Assessment of dermal papillary and microvascular parameters in psoriasis vulgaris using in vivo reflectance confocal microscopy

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Abstract. In vivo reflectance confocal microscopy (RCM) is a modern, non-invasive imaging technique, which allows for real-time examination of the upper layers of the skin at a resolution similar to that of classic microscopy. In addition, it has the advantage of real-time evaluation of blood flow and dynamic monitoring of cutaneous changes while preserving tissue integrity. The present study reported on the in vivo RