[1], Priscila Alves Maranhão[1], Luiz Guilherme Kraemer-Aguiar[1,3], Eliete Bouskela[1]

1 Laboratório de Pesquisas Clínicas e Experimentais em Biologia Vascular, Centro Biomédico, Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Rio de Janeiro, Brazil, 2 Departamento de Dermatologia, Universidade Federal do Estado do Rio de Janeiro (Uni-Rio), Rio de Janeiro, Rio de Janeiro, Brazil, 3 Ambulatório de Obesidade, Policlínica Piquet Carneiro, Departamento de Medicina Interna, Faculdade de Ciências Médicas, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

* mgcsouza@gmail.com

Abstract

Leprosy is a chronic granulomatous infection of skin and peripheral nerves caused by *Mycobacterium leprae* and is considered the main infectious cause of disability worldwide. Despite the several studies regarding leprosy, little is known about its effects on microvascular structure and function *in vivo*. Thus, we have aimed to compare skin capillary structure and functional density, cutaneous vasomotion (spontaneous oscillations of arteriolar diameter), which ensures optimal blood flow distribution to skin capillaries) and cutaneous microvascular blood flow and reactivity between ten men with lepromatous leprosy (without any other comorbidity) and ten age- and gender-matched healthy controls. Orthogonal polarization spectral imaging was used to evaluate skin capillary morphology and functional density and laser Doppler flowmetry to evaluate blood flow, vasomotion and spectral analysis of flowmotion (oscillations of blood flow generated by vasomotion) and microvascular reactivity, in response to iontophoresis of acetylcholine and sodium nitroprusside. The contribution of different frequency components of flowmotion (endothelial, neurogenic, myogenic, respiratory and cardiac) was not statistically different between groups. However, endothelial-dependent and -independent vasodilatations elicited by acetylcholine and sodium nitroprusside iontophoresis, respectively, were significantly reduced in lepromatous leprosy patients compared to controls, characterizing the existence of microvascular dysfunction. These patients also presented a significant increase in the number of capillaries with morphological abnormalities and in the diameters of the dermal papilla and capillary bulk when compared to controls. Our results suggest that lepromatous leprosy causes severe microvascular dysfunction and significant alterations in capillary structure. These structural and functional

changes are probably induced by exposure of the microvascular bed to chronic inflammation evoked by the *Mycobacterium leprae*.

Introduction

Leprosy is a chronic granulomatous infection of skin and peripheral nerves caused by the *Mycobacterium leprae* [1]. This obligatory intracellular pathogen causes nerve damage that affects sensory, motor and autonomic fibers resulting in disabilities and deformities [2,3]. In the last 50 years the prevalence of leprosy has reduced; however, the transmission still occurs and it remains an important public health problem worldwide [4]. According to the World Health Organization, only in 2011, the global incidence of leprosy was 219,075 with India and Brazil leading the number of new cases [5].

Despite the several studies regarding leprosy epidemiology, immunological reactions, peripheral nerve infection and clinical features, little is known about its effects on the microcirculation *in vivo*.

The microcirculation is the part of the vascular bed that encompasses vessels with diameters inferior to100 μm (arterioles, capillaries and venules), where delivery of oxygen and nutrients to tissues and removal of cellular waste as well as the control of peripheral vascular resistance occur [6,7].

Arterioles display spontaneous rhythmic variations of vessel lumen, the so called vasomotion [8] that elicits blood flow oscillations, termed flowmotion, [9] and regulates microvascular blood flow distribution in the skin microvasculature [7].

Investigation of human vasomotion and its consequent flowmotion is possible through the spectral analysis of skin laser Doppler flowmetry (LDF) signal [9]. Moreover, LDF can also be used to investigate endothelial and microvascular function when associated to iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). The iontophoresis allows the transdermal delivery of these vasodilators across the skin using a weak current [10,11]. The subsequent increase in blood flow is, then, recorded by the LDF system.

The analysis of the frequency interval of LDF signal enables the evaluation of five different mechanisms that control flowmotion: endothelial (0.01–0.02 Hz), neurogenic (0.02–0.06 Hz), myogenic, related to vascular smooth muscle cells (VSMC) (0.06–0.15 Hz), respiratory (0.15–0.4 Hz) and cardiac, associated to heart frequency (0.4–1.6 Hz) [12].

The assessment of endothelial and microvascular function in cutaneous microvascular bed constitutes an important noninvasive tool for precocious detection of cardiovascular risk in clinical settings. Since microvascular dysfunction is a systemic process that occurs in a similar manner in the entire body [13,14] it is possible to consider that the impairment of microvascular function observed in the skin microvasculature also occurs in the coronary microcirculation, for example.

The orthogonal polarization spectral (OPS) imaging technique allows real time assessment of the skin microcirculation up to 3 mm in depth [15,16,17,18,19] *in vivo*. This technique enables the evaluation of functional capillary density, capillary morphology, dermal papilla diameter, capillary bulk diameter and capillary diameter.

The present study aimed to assess, *in vivo*, functional and morphological microcirculatory variables in patients with lepromatous leprosy (LL) and healthy age- and gender-matched controls by means of LDF (associated or not to ACh and SNP iontophoresis) and OPS imaging technique in order to evidence microvascular differences between these two groups.

Materials and methods

This is a cross-sectional study approved by the Ethics Committee of the Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro (2405-CEP/HUPE) performed according to principles outlined in the Declaration of Helsinki (Clinical Trials.gov registration no. NCT02085317).

Subjects

Ten LL patients under proper treatment were recruited and compared to 10 age- and gender-matched healthy controls. All participants of the study have met the following inclusion criteria and signed the written informed consent.

Inclusion criteria. Males with or without lepromatous leprosy, with ages between 20 and 60 years old, body mass index (BMI) between 18 and 29.9 kg/m^2, Fitzpatrick's phototype between I and IV [20], able to follow given directions and to attend microvascular assessments and be under treatment for leprosy (for LL patients only) were included in the study.

