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Morphological characteristics of microcirculation in oral lichen planus involving the lateral border of the tongue

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Abstract: Oral lichen planus (OLP) is a chronic mucosal condition commonly encountered in dental practice. Lichen planus is believed to represent an abnormal immune response in which epithelial cells are recognized as foreign, secondary to changes in the antigenicity of the cell surface. It has various oral manifestations. The aim of the study was to evaluate the morphological characteristics of microcirculation of lingual lichen planus (LLP). Twenty patients (10 patients with LLP and 10 healthy patients) were examined by means of videocapillaroscopy. The left margin of the lingual mucosa was examined in each patient. The capillary loop length, loop diameter, and capillary density were analyzed on every capillaroscopic image. The results obtained using videocapillaroscopic software were subjected to statistical analysis. The density, loop length, and total diameter showed statistically significant differences. A remarkable increase in capillary density was shown. The microcirculation of LLP can be studied "in vivo" with the help of videocapillaroscopy, and it could be an indicator of the evolutionary condition of LLP, useful for the evaluation of the evolution or regression phases of the disease. (J Oral Sci 51, 193-197, 2009)

Keywords: oral; lichen; tongue; capillaroscopy.

Introduction

Oral lichen planus (OLP) is a chronic mucosal condition commonly encountered in dental practice. Lichen planus is believed to represent an abnormal immune response in which epithelial cells are recognized as foreign, secondary to changes in the antigenicity of the cell surface. It has various oral manifestations, the reticular form being the most common. The erosive and atrophic forms of OLP are less common, yet are most likely to cause symptoms. The rate of malignant evolution of lichen planus is currently controversial. It is reported that 1% to 1.5% of the patients are likely to undergo this evolution after 10 years. Capillaroscopy is a method for studying microcirculation, which permits the observation of small vessels in vivo with the use of a microscope (1). The choice of this examination for diagnostic evaluation and monitoring of lingual lichen planus (LLP) as a chronic inflammatory condition with immunologic pathogenesis is very important, since it reveals the presence of vascular modifications in patients affected by this pathology compared to a control group (2). Such modifications in the vascular pattern (3-10) might be useful for diagnostic monitoring of the disease. The aim of this study was to evaluate possible differences in the vascular pattern between healthy patients and patients with LLP.

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Materials and Methods

Twenty patients were examined: 10 patients with LLP (9 females, 1 male; mean age: 64.11 ± 2.5 (mean ± SD); range = 42-72) and 10 healthy patients (9 females and 1 male; mean age: 63.14 ± 2.4 (mean ± SD); range = 39-69) (Table 1). All the patients gave their informed consent according to the Italian law on privacy and treatment of personal data. The patients having elementary white and/or red lesions with suspected OLP, regardless of the clinical aspect, were subjected to incision biopsy in the area of the lingual mucosa which clinically better represented the lesion and, if possible, included margins of healthy tissue

Table 1 Demographic characteristics of the patients included in the study. The difference between LLP and the control groups was evaluated with the Mann-Whitney *U*-test

	LLP group (N = 10)	Control group (N = 10)	Significance
Age (mean ± SD)	64.11 ± 2.5	63.14 ± 2.4	NS
Range	42-72	39-69	/

NS = Not significant; SD = Standard deviation

(11).

The patients with LLP included in the study were selected according to definite histopathological criteria: 1) Signs of keratinocyte alteration of the squamous epithelium basal layer had to be present, with a saw-toothed epithelium-connective tissue interface; and 2) the maturity of the different epithelial layers had to be normal; therefore, the absence of dysplastic phenomena was fundamental. Patients with lesions which did not reflect the above strict histological criteria were not included in the study. The control group patients did not show any systemic pathology which could alter oral cavity microcirculation (diabetes, hypertension, Sjögren's syndrome, rheumatoid arthritis, etc.). Besides, the patients on systemic medication (anti-hypertensives, oral hypoglycemic drugs, anti-inflammatory) or those with amalgam or composite fillings in direct contact with the lesions were not included in the two groups. Videocapillaroscopy was carried out with the patients in a sitting position, with the same light source, at the same temperature (23°C), in the morning, by the same operator and repeated various times for each examined area (12,13). The left margin mucosa of the tongue was examined in each patient, both in the control and in the LLP groups. To identify microangiotectonics and define its type and group, a ×200 magnification optics was used, whose characteristics make it the ideal working objective for normal diagnostic requirements.

