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Microcirculatory Abnormality Quantification in Patients with Systemic Sclerosis Using Labial Video Capillaroscopy

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Abstract: The aim of this study is to verify labial microcirculation differences among healthy subjects and those with systemic sclerosis (SSc). 35 healthy patients and 35 patients suffering from systemic sclerosis were examined. Labial capillaroscopy was used to investigate the characteristics of microcirculation. For each patient we evaluated visibility, course, tortuosity and the possible presence of microhaemorrhages, average calibre of capillary loops and the number of visible capillary loops per square millimetre. The investigation of the labial mucous was simple, non invasive and repeatable for each patient. In SSc patients it was possible to observe a wide vascular architectural disorganisation, anarchical capillary orientation, morphologic anomalies of the capillary loops, loosening of the U shape, reduced length and increased capillary diameter. This study shows that capillary alterations in patients suffering from SSc, are not restricted to the naifold bed but also occur in the labial mucous microcirculation.

Key words: Oral mucous, capillaroscopy, systemic sclerosis

INTRODUCTION

Capillaroscopy is a non-invasive diagnostic technique fundamental for viewing peripheral circulation and for studying microangiopathies which are the manifestations of numerous pathologies, both at diagnosis and during the monitoring phase of the disease[1,2].

The alterations in the capillaroscopic picture can represent, in fact, the only documentary evidence of an incipient disease. Compared to other pathologies, in which microangiopathies represent exclusively a complication (diabetes mellitus), some collagenopathies, may present microcirculation alterations even in clinically healthy areas, these alterations have been documented by histological examinations during the early stages of this disease[3-5].

Microangiopathy evaluation can be of valuable help in giving prognostic indications within a therapeutic intervention. Since capillaroscopy is non-invasive, unexpensive, easily repeatable and comparable even after years, it permits the monitoring in time of any disease compromising microcirculation[6,7].

Even though the methods used for investigation did not permit a closer study, capillary study remotes to the distant past: only recently, it has been possible to investigate microcirculation in depth with greater diagnostic possibilities.

In the past, the fundamental technique for understanding the semiotics of the capillaries was the vascular fingernail bed examination[5-7]. However the observation, *in vivo*, of the fingernail fold microcirculation is not always easy due to several factors, such as the peculiar anatomical conformation of the fingernail fold, reducing the transparency of the skin[8-10].

Looking at the wide field of clinical literature, it is clear how a capillaroscopy of labial mucous could represent an interesting complementary or alternative technique to fingernail capillaroscopy[3,11-13].

At first, the oral capillaroscopic investigation was carried out with a reflecting microscope connected to a camera. This technique was invalidated due to the fact that the observation was limited to one or at most two operators and that, being an *in vivo* study, the image was never completely still, which made the photographic documentation difficult. Today, the optical probe videomicroscope permits the investigation under uniform lighting at variable magnifications, viewing the capillaries directly on a monitor[5]. Literature concerning capillaroscopy has documented how the various investigation centres differ from one another as to the data concerning position, form, capillary calibre and architectural framework[1,2].

The aim of our study is to compare the labial microcirculation in healthy subjects and in patients suffering from SSc.

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MATERIALS AND METHODS

35 healthy subjects (3 males and 32 females; mean±SD age: 61.23±4.4 years; range: 38-79 years) and 35 patients with SSc (3 males and 32 females; mean±SD age: 61.89±7.7 years; range: 35-72 years) were examined in our laboratory; their demographic characteristics are summarised in Table 1. Healthy subjects were included in the study only if the accurate exam of their medical history and the objective examination of their oral mucosa revealed that they were non smokers. All subjects provided informed consent for the processing and use of their personal medical data in scientific papers, according to the Italian law. All patients suffering from systemic sclerosis fulfilled the diagnostic criteria for scleroderma[14]. Twenty patients were classified as having Limited Cutaneous Sclerosis (LCS) (skin involvement limited to hands, forearms, face) and 15 had Diffuse Cutaneous Sclerosis (DCS) according to the guidelines of Le Roy *et al.*[15]. They were examined by computerised videomicroscopic techniques and related software (Videocap 200-DS medigroup MI)[12,5]. The optical probe videomicroscope is composed of a main unit, to which an optical probe with video-optical terminal is connected and by a high resolution colour monitor to view the examined area. The main unit is made of: - a cold halogen light source emitted by a 100W lamp provided with an electronic device which controls light intensity;-a processing unit for the high definition video signal (420,000 pixels) provided with a colour calibration device.

The probe is equipped with a video-optical terminal containing a high definition video sensor, on which different variable magnification optics from 10X to 1000X can be applied. A technological characteristic of the video-optical terminal is the possibility to focus directly from the handpiece[16,17]. Image digitalization allows for the analysis of the fundamental parameters of microcirculation (calibre and vessel length) and the calculation of the number of capillaries per mm2 of the mucosa examined. The capillaroscopic investigation was carried out with the patients in a sitting position, with the same light source, at the same room temperature (23°C), in the morning, by the same operator and repeated twice for each examined area. The examined area was always the same for each patient:-the fraenum area of the lower lip. Two independent observers examined all the images. The intraobserver and interobserver variability was assessed with the two observers evaluating twice the same randomly selected images.