Exclusion criteria. Females, subjects with previously confirmed diagnostic of hypertension, diabetes mellitus, BMI $\geq$30 kg/m^2, past or present history of tabagism and ages under 20 and over 60 years old were excluded.

Study recruitment. The recruitment occurred between July 20th, 2009 and April 27th, 2012. For LL group, 112 patients were recruited, but only 61 patients came to the laboratory for medical appointment and examinations. From this total, 51 were considered ineligible for the study due to its rigid exclusion criteria. Obesity *per se* was the most common cause for exclusion (18 patients), obesity and hypertension accounted for exclusion of 13 patients, obesity and type II diabetes mellitus were responsible for 9 exclusions and tabagism was the cause of 11 exclusions.

For the control group, 13 volunteers were recruited. However, one was smoker and two were obese and, therefore, ineligible for the study.

Microvascular assessment

Microvascular assessments occurred from September 8th, 2009 to June 5th, 2012. All participants were asked to arrive at the laboratory after 12 h overnight fast and to abstain from caffeine and alcohol during the last 24 hours. They were accommodated in an acclimatized room (23±1˚C) during 20 minutes before microvascular evaluations. All subjects had their anthropometric variables assessed and blood pressure evaluated before the examination to ensure that they met the prerequisites for inclusion.

Skin microvascular blood flow and vasomotion. Skin blood perfusion and vasomotion was evaluated by a LDF apparatus (PeriFlux System PF5000, Perimed AB, Stockholm, Sweden) consisting of a transmission of low-power laser light (780 nm) to the tissue through a fiber optic probe that penetrates 0.4–1.0 nm. The light penetration allows the assessment of net red blood cell flow in arbitrary perfusion units (PU) that corresponds to the concentration of moving blood cells and their velocity, in arterioles, capillaries and venules and in anastomosis of deeper blood vessels in dermal layers, within an area of 1 mm^2 [21]. The LDF signal was recorded continuously during 20 min by an interfaced computer equipped with Perisoft software (PSW 2.50, Perimed AB, Stockholm, Sweden) in order to assess skin blood flow and vasomotion. For these measurements a probe was positioned at the dorsum of the left wrist.

For the fast Fourier transform analysis of LDF signal, the Perisoft software (PSW version 2.50, Perimed AB, Stockholm, Sweden) was used to determine the contribution of different frequency components of flowmotion through the variability of the LDF signal. The frequency spectrum between 0.01 and 1.6 Hz was divided into five frequency intervals: endothelial (0.01–

0.02 Hz), neurogenic (0.02–0.06 Hz), myogenic, related to VSMC activity (0.06–0.15 Hz), respiratory (0.15–0.4 Hz) and cardiac, associated to heart frequency (0.4–1.6 Hz) [12,22]. Mean total amplitude value of the total spectrum as well as the mean amplitude values of each frequency interval were recorded and normalized (absolute amplitude at a particular frequency interval divided by the mean amplitude of the entire spectrum) [23]. Normalized results were then compared between controls and LL patients.

Iontophoresis of acetylcholine and sodium nitroprusside. Endothelium-dependent and -independent vasodilatations were evaluated by LDF combined to iontophoresis of ACh and SNP, respectively. ACh (Acetylcholine, Sigma-Aldrich, Saint Louis, MO, USA) solution at 1% was delivered by nine iontophoretic pulses of 0.1 mA during 20 s with a 60 s interval to the middle phalanx of the second left finger using an anodal current. On the other hand, SNP (sodium nitroprusside, Niprid® 10mg/ml—Biolab, São Paulo, Brazil) was delivered by seven iontophoretic pulses of 0.2 mA during 20 s with a 180 s interval to the middle phalanx of the third left finger using a cathodal current.

During ACh and SNP iontophoresis, it was possible to evaluate cutaneous blood perfusion (in perfusion units—PUs) at baseline and plateau and vasodilatation expressed in absolute values (difference between plateau and baseline in PUs) and in percentage (% of increase from baseline to plateau). These values were compared between controls and LL patients.

Orthogonal Polarization Spectral (OPS) imaging assessment. After acclimatization, cutaneous microcirculation of patients and controls were assessed by OPS imaging (Cytoscan, Cytometrics Inc, Philadelphia, PA, USA) at three different points of the skin lesion area (for LL patients) and of healthy skin area (for controls), according to criteria recommended by De Backer [24]. Images were recorded for 10 seconds at each point and evaluated afterwards using the Cap-Image v7.2 software.

Using OPS imaging, we have evaluated FCD (number of capillaries with flowing red blood cells/mm^2), DPD (μm, to quantify edema), CD (μm, to detect capillary enlargement), CBD (μm, to assess its degree of change) and CM (percentage of abnormal capillaries per field) of the participants. These variables have already been evaluated in other studies of our group [18,25,26,27].

Statistical analysis

Clinical and anthropometric variables are presented as mean ± SD and unpaired t test was used to assess statistical differences between them. Results of microcirculatory variables were presented as median [interquartile range] and Mann-Whitney U test was used for comparisons between groups. For all statistical analysis, the Graph Pad Prism 5.0 software (Graph Pad Software Inc., San Diego, CA, USA) was used. P value of less than 0.05 was considered significant.

Results

Characteristics of control and lepromatous leprosy groups

Table 1 presents anthropometric and clinical characteristics of control and lepromatous leprosy groups. Healthy participants and LL patients did not have obesity or hypertension and there was no significant difference between groups in age, height, weight, BMI and systolic blood pressure (SBP). Although all patients were normotensive, the diastolic blood pressure (DBP) in LL patients was statistically higher compared to controls.

Skin microvascular blood flow and flowmotion

At rest, skin blood perfusion was not significantly different between controls and LL patients. Spectral analysis of flowmotion frequency components (endothelial, neurogenic, myogenic,

Table 1. Clinical and anthropometrical characteristics of groups (mean ±SD).

