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Microvascular abnormalities in patients with rheumatoid arthritis

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Summary

Microvascular involvement represents one of the first apparent steps in many autoimmune diseases such as rheumatoid arthritis (RA). Early in the disease, peripheral microangiopathy may be easily recognized and studied by videocapillaroscopy. The aim of this study has been to observe the differences in labial microcirculation between healthy patients and patients suffering from RA.

A total of 30 healthy patients and 30 patients suffering from RA were examined. The patients with conditions known to compromise microcirculation, such as diabetes, hypertension, or some pharmacological treatments were not included in the study. All the patients were non-smokers. Labial capillaroscopy was used to investigate the characteristics of microcirculation.

Visibility, course, tortuosity, as well as the possible presence of microhemorrhages, the average caliber of the capillary loops and the number of visible capillary loops per square millimeter were evaluated for each patient.

The investigation was simple, non-invasive, and repeatable for each patient.

In patients suffering from RA, it was possible to observe a reduced caliber of capillaries, as well as greater elongated capillaries, in comparison to controls.

This study shows that capillary alterations in patients suffering from RA occur in labial mucosa microcirculation; such evidence could be extremely important in the diagnosis of suspected RA.

Introduction

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease characterized by the accumulation and persistence of an inflammatory infiltrate in the synovial membrane that leads to

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synovitis and the destruction of the joint architecture resulting in impaired function. As a systemic disease, RA has extra-articular manifestations in systems such as the pulmonary, ocular, vascular, and other organs or structures that may be affected by the inflammatory process (Bartold et al., 2005).

Histologically, vascular lesions (vasculitis and accelerated artherosclerosis) have been found in 25% of patients with RA (Granier et al., 1986; Grassi et al., 1984; Kabasakal et al., 1996; Pache et al., 2002).

Capillaroscopy seems to be able to differentiate between morphological and functional abnormalities due to the inflammatory process in RA at the microvascular level (Kuryliszyn-Moskal, 1998; Nagy and Czirjak, 2004; Zaric et al., 1981).

Oral capillaroscopy is a non-invasive method of assessing mucous microvasculature, contributing to quality of differential diagnosis and prognosis of several autoimmune disorders (Lova et al., 2002; Miniati et al., 2001; Scardina, 2005; Scardina and Messina, 2004; Scardina et al., 2004, 2005).

Since capillaroscopy is non-invasive, inexpensive, easily repeatable, and comparable even after years, it permits the monitoring over time of any disease compromising microcirculation (Del Guercio and Piovella, 1995). The aim of this study has been to investigate the labial microcirculation in patients suffering from RA and to evaluate whether microcirculation alterations may play a part in the diagnosis of RA.

Materials and methods

Thirty healthy patients (10 men and 20 women; mean $\pm$ SD age: 61.23 $\pm$ 4.4 years; range: 38–79 years) and 30 patients with RA (10 men and 20 women; mean $\pm$ SD age: 62.01 $\pm$ 4.7 years; range: 37–70 years) (disease duration mean $\pm$ SD: 9.75 $\pm$ 1.27 years) were examined in our laboratory. No significant differences relating to age were detected between the healthy and the RA patients ($P > 0.01$, Mann–Whitney (MW) test).

All the patients were included in the study only if an accurate examination of their medical history revealed that they were non-smokers. The patients who showed conditions known to compromise microcirculation, such as diabetes, hypertension, or certain pharmacological treatments, were not included in the study. Similarly, RA patients with these conditions and relevant medications were also excluded.

All the patients gave their informed consent for the processing and use of their personal medical data in scientific papers, according to Italian law.

All the patients suffering from RA fuled the American Rheumatism Association diagnostic criteria (Arnett et al., 1988). They had no symptoms of secondary Sjogren's syndrome.

Fifteen out of 30 patients were rheumatoid factor positive and 15 were rheumatoid factor negative; 17 out of 30 patients were ANA positive and 13 were ANA negative; and 24 out of 30 patients were RANA positive and 6 were RANA negative.

Oral capillaroscopy was performed by the panoramic technique. In this study, qualitative and quantitative methods were applied. They were examined using computerized videomicroscopic techniques and related software (DS Medigroup, Milan, Italy). The optical probe videomicroscope is composed of a main unit, to which an optical probe with video-optical terminal is connected, and a high-resolution color monitor to view the examined area. The main unit consists of: a cold halogen light source emitted by a 100 W lamp equipped with an electronic device to control light intensity and a processing unit for the high-definition video signal (420,000 pixels) equipped with a color calibration device. The probe is equipped with a video-optical terminal containing a high-definition video sensor, via which different variable magnification optics from 10 to 1000 $\times$ can be applied. A technological characteristic of the video-optical terminal is the possibility to focus directly from the hand piece.