Table 1: Demographic characteristics of subjects enrolled in the study

	Healthy subjects (n= 35)	Scleroderma subjects (n= 35)	Significance[a]
M/F ratio	3/32	3/32	
Age (mean±SD)	61.23±4.4	61.8±7.7	NS
Range	38-79	35-72	

SD Standard Deviation [a]Differences between healthy and scleroderma patients were tested by Mann-Whitney U test; NS Not significant (p>0.01)

The following static parameters were used:

- Nonparametric data: capillary loop visibility (score from 1 to 4): 1) simple focusing - within 30 seconds from the beginning of the examination; 2) average focusing - over 30 seconds and within 2 min; 3) difficult focusing - over 2 min; 4) impossible focusing; orientation regarding the surface (score A, B or AB): A) capillary loop course parallel to the surface; B) capillary loop course perpendicular to the surface; AB) both parallel and perpendicular; capillary tortuosity (score from 0 to 3): 0) absence of crossing in the capillary loops; 1) presence of crossing; 2) greater presence of crossing; 3) complete distortion of the capillary loops; microhaemorrhages (score 0 or 1): 0) absence; 1) presence; characteristics of the capillary loops (score 0 or 1): 0) absence; 1) presence.
- Parametric data: number of visible capillary loops in every square millimetre (value obtained from the average of the two observations for each examined area); capillary loop calibre (values obtained from the average of the two observations for each examined area).

Regarding the parametric data, it must be underlined that they originate from the software connected to the videocapillaroscope. The system is properly calibrated: to every optical magnification corresponds an exact metric pixel value in the digitalized image; therefore, the capillary calibre can be measured with considerable precision. All patients involved in the study kept a dietary diary for a period of three months, in order to evaluate any dietary differences possibly affecting the oral capillary pattern observed. The statistical significance of the differences between healthy and SSc subjects was checked with the Student's t test for independent samples regarding parametric data and with the Mann-Whitney test (MW test) regarding nonparametric data. The level of significance was set to $p<0.05$. Data analysis was carried out with StatView 5.0.1 (SAS Institute Inc., Cary, NC).

The results obtained from each examined area represent the average values of the two observations.

RESULTS

Intrasubject variability satisfied the priori hypothesis of a limited dispersion. For the parametric data, variability ranged from +2% to -2% regarding the mean value. For the non-parametric data, 1 score point difference at most was observed. No significant differences were detected between healthy and scleroderma subjects in age ($p>0.01$, MW test).

Labial microcirculation: The visibility of the microcirculation was simple in both patient and control group. The microcirculation architecture in healthy subjects was characterised by a network of capillaries in polygonal mesh and a parallel orientation (type A) regarding the surface. The microcirculation architecture in 18 SSc patients was characterised by an anarchical arrangement of the capillary major axis (12 patients with DCS and 6 patients with LCS).

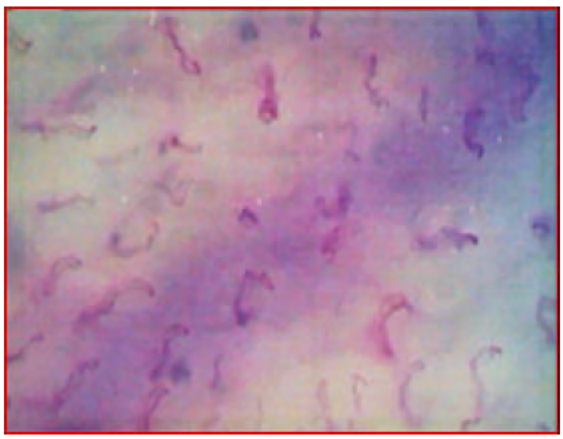

Fig. 1: Microcirculation characteristics close to the labial mucosa in healthy subjects (magnification: 200X)

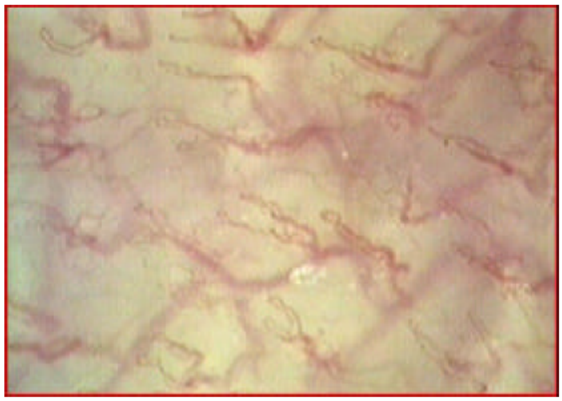

Fig.2: Microcirculation characteristics close to the labial mucosa in AR patients (magnification: 200X)

The tortuosity of the capillaries in healthy subjects showed a score=0 in 24 patients; score=1 in 7 patients; score =2 in 3 patients; score=3 in 1 patient. In patients with SSc, capillaries tortuosity showed score=0 in 10 patients (9 with lcs and 1 with dcs); score= 1 in 9 patients (5 with lcs and 4 with dcs); score=2 in 10 patients (5 with lcs and 5 with dcs); score 3 in 6 patients (1 with lcs and 5 with dcs) ($p<.001$). Rare microhemorrhages (score=1) were observed in 6 healthy patients. These were identified as reddish stains, that could have been caused by possible microtraumas. No microhemorrhages were observed in SSc patients .