	Control group (n = 10)	LL group (n = 10)	*P* value
Age (years)	32.7±1.6	33.5±1.8	0.22
Weight (kg)	69.3±9.8	70.1±6.0	0.83
Height (m)	1.73±0.06	1.71±0.05	0.39
BMI (kg/m^2)	23.1±2.6	24.1±1.8	0.37
SBP (mmHg)	111.2±8.5	117.6±5.6	0.06
DBP (mmHg)	71.2±7.7	78.8±6.0	0.02

LL group—Lepromatous leprosy group; BMI—body mass index, SBP—systolic blood pressure; DBP—diastolic blood pressure.

respiratory and cardiac) did not show any significant differences between controls and LL patients. These results are depicted on Table 2.

Iontophoresis of acetylcholine and sodium nitroprusside

As demonstrated on Table 3, there are no significant differences in blood flow between controls and LL patients, before ACh and SNP iontophoresis.

During ACh iontophoresis, controls presented a significant increase in microvascular blood flow in relation to LL patients, reaching the plateau with significantly fewer ACh doses (Fig 1). Absolute and percentage values of blood flow, reflecting endothelial-dependent vasodilatation, were significantly higher in controls compared to LL patients (Table 3).

During SNP iontophoresis, controls presented a significant increase in microvascular blood flow in relation to LL patients, reaching the plateau with significantly fewer SNP doses (Fig 2). Absolute and percentage values of blood flow, corresponding to endothelial-independent vasodilatation, were significantly greater in controls compared to LL patients (Table 3).

Orthogonal Polarization Spectral (OPS) imaging assessment

Controls and LL patients did not present significant differences concerning FCD and CD. However, LL patients presented a significant increase in DPD, CBD and CM compared to controls (Table 4).

Discussion

The novel findings of the present study are: (a) LL patients present a significant impairment of endothelium-dependent and -independent microvascular reactivity in response to ACh and

Table 2. Skin microvascular perfusion and flowmotion spectral analysis in controls and in Lepromatous Leprosy (LL) patients measured by laser Doppler flowmetry.

	Controls (n = 10)	LL patients (n = 10)	*P* value
Total Spectrum (PU)	5.587 [4.545–7.648]	6.760 [5.051–9.201]	0.393
Vasomotion (PU/Hz)			
Endothelial	0.4457 [0.3793–0.5214]	0.4077[0.2277–0.4844]	0.481
Neurogenic	0.2824 [0.2479–0.3358]	0.2557 [0.2446–0.3295]	0.579
Myogenic	0.1477[0.1053–0.2162]	0.1624 [0.1062–0.2955]	0.684
Respiratory	0.04968 [0.03397–0.1035	0.06111 [0.03390–0.1314]	0.529
Cardiac	0.04462[0.02984–0.06420]	0.06975 [0.05322–0.09371]	0.089

Data are expressed as median [interquartile range]. PU: perfusion units.

Table 3. Microvascular measurements before and during iontophoresis using laser Doppler flowmetry.

	Controls (n = 10)	LL patients (n = 10)	*P* value
ACh-mediated vasodilation			
Baseline skin perfusion, PU	45.52 [32.20–57.73]	46.94 [28.69–58.85]	1.000
Plateau, PU	203.4 [165.5–255.4]	70.01 [42.73–78.25]	0.0002
Number of doses to reach Plateau	5.50[5.00–7.00]	8.50[8.00–9.00]	0.0003
Absolute increase, PU	147.5 [116.6–213.8]	12.40 [8.550–30.15]	0.0001
Percentage increase, %	287.4 [247.9–509.8]	46.40 [17.90–83.40]	0.0001
SNP-mediated vasodilation			
Baseline skin perfusion, PU	29.76 [22.81–56.98]	30.70 [19.59–42.48]	1.000
Plateau, PU	145.8 [121.5–194.0]	45.44 [32.35–64.06]	0.0001
Number of doses to reach Plateau	5.00[4.00–5.25]	6.50[6.00–7.00]	0.0005
Absolute increase, PU	119.1 [85.88–163.7]	13.25 [5.250–21.55]	0.0001
Percentage increase, %	376.5 [257.4–564.8]	55.90 [32.98–78.40]	0.0001

Data are expressed as median [interquartile range]. LL: lepromatous leprosy. ACh: acetylcholine. SNP: sodium nitroprusside. PU: perfusion units.

https://doi.org/10.1371/journal.pone.0175743.t003

SNP iontophoresis, respectively, when compared to controls; (b) LL patients did not present significant changes in none of the five frequency components of flowmotion (endothelial, neurogenic, myogenic, respiratory and cardiac), in comparison to controls and; (c) LL patients presented significant alterations in capillary structure, such as increased DPD, CBD and CM, when compared to controls.

This is the first time, to our knowledge, that an investigation evaluates *in vivo* the impact of LL on structure and function of cutaneous microcirculation. There are, however, two other studies that investigated microvascular blood flow but not microvascular function *per se*. In one study, microvascular blood flow of skin lesions was evaluated by LDF in leprosy patients (not in LL patients) to demonstrate that this noninvasive technique can be useful and clinically acceptable to determine the severity of the hyperemia response, an early indicator of reversal reaction during chemotherapy in these patients [28]. In the other study, the vasomotor reflex, defined by the vasoconstrictor response to autonomic stimuli, was assessed in finger and toe tips by laser Doppler flow temperature technique in order to show skin microvascular dysautonomia in leprosy patients [29].

Turkel and coworkers [30] analyzed, by electron microscopy, the microvascular structure of skin biopsies of LL patients and reported ultrastructural changes in the dermal microvasculature that included: endothelial cell swelling and hypertrophy, increased endothelial cytoplasmic processes, presence of phagocytized, membrane-bound intra-endothelial *M. leprae* and perivascular dermal inflammatory infiltrate formed by lymphocytes, macrophages and mast cells. Another study, using immunohistochemical staining, showed that LL patients presented dense and tortuous mesh of microvessels among *M. leprae*-glutted macrophages [31]. Based on these observations and considering that endothelial dysfunction precedes structural changes in blood vessels [10] we have speculated that endothelial dysfunction occurs in early stages of LL.

Endothelial cells are key regulators of vascular homeostasis [32]. Due to its localization between blood and VSMC, the endothelium perceives physical and chemical *stimuli* from blood, vascular wall and interstitium and respond with expression and release of several molecules involved in tone regulation, cellular adhesion, coagulation, fibrinolysis, VSMC proliferation and vascular wall inflammation [33].
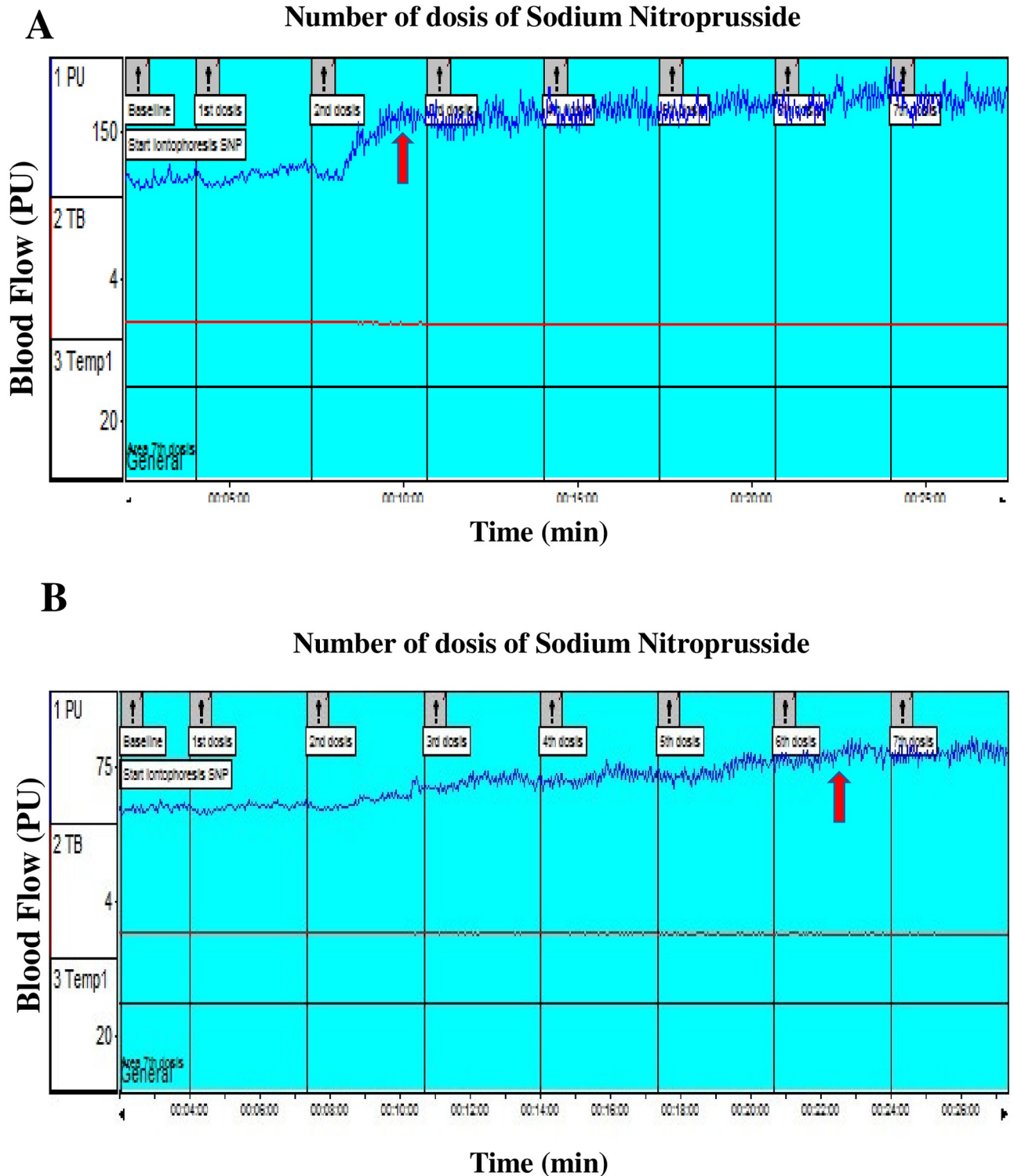
A

B
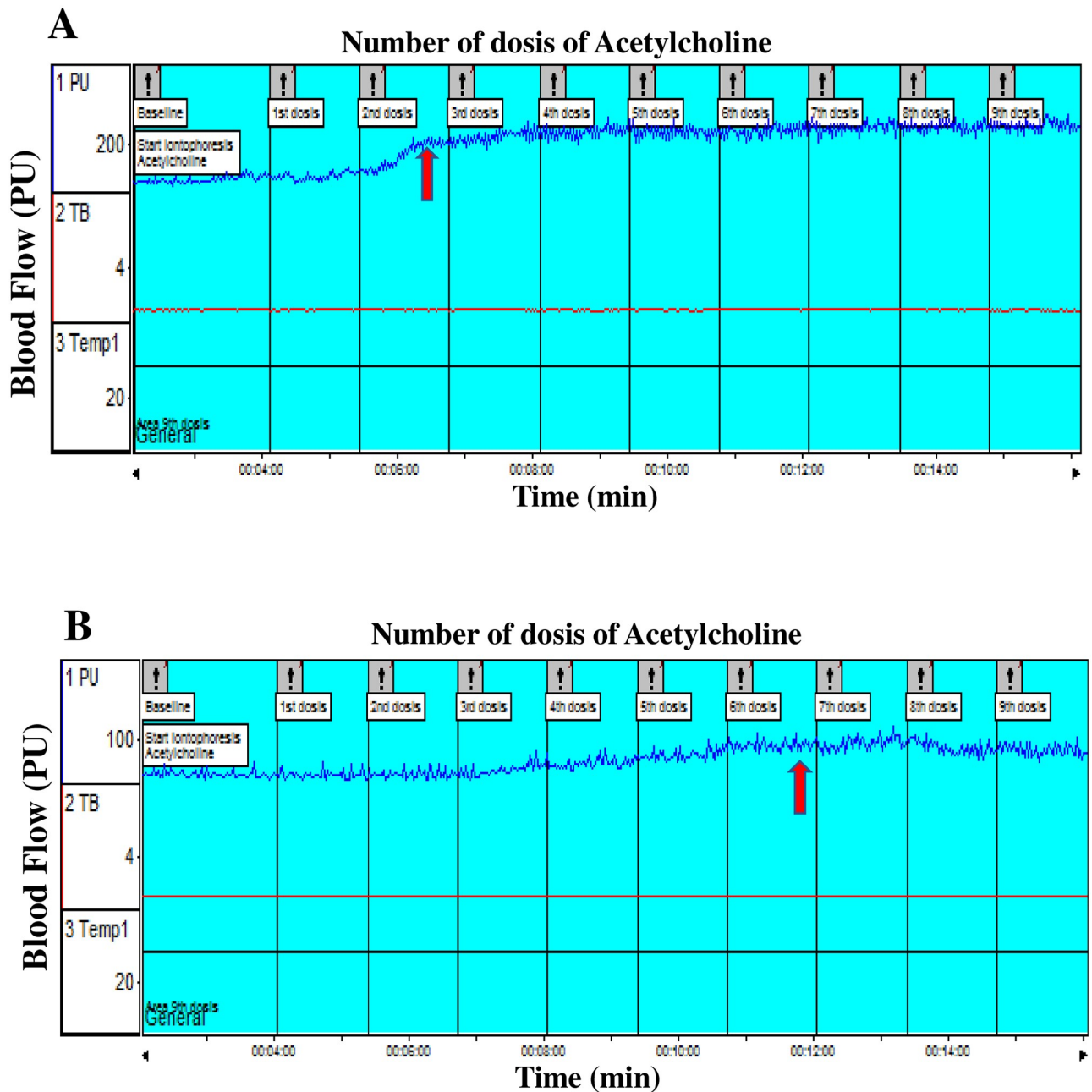
Fig 1. Acetylcholine intophoresis. Microvascular skin blood flow during acetylcholine iontophoresis (A) in controls and (B) in lepromatous leprosy patients. The arrows indicate where the plateau is reached.

Endothelial dysfunction is characterized by decreased bioavailability of NO with concomitant increase of endothelium-derived vasoconstrictors, resulting in reduction of endothelium dependent vasodilatation [10,34].

In our study, LL patients presented significant reduction of endothelium- dependent vasodilatation, evidenced by significant impairment of ACh-induced increase in blood flow during iontophoresis in comparison to control group. ACh promotes the release of endothelium-dependent vasodilators including NO [35] produced via endothelial nitric oxide synthase

Fig 2. Iontophoresis of sodium nitroprusside. Microvascular skin blood flow during sodium nitroprusside iontophoresis (A) in controls and (B) in lepromatous leprosy patients. The arrows indicate where the plateau is reached.

Table 4. Orthogonal polarization spectral imaging of skin microcirculation.

	Controls (n = 10)	LL patients (n = 10)	*P* value
FCD (capillaries/mm^2)	38.38 [36,02–41.95]	39.34 [34.98–46.10]	0.684
DPD (µm)	85.73 [81.12–92.59]	100.4 [90.22–112.7]	0.005
CBD (µm)	53.01[47.35–56.28]	58.85 [53.99–72.07]	0.043
CD (µm)	7.000 [6.538–7.525]	8.033 [6.742–9.542]	0.123
CM (%)	6.850 [2.938–10.83]	35.50 [8.750–73.42]	0.014

Data are expressed as median [interquartile range]. LL: lepromatous leprosy. FCD: functional capillary density (number of capillaries with flowing red cells/ mm^2). DPD dermal papilla diameter (µm). CBD, capillary bulk diameter (µm). CD: capillary diameter (µm). CM: capillary morphology (% of abnormal capillaries).

https://doi.org/10.1371/journal.pone.0175743.t004

(eNOS or NOS3) activation. Once produced by the endothelium, NO diffuses toward VSMC where it induces cGMP production [36]. Increased levels of cGMP reduce intracellular Ca^{2+} concentration [37] as well as the sensitivity of the contractile apparatus to Ca^{2+} [38], causing relaxation of VSMC.

The significant reduction of ACh-mediated increase in blood flow in LL group, during iontophoresis, can also be explained by the impairment of Lewis triple-flare response (also called nerve axon reflex-mediated vasodilatation). In this mechanism, ACh stimulates the release of the vasodilators calcitonin gene-related peptide and substance P by nociceptive C-type fibers of the somatic peripheral nervous system in the epidermis [14]. Since these fibers are the earliest to be affected by *M. leprae* [39], this mechanism of vasodilatation could be compromised in LL patients.

The present study also demonstrated significant impairment of endothelium-independent vasodilatation, during SNP iontophoresis. SNP, an exogenous donor of NO, reacts with sulfhydryl containing compounds in the tissues [40] to produce and release NO, stimulating VSMC relaxation [10].

The observed significant impairment of ACh and SNP-induced increase in blood flow, demonstrated that LL severely inhibited endothelial-dependent and -independent vasodilatory mechanisms, characterizing the presence of microvascular dysfunction in these patients. Since microvascular dysfunction is a systemic phenomenon that occurs in similar way in the entire body [13,14] we can speculate that the impairment of microvascular function observed in the skin microvasculature occurs in other microvascular beds, including the coronary microcirculation.