Image digitalization allows for the analysis of the fundamental parameters of microcirculation (caliber and vessel length), and the calculation of the number of capillaries per mm^2 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 mucosa of the lower lip.

Two independent observers examined all the images. The intraobserver and interobserver variability was assessed with the two observers evaluating the same randomly selected images twice.

The following static parameters were used:

(A) *Non-parametric data*: capillary loop visibility (marks from 1 to 4): (1) simple focusing – within 30 s from the beginning of the examination; (2) average focusing – over 30 s and within 2 min; (3) difficult focusing – over 2 min; (4) impossible focusing; orientation to the surface (marks 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 (marks from 0 to 3): (0) absence of crossing in the capillary loops; (1) presence of crossing; (2) more abundant crossing; (3) complete distortion of the capillary loops; microhemorrhages (marks 0 or 1): (0) absence; (1) presence; characteristics of the capillary loops (marks 0 or 1): (0) absence; (1) presence.

(B) *Parametric data*: number of visible capillary loops per square millimeter (value obtained from the average of the two observations for each examined area); capillary loop caliber (values obtained from the average of the two observations for each examined area).

As regards the parametric data, it must be underlined that they originate from the software connected to the videocapillaroscope. The system is specifically calibrated: an exact metric pixel value to every optical magnification corresponds in the digitalized image; therefore, the capillary caliber can be measured with a high degree of precision.

All the patients involved in the study kept a dietary diary for a period of three months, to evaluate any dietary differences which could affect the periodontal capillary pattern observed.

The statistical significance of the differences between the healthy and the RA patients was checked with the Student's *t* test for independent samples with regard to parametric data, and with the MW test with regard to non-parametric 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 a priori hypothesis of limited dispersion. For the parametric data, variability ranged from +2% to −2% with respect to the mean value. For the non-parametric data, 1 mark difference was observed at most.

Microcirculation visibility was clear both in the patients and in the control.

Microcirculation architecture in the healthy and in the RA patients was characterized by a network of capillaries in polygonal mesh with parallel orientation (type A) to the surface.

Capillary tortuosity in the healthy patients obtained a mark of: 0 in 12 patients, 1 in 9 patients, 2 in 7 patients, 3 in 2 patients. In the RA patients, capillary tortuosity was: 0 in 10 patients, 1 in 7 patients, 2 in 5 patients; 3 in 10 patients (mark 0: NS; mark 1: NS; mark 2: NS; mark 3: S; X^2+Fisher's exact *P* value). Rare microhemorrhages (mark 1) were observed in 2 healthy patients. These were identified as reddish stains, possibly caused by microtraumas. No microhemorrhages were observed in the RA patients.

The caliber of the visible capillary loops was $14.02\pm2.5\,\mu m$ (mean $\pm$ SD) in the healthy patients and $9.56\pm2.7\,\mu m$ (mean $\pm$ SD) in the RA patients (S, MW test). The number of visible capillaries was 8.10 ± 1.67 (mean $\pm$ SD) in the healthy patients and 13.98 ± 1.34 (mean $\pm$ SD) in the RA patients (S, MW test). The length of the visible capillary loops was $179.74\pm2.6\,\mu m$ (mean $\pm$ SD) in the healthy patients and $296.88\pm2.9\,\mu m$ (mean $\pm$ SD) in the RA patients (S, MW test) (Figs. 1 and 2).

No significant differences in the dietary habits of the patients were detected.

Discussion

RA is a chronic inflammatory autoimmune disease associated with a wide range of extra-articular manifestations. Recent studies emphasize a key inflammatory role of the endothelial cells, either by overexpression of inflammatory mediators or by the proliferation of new blood vessels, in the disease process leading to the systemic organ involvement (Kuryliszyn-Moskal et al., 2005).

The existence of a specific capillaroscopic pattern in RA patients is a matter of debate. Schumacher et al. (1968) excluded the presence of specific alterations in RA. Other authors found

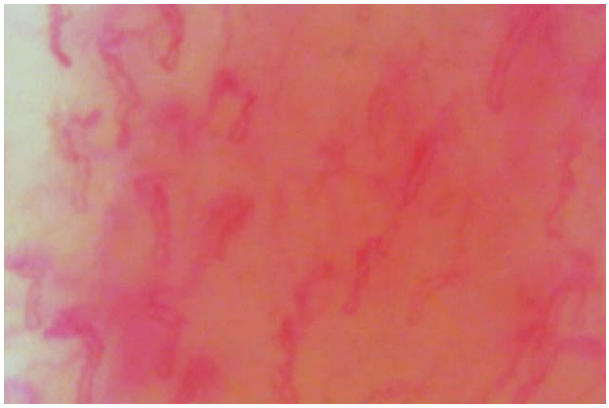

Figure 1. Microcirculation characteristics close to lower lip in healthy patients (magnification: 200–400 ×).