Loop visibility is a morphological parameter which indicates the difficulty of capillary focusing. It was evaluated as follows: 1) mark 1: simple to focus (less than 30 s from the beginning of the examination); 2) mark 2: medium simplicity (from 30 s to 2 min from the beginning of the examination); 3) mark 3: difficult to focus (more than 2 min from the beginning of the examination); and 4) mark 4: impossible to focus. This was followed by a morpho-functional evaluation of microcirculation, paying particular attention to the following parameters: 1) capillary loop length; 2) loop diameter; and 3) capillary density (14). Capillary density (number of loops per mm^2) was calculated for every patient and for each image obtained by videocapillaroscopy; then, an average value was calculated for every patient. Capillary density is the parameter which permitted the evaluation of the possible angiogenesis process in LLP patients with the use of videocapillaroscopy. A minimum of 6 capillary loops were examined for every image, and capillary loop length and total loop diameter were measured on each of them. A qualitative investigation was also carried out, to evaluate the level of discomfort experienced by the patients during the clinical procedures of videocapillaroscopy. On the whole, according to the patients, the capillaroscopic investigation was well tolerated.

Statistical analysis

The ordinal, non-parametric data was analyzed by the Mann-Whitney *U*-test using *P.A.S.T.* software v. 1.53, a freeware developed in 1995 by Ryan PD, Harper DAT and Whalley JS, updated to the latest version in September 2006. The value of significance was $P < 0.05$.

Results

The lingual mucosa was characterized by a microcirculation with an arrangement corresponding to type III of Curri classification. The loop pattern was constantly parallel to the surface. This particular pattern of the capillaries belonging to what is called the "sub epithelial plexus", proved to be very useful for determining what is probably the most important parameter, since it is related to the neoangiogenetic phenomenon, namely capillary density.

Microcirculation architecture in the healthy and LLP patients was characterized by a network of capillaries in a polygonal mesh oriented parallel to the surface. In normal oral mucosa and LLP, micro vessels were mainly located just underneath the epithelium. After counting these micro vessels, we determined the mean ± SD of micro vessel density. The loop diameter was 0.03 ± 0.0044 mm (mean ± SD) in healthy subjects and 0.07 ± 0.0066 mm (mean ± SD) in patients with LLP ($P < 0.001$, Mann-Whitney *U*-

Table 2 Lichen/Control parameters

Examined parameter	LLP	Control	Significance
Density per mm^2 (mean ± SD)	17.2 ± 2.9	8.2 ± 1.2	S
Loop length mm (mean ± SD)	0.13 ± 0.10	0.11 ± 0.9	NS
Loop diameter mm (mean ± SD)	0.07 ± 0.0066	0.03 ± 0.0044	S

The differences between LLP and the control groups were evaluated with the Mann-Whitney *U*-test. NS = Not significant ($P > 0.001$); S = Significant; SD = Standard deviation.