The analysis of frequency components of flowmotion (endothelial, neurogenic, myogenic, respiratory and cardiac) did not show statistical differences between LL patients and controls.

OPS imaging demonstrated that LL promotes significant alterations in capillary architecture evidenced by increased DPD, CBD and CM compared to controls. Since a significant rise of DPD indicates the onset of the inflammatory process [41], we believe that these structural changes are consequence of immune reactions induced by the disease.

Due to inflammation of peripheral autonomic nerves promoted by *M. leprae*, we should expect a significant reduction of the neurogenic component of flowmotion in LL patients, which was not observed even in LL patients with dermal anesthesia. A possible explanation for this could be the diffuse alteration in innervation in LL patients [42]. Thus, we presume that the peripheral autonomic nerves in the skin areas analyzed were not compromised by *M. leprae* infection yet.

Another possible reason could be the low reproducibility of the method [43], which demands higher number of participants, in special LL patients, to show possible statistical

differences. LL patients, due to social stigma, had difficulties to accept the study invitation and to attend the examinations (we invited 112 patients and only 61 came to the Laboratory). Furthermore, it was difficult to have LL patients eligible for the study due to rigorous inclusion criteria.

On the other hand, despite the small number of LL patients, we have shown an impairment of microvascular reactivity in response to ACh and SNP iontophoresis in the LL group with very expressive statistical significance and significant differences in CBD, DPD and CM in LL patients compared to controls. We considered these data enough to demonstrate the presence of microvascular dysfunction and structural abnormalities in capillaries in LL and decided not to insist in the recruitment of new patients due to the difficulties mentioned above.

In order to exclude any confounding factors from our analysis we did not include hypertensive, diabetic, obese, aged patients and smoker individuals in the study since hypertension [44,45], diabetes mellitus [44,46] obesity [47], age [48,49] and tobacco [50,51,52,53] are factors that alter microvascular reactivity and blood flow evaluated by LDF. OPS imaging has also shown microvascular changes in smokers [54] and hypertensive patients [46].

The interesting findings of this study encouraged our group to investigate whether the impaired microvascular reactivity and capillary morphology abnormalities, observed in LL patients, could also be found in other forms of leprosy, especially in tuberculoid leprosy, the other polar form of the disease.

In conclusion, our results suggest that lepromatous leprosy causes severe microvascular dysfunction and significant alterations in capillary structure. The microcirculatory structural and functional changes found in these patients are probably induced by exposure of the microvascular bed to chronical inflammation evoked by the *Mycobacterium leprae*.

Supporting information

S1 Table. Anthropometric and clinical characteristics of the control group.
(DOCX)

S2 Table. Anthropometric and clinical characteristics of lepromatous leprosy group.
(DOCX)

S3 Table. Absolute amplitude of vasomotion frequency components. Controls.
(DOCX)

S4 Table. Absolute amplitude of vasomotion frequency components. Lepromatous leprosy patients.
(DOCX)

S5 Table. Acetylcholine iontophoresis. Controls.
(DOCX)

S6 Table. Acetylcholine iontophoresis. Lepromatous leprosy patients.
(DOCX)

S7 Table. Sodium nitroprusside iontophoresis. Controls.
(DOCX)

S8 Table. Sodium nitroprusside iontophoresis. Lepromatous leprosy patients.
(DOCX)

S9 Table. Orthogonal polarized spectral imaging. Controls.
(DOCX)

S10 Table. Orthogonal polarized spectral imaging. Lepromatous leprosy patients. (DOCX)

Author Contributions

Conceptualization: CT OL EB.

Formal analysis: FS.

Funding acquisition: EB.

Investigation: CT PM.

Methodology: CT OL LGKA.

Project administration: CT OL EB.

Resources: CT.

Supervision: EB.

Validation: CT OL PM LGKA.

Visualization: CT.

Writing – original draft: MdGS.

Writing – review & editing: MdGS EB.

References

1. Walker SL, Lockwood DNJ. Leprosy. Clin Dermatol. 2007; 25:165–172. https://doi.org/10.1016/j.clindermatol.2006.05.012 PMID: 17350495

2. Britton WJ, Lockwood DN. Leprosy. Lancet. 2004; 363:1209–1219. https://doi.org/10.1016/S0140-6736(04)15952-7 PMID: 15081655

3. Agrawal A, Pandit L, Dalal M, Shetty JP. Neurological manifestations of Hansen's disease and their management. Clin Neurol Neurosurg. 2005; 107:445–454. https://doi.org/10.1016/j.clineuro.2005.03.007 PMID: 16202816

4. Rodrigues LC, Lockwood DNJ. Leprosy now: epidemiology, progress, challenges, and research gaps. Lancet Infect Dis. 2011; 11: 464–470. https://doi.org/10.1016/S1473-3099(11)70006-8 PMID: 21616456

5. World Health Organization. WHO Weekly Epidemiological Record: Global leprosy situation. World Health Organization. 2012; 87:317–328. http://www.who.int/wer/5/8/16.

6. Kraemer-Aguiar LG, Laflor CM, Bouskela E. Skin microcirculatory dysfunction is already present in normoglycemic subjects with metabolic syndrome. Metabolism. 2008; 57:1740–6. https://doi.org/10.1016/j.metabol.2008.07.034 PMID: 19013299

7. Jonk AM, Houben AJ, Schaper NC, de Leeuw PW, Serné EH, Smulders YM et al. Meal-related increases in microvascular vasomotion are impaired in obese individuals: a potential mechanism in the pathogenesis of obesity-related insulin resistance. Diabetes Care. 2011; 34 Suppl 2: S342–S348.