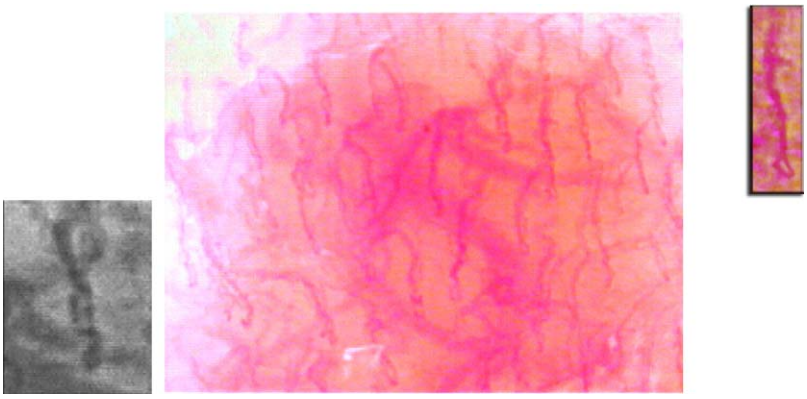

Figure 2. Microcirculation characteristics close to lower lip in RA patients (magnification: 200–400 ×).

aspecific capillaroscopic alterations. According to these authors, abnormalities consisted of elongated and tiny loops, microhemorrages, capillary low density, and subpapillar venous plexus visibility alterations. Although they do not seem to be pathognomonic for RA, they do appear to be sufficiently characterized as to differentiate patients with RA from normal controls (Ohtsuka et al., 1995; Redish et al., 1970; Weyand, 2000; Zaric et al., 1981).

Vascular injury is considered to be a key finding in the pathogenesis of RA. Manifestations are varied depending on the vessel size and the organ system involved (Chatterjee and Kupsky, 2005).

The hypotheses on the pathogenic mechanism of vascular damage involve the autoantibody and circulating immune complex precipitation in the vessel walls, considered the main cause of damage. Endothelial cells and their products participate in the initiation and maintenance of tissue damage (Kahaleh, 1990; Witkowska et al., 2003).

The vascular damage is also correlated to the production of osteopretogerin (OPG) by endothelial cells. OPG has been reported to be required for endothelial cell survival and growth. In addition, OPG knock-out mice have been shown to develop microvascular damage, suggesting that vascular endothelial expression of OPG may have a role in vascular homeostatis (Bartold et al., 2005; Min et al., 2000).

Our study is basically characterized by the originality of the investigation site chosen to evaluate any microcirculation damage.

To our knowledge, the labial mucosa microcirculation has never been examined in patients suffering from RA.

On the contrary, the oral, and particularly the labial microcirculation have been studied for other purposes using contact optical probe videocapillaroscopy. These studies show that oral capillaroscopy is mainly characterized by easy, fast execution, as well as by excellent microcirculation visibility (Lova et al., 2002; Miniati et al., 2001; Scardina, 2005).

Capillaroscopy is a non-invasive diagnostic technique, fundamental in viewing peripheral circulation and in studying microangiopathies, which are the manifestations of numerous pathologies in both the diagnostic and monitoring phases of the disease (Scardina et al., 2005).

Compared to other pathologies, in which microangiopathies exclusively represent a complication (diabetes mellitus), some diseases may show microcirculation alterations even in clinically healthy areas; these alterations have been documented by histological examinations in the early stages of such disease (Haak et al., 1998; Halfoun et al., 2003; Maricq and Le Roy, 1973).

Following up such observations, our study was aimed at evaluating whether any microcirculation modifications could be detected in the labial mucosa in patients suffering from RA.

The results allow us to state that, in these patients, the labial microcirculation is characteristic. The main factor emerges from both parametric and non-parametric observations, such as capillary morphology. According to the authors, this factor is very interesting and useful, since it permits an immediate diagnostic doubt.

In fact, lengthened capillaries are, for example, immediately detectable, and this can give rise to diagnostic doubt. The latter must be obviously supported by specific diagnostic investigations for the disease.

Therefore, because it is immediate and non-surgical, labial capillaroscopy in patients suffering from RA can represent a new approach to the diagnosis. The presented data suggest that videocapillaroscopy might nowadays be considered an early, non-expensive, diagnostic tool for RA and, possibly, for other autoimmune diseases.

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