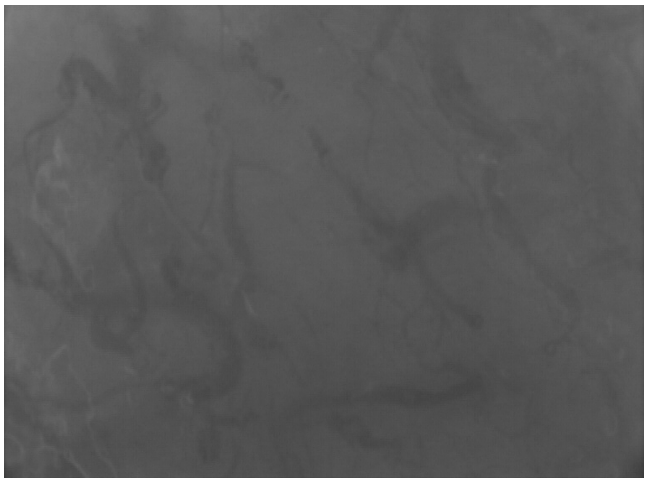

Fig. 1 Lingual microcirculation in an LLP patient.

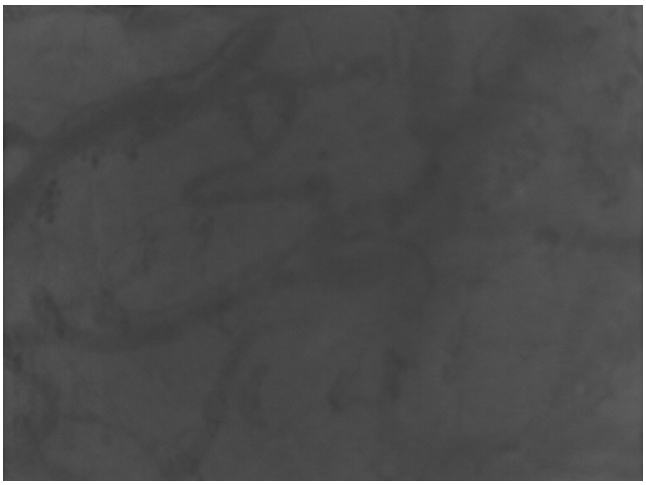

Fig. 2 Lingual microcirculation in a healthy patient.

test). The number of capillaries visible was 8.2 ± 1.2 (mean ± SD) in healthy subjects and 17.2 ± 2.9 (mean ± SD) in patients with LLP ($P < 0.001$, Mann-Whitney *U*-test). The micro vessel density in LLP was significantly higher than that in normal tissues. The loop length was 0.11 ± 0.9 mm (mean ± SD) in healthy subjects and 0.13 ± 0.10 mm (mean ± SD) in patients with LLP ($P < 0.001$, Mann-Whitney *U*-test). It must be stressed that there was never a sharp distinction between the normal condition (control group) and pathological one (LLP group), borderline conditions were frequently observed. The density showed significant differences between the two groups examined (Table 2).

Discussion

The oral mucosa is a window through which we can observe *in vivo* the vascular bed. This study investigated the morphology of LLP through oral capillaroscopy. The importance of microcirculation, from a clinical point of view, is due to the fact that the capillaries represent a vascular area where trophic-metabolic exchanges between blood and tissue take place, thus leading to a critical role in organ function. Oral capillaroscopy is mainly indicated in the early detection of scleroderma microangiopathy and in connective tissue diseases. It is also used in diseases whose pathogenesis is due to an anatomical or functional anomaly of microcirculation. The non-invasiveness, reproducibility, low cost, and considerable sensitivity make this technique very useful in the early diagnosis and monitoring of microangiopathies.

Lichen planus is a relatively common disorder, estimated to affect 0.5% to 2.0% of the general population. It is a chronic inflammatory disease that affects mucosal and cutaneous tissues. It occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment (15).