8. Bouskela E, Grampp W. Spontaneous vasomotion in hamster cheek pouch arterioles in varying experimental conditions. Am J Physiol. 1992; 262(2 Pt 2):H478–H485. PMID: 1539706

9. Rossi M, Carpi A, Galetta F, Franzoni F, Santoro G. The investigation of skin blood flowmotion: a new approach to study the microcirculatory impairment in vascular diseases? Biomed Pharmacother. 2006; 60:437–442. https://doi.org/10.1016/j.biopha.2006.07.012 PMID: 16935461

10. Turner J, Belch JJ, Khan F. Current concepts in assessment of microvascular endothelial function using laser Doppler imaging and iontophoresis. Trends Cardiovasc Med. 2008; 18:109–16. https://doi.org/10.1016/j.tcm.2008.02.001 PMID: 18555183

11. Tesselaar E, Sjöberg F. Transdermal iontophoresis as an in-vivo technique for studying microvascular physiology. Microvasc Res. 2011; 81:88–96. https://doi.org/10.1016/j.mvr.2010.11.002 PMID: 21070791

12. Stefanovska A, Bracic M, Kvernmo HD. Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. IEEE Trans Biomed Eng. 1999; 46:1230–1239. PMID: 10513128

13. Sax FL, Cannon RO, Hanson C, Epstein SE. Impaired forearm vasodilator reserve in patients with microvascular angina. Evidence of a generalized disorder of vascular function? N Engl J Med. 1987; 317:1366–70. https://doi.org/10.1056/NEJM198711263172202 PMID: 3683470

14. Abularrage CJ, Sidawy AN, Aidinian G, Singh N, Weiswasser JM, Arora S. Evaluation of the microcirculation in vascular disease. J Vasc Surg. 2005; 42:574–81. https://doi.org/10.1016/j.jvs.2005.05.019 PMID: 16171612

15. Cerny V, Turek Z, Parizkova R. Orthogonal polarization spectral imaging. Physiol Res. 2007; 56:141–147. PMID: 16555953

16. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nat Med. 1999; 5:1209–1212. https://doi.org/10.1038/13529 PMID: 10502828

17. Langer S, Born F, Hatz R, Biberthaler P, Messmer K. Orthogonal polarization spectral imaging versus intravital fluorescent microscopy for microvascular studies in wounds. Ann Plast Surg. 2002; 48:646–653. PMID: 12055436

18. Lupi O, Semenovitch IJ, Treu C, Bottino D, Bouskela E. Evaluation of the effects of caffeine in the microcirculation and edema on thighs and buttocks using the orthogonal polarization spectral imaging and clinical parameters. J Cosmet Dermatol. 2007; 6:102–107. https://doi.org/10.1111/j.1473-2165.2007.00304.x PMID: 17524126

19. Schiessler C, Schaudig S, Harris AG, Christ F. Orthogonal polarization spectral imaging-a new clinical method for monitoring of microcirculation. Anaesthesist. 2002; 51:576–579. PMID: 12243045

20. Treu CM, Lupi O, Bottino DA, Bouskela E. Sidestream dark field imaging: the evolution of real-time visualization of cutaneous microcirculation and its potential application in dermatology. Arch Dermatol Res. 2011; 303:69–78. https://doi.org/10.1007/s00403-010-1087-7 PMID: 20972572

21. Buss C, Kraemer-Aguiar LG, Maranhão PA, Marinho C, de Souza Md, Wiernsperger N et al. Novel findings in the cephalic phase of digestion: a role for microcirculation? Physiol Behav. 2012; 105:1082–108. https://doi.org/10.1016/j.physbeh.2011.12.004 PMID: 22197630

22. Serné EH, IJzerman RG, Gans RO, Nijveldt R, De Vries G, Evertz R, et al. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. Diabetes. 2002; 51:1515–1522 PMID: 11978650

23. Kvernmo HD, Stefanovska A, Bracic M, Kirkebøen KA, Kvernebo K. Spectral analysis of the laser Doppler perfusion signal in human skin before and after exercise. Microvasc Res. 1998; 56:173–82. https://doi.org/10.1006/mvre.1998.2108 PMID: 9828155

24. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, et al. How to evaluate the microcirculation: report of a round table conference. Crit Care. 2007; 11: R101. https://doi.org/10.1186/cc6118 PMID: 17845716

25. Lupi O, Semenovitch I, Treu C, Bouskela E. Orthogonal polarization technique in the assessment of human skin microcirculation. Int J Dermatol. 2008; 47:425–431. https://doi.org/10.1111/j.1365-4632.2008.03694.x PMID: 18412856

26. Virgini-Magalhaes CE, Porto CL, Fernandes FF, Dorigo DM, Bottino DA, Bouskela E. Use of microcirculatory parameters to evaluate chronic venous insufficiency. J Vasc Surg. 2006; 43:1037–1044. https://doi.org/10.1016/j.jvs.2005.12.065 PMID: 16678701

27. Lascasas-Porto CL, Milhomens AL, Virgini-Magalhaes CE, Fernandes FF, Sicuro FL, Bouskela E. Use of microcirculatory parameters to evaluate clinical treatments of chronic venous disorder (CVD). Microvasc Res. 2008; 76:66–72. https://doi.org/10.1016/j.mvr.2008.02.002 PMID: 18423497

28. Agusni I, Beck JS, Potts RC, Cree IA, Ilias MI. Blood flow velocity in cutaneous lesions of leprosy. Int J Lepr Other Mycobact Dis. 1988; 56:394–400. PMID: 2971095

29. Wilder-Smith E, Wilder-Smith A, Van Brakel WH, Egger M. Vasomotor reflex testing in leprosy patients, healthy contacts and controls: a cross-sectional study in western Nepal. Lepr Rev. 1996; 67:306–17. PMID: 9033201

30. Turkel SB, Van Hale HM, Rea TH. Ultrastructure of the dermal microvasculature in leprosy. Int J Lepr Other Mycobact Dis. 1982; 50:164–71. PMID: 6889577