The calibre of the visible capillary loops was 14.92±2.3 μm (mean±SD) in healthy subjects and 20.7±2.5 μm (mean±SD) in patients with systemic sclerosis ($p<.001$,MW test). The number of capillaries visible was 7.87±1.92 (mean±SD) in healthy subjects and 4.1±1.8 (mean±SD) in patients with systemic sclerosis ($p<.001$, MW test) (Fig. 1,2). No significant differences in the alimentary habits of patients were detected. The results of the labial observations are summarised in Table 2.

DISCUSSION

Capillaroscopy is a very stimulating method for studying microcirculation, because of the possibility of studying small vessels *in vivo* by means of a microscope. Today, it is becoming more reliable thanks to the improvement of the observation tools (photography, videomicroscopy)[13,18-20]. The morphological study of microcirculation is of fundamental importance; in fact, the microvascular bed is directly involved both in autoimmune etiopathogenesis pathologies and in acute and chronic inflammatory etiopathogenesis pathologies[21,22].

The value of capillaroscopic investigation as a diagnostic means for peripheral microcirculation damage is confirmed by numerous studies. With the use of this method, Halfoun demonstrated that diabetic patients have capillary flow regulation abnormalities (4); with the same investigation technique, Haak demonstrated that, in diabetic patients, the nervous reflex arcs are impaired (3). It must be mentioned, however, that other studies have used capillaroscopic investigation to evaluate microcirculation damage not as a complication of disease (diabetes), but as its beginning and therefore for diagnosis (SSc)[16,21,23,24].

Prominent SSc vascular abnormalities are noted in capillaries and small blood vessels. Affected capillaries are characterized by distorted and irregular loops. The

Table 2: The characteristics of the microcirculation of labial mucous in healthy subjects and scleroderma patients

	Healthy subjects		Scleroderma patients	
	Score	Subjects	Score	Subjects
Visibility of the capillary loops	1	89%	1	9 0%
	2	7%	2	10%
	3	4%		
	4	0%		
Orientation regarding the surface	A	100%	A	100%
Tortuosity	0	24	0	10
	1	7	1	9
	2	3	2	10
	3	1	3	6
Microhemorraghes	1	6	0	100%
Calibre of the capillary loops (mean±SD) Significance[a]=S[b]	14.92±2.3µm		20.7±2.5 µm	
N°/mm^2 (mean+SD) Significance[a]=S[c]	7.87±1.92		4.1±1.8	

SD Standard Deviation; [a]Differences between healthy and scleroderma patients were tested by Mann-whitney U test; S significant; [b]p<0.001; [c]p<0.001

changes include reduced numbers of capillaries and the presence of desertification. The early appearance of giant capillaries and haemorrages is of great relevance for the early diagnosis of the SSc. The naifold observation of loss of capillaries, vascular architectural disorganisation represents the clearest aspect of advanced SSc microvascular damages. On the ultrastructural level the earliest changes consist of large gaps between endothelial cells, vacuolisation of endothelial cytoplasm, an increase in the number of basal lamina-like layers and disruption of endothelial cell cytoplasmic membranes. Our study shows that capillaroscopy is a reliable method for studying labial microcirculation. The advantages of oral mucous as a capillaroscopic examination area, result in a satisfying evaluation of microcirculation for the excellent mucous transparency, especially at the labial level; in an easily approachable exam area; in the lack of local mechanical or chemical microcirculation stimulation and in a reduced susceptibility to the "cold stress" due to the contact of the mucous to the probe[7,19]. This study demonstrates that capillaroscopic alterations during SSc, are not restricted exclusively to the naifold bed, but result even in the oral mucous peripheral circulation. Labial capillaroscopy in patients with systemic sclerosis revealed significant microvascular changes regarding the controls. Labial capillaroscopic pattern in patients with SSc is recognisable by a direct evaluation and is characterised by:

- wide architectural disorganisation, anarchical orientation of the capillaries and by a non homogeneous capillary density;
- loosening of the U shape capillaries with a high degree of heterogeneity in shapes characterised by reduced length and increased diameter.

However, based on our study, in order to obtain a diagnosis the mesuration of the capillaries calibre and number per square millimetre is important. The difficulties that have limited the use of oral capillaroscopy to an experimental context now appear anachronistic in the light of the introduction of modern videocapillaroscopy[2,10,25,26].

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