Erosive and atrophic OLP is the second most common type. It presents as a mix of erythematous and ulcerated areas surrounded by finely radiating keratotic striae. The lesions of erosive lichen planus migrate over time and tend to be multifocal. Patients with this form often present with symptoms ranging from episodic pain to severe

discomfort that can interfere with normal masticatory function. In the bullous form, intraoral bullae are observed on the buccal mucosa and the lateral borders of the tongue; the bullae rupture soon after they appear, which results in the classic appearance of erosive form. Some studies indicate an increased risk of squamous cell carcinoma in patients with lichen lesions. This increased risk appears most commonly with the erosive and atrophic forms and in cases of lesions of the lateral border of the tongue. A review of previously published studies concluded that the risk of developing squamous cell carcinoma in patients with OLP is approximately 10 times higher than that in the unaffected general population. The study of the microcirculation in LLP could be important in the interception of the evolution of LLP (16). Although many steps are associated with the progression of the disease, neoangiogenesis is an essential factor. Angiogenesis, the formation of new microvasculature, is an important component in many biological processes, both in physiological conditions, such as proliferating endometrium, corpus luteum formation, and embryogenesis, and in pathological conditions, such as rheumatoid arthritis, diabetic retinopathy, inflammatory diseases and neoplastic disease. The process of angiogenesis involves several sequential steps, including degradation of the basement membrane of the parent vessel and extracellular matrix, locomotion of endothelial cells toward a tumor implant, mitosis, lumen formation, development of sprout loops and of a new basement membrane, and finally, recruitment of pericytes. Each of these distinct steps is regulated differentially. The induction of angiogenesis depends on a balance between positive and negative angiogenic factors. We analyzed the microvessel density in LLP. A number of studies have compared normal tissues with either premalignant or malignant lesions and have shown significantly higher vascularity in dysplasia and cancer. The difficulty in studying angiogenesis in humans is the lack of direct methods for measuring angiogenic activity. A commonly used indirect method consists of measuring the density of the microvasculature in histological sections and considering this measurement to represent the angiogenic status of the lesions (17,18).

To our knowledge, our research is the first scientific study in which videocapillaroscopy was used for the evaluation of the morphology and vascular density of microcirculation in LLP patients. Capillaroscopy applied to lingual mucosa is a reliable method, useful for OLP study and monitoring, if combined with the current conventional methods. Our results showed an increased capillary density in LLP, along with an increased capillary diameter. The increased capillary diameter and density can be easily identified and are correlated to the inflammatory characteristic of this disease. The loops examined showed a variable pattern; beside those with the typical stirrup shape, there were others with a hairpin, comma and corkscrew shape, as well as rare micro haemorrhages in the form of reddish spots, which could be traced back to possible micro traumas. A pathological condition is characterized by an apparent architecture disorder and/or by the presence of morphological anomalies.

This method might also be useful to check the effectiveness of a selected therapeutic protocol (17). It could permit comparison of the characteristics of pre- and post-treatment local microcirculation, as well as an objectification or parameterization of an aspect of the program, often relying on patient information (symptoms) or on clinician's evaluation (18). The capillaroscopic patterns give us a snapshot of the lingual mucosa *in vivo*, showing alterations, either significant or not, especially of parameters like vessel diameter and capillary density. The choice of this examination for diagnostic evaluation and monitoring of LLP as a chronic inflammatory condition with immunologic pathogenesis is fundamental, since it reveals the vascular pattern in patients affected by this pathology compared to a control group. Such modifications in the vascular pattern might play an important role in the comprehension of the evolutionary mechanism of OLP; the observation of these changes might be useful for diagnostic monitoring of the disease. The angiogenesis and inflammatory aspect of OLP can be observed through the "in vivo" observation of sub epithelial plexus. The capillaroscopy revealed that the loop diameter and loop density underwent a significant increase in LLP. These parameters could be monitored for evaluation of proper management of these patients. In the final analysis, thanks to videocapillaroscopy, the lingual mucosa could act as a reliable indicator of the evolutionary condition of inflammatory pathologies such as LLP, allowing the oral physician to evaluate *in vivo* the effectiveness of ongoing pharmacological therapies, the patient's state of health, the evolution or regression phases of a certain pathology, and all those microstructure alterations of the vascular system useful for predicting and monitoring the progress in time of a systemic and/or topical pathological condition based on an angiogenesis process (19). We believe that oral capillaroscopy may represent an additional tool useful in the follow-up of LLP.

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