31. Antunes SL, Motta E, de Almeida SM, Gallo ME, Nery JA, Lenzi HL. Distinct patterns of microvasculature in the cutaneous lesions of leprosy. Int J Lepr Other Mycobact Dis. 2000; 68:143–51. PMID: 11036494

32. Michiels C. Endothelial cell functions. J Cell Physiol. 2003; 196:430–43. https://doi.org/10.1002/jcp.10333 PMID: 12891700

33. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007; 115:1285–95. https://doi.org/10.1161/CIRCULATIONAHA.106.652859 PMID: 17353456

34. Esper RJ, Nordaby RA, Vilariño JO, Paragano A, Cacharrón JL, Machado RA. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol. 2006; 5:4. https://doi.org/10.1186/1475-2840-5-4 PMID: 16504104

35. Vanhoutte PM, Scott-Burden T. The endothelium in health and disease. Tex Heart Inst J. 1994; 21:62–7. PMID: 8180513

36. Ignarro LJ, Kadowitz PJ. The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. Annu Rev Pharmacol Toxicol. 1985; 25:171–91 https://doi.org/10.1146/annurev.pa.25.040185.001131 PMID: 2988418

37. Cohen RA, Weisbrod RM, Gericke M, Yaghoubi M, Bierl C, Bolotina VM. Mechanism of nitric oxide-induced vasodilation: refilling of intracellular stores by sarcoplasmic reticulum Ca^{2+} ATPase and inhibition of store operated Ca^{2+} influx. Circ Res.1999; 84: 210–219. PMID: 9933253

38. Ungvari Z, Koller A. Selected contribution: NO released to flow reduces myogenic tone of skeletal muscle arterioles by decreasing smooth muscle Ca^{2+} sensitivity. J Appl Physiol (1985). 2001; 91:522–7.

39. Scollard DM, Truman RW, Ebenezer GJ. Mechanisms of nerve injury in leprosy. Clin Dermatol 2015; 33:46–54. https://doi.org/10.1016/j.clindermatol.2014.07.008 PMID: 25432810

40. Grossi L, D'Angelo S. Sodium nitroprusside: mechanism of NO release mediated by sulfhydryl-containing molecules. J Med Chem. 2005; 48:2622–6. https://doi.org/10.1021/jm049857n PMID: 15801852

41. Fagrell B. Structural changes of human capillaries in chronic arterial and venous finsufficiency. Bibl Anat 1981; 20:645–8.

42. Wilder-Smith EP, Wilder-Smith AJ, Nirkko AC. Skin and muscle vasomotor reflexes in detecting autonomic dysfunction in leprosy. Muscle Nerve. 2000; 23:1105–12. PMID: 10883006

43. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol Sci. 2006; 27:503–8. https://doi.org/10.1016/j.tips.2006.07.008 PMID: 16876881

44. de Jongh RT, Serne EH, IJzerman RG, Stehouwer CD. Microvascular function: a potential link between salt sensitivity, insulin resistance and hypertension. J Hypertens. 2007; 25:1887–1893. https://doi.org/10.1097/HJH.0b013e32825e1db7 PMID: 17762653

45. Struijker-Boudier HA, Rosei AE, Bruneval P, Camici PG, Christ F, Henrion D, et al. Evaluation of the microcirculation in hypertension and cardiovascular disease. Eur Heart J. 2007; 28:2834–2840. https://doi.org/10.1093/eurheartj/ehm448 PMID: 17942581

46. Quattrini C, Harris ND, Malik RA, Tesfaye S. Impaired skin microvascular reactivity in painful diabetic neuropathy. Diabetes Care. 2007; 30:655–659. https://doi.org/10.2337/dc06-2154 PMID: 17327336

47. de Jongh RT, Serné EH, IJzerman RG, Jørstad HT, Stehouwer CD. Impaired local microvascular vasodilatory effects of insulin and reduced skin microvascular vasomotion in obese women. Microvasc Res. 2008; 75:256–262. https://doi.org/10.1016/j.mvr.2007.08.001 PMID: 17920639

48. Li L, Mac-Mary S, Marsaut D, Sainthillier JM, Nouveau S, Gharbi T, et al. Age-related changes in skin topography and microcirculation. Arch Dermatol Res. 2006a; 297:412–416.

49. Li L, Mac-Mary S, Sainthillier JM, Nouveau S, de Lacharriere O, Humbert P. Age-related changes of the cutaneous microcirculation in vivo. Gerontology. 2006b; 52:142–153.

50. Monfrecola G, Riccio G, Savarese C, Posteraro G, Procaccini EM. The acute effect of smoking on cutaneous microcirculation blood flow in habitual smokers and nonsmokers. Dermatology. 1998; 197:115–118. PMID: 9732157

51. Pellaton C, Kubli S, Feihl F, Waeber B. Blunted vasodilatory responses in the cutaneous microcirculation of cigarette smokers. Am Heart J. 2002; 144:269–274. PMID: 12177644

52. IJzerman RG, Serne EH, van Weissenbruch MM, de Jongh RT, Stehouwer CD. Cigarette smoking is associated with an acute impairment of microvascular function in humans. Clin Sci (Lond). 2003; 104:247–252.

53. Edvinsson ML, Andersson SE, Xu CB, Edvinsson L. Cigarette smoking leads to reduced relaxant responses of the cutaneous microcirculation. Vasc Health Risk Manag. 2008; 4:699–704. PMID: 18827920

54. Lindeboom JA, Mathura KR, Harkisoen S, van den Akker HP, Ince C. Effect of smoking on the gingival capillary density: assessment of gingival capillary density with orthogonal polarization spectral imaging. J Clin Periodontol. 2005; 32:1208–1212. https://doi.org/10.1111/j.1600-051X.2005.00854.x PMID: 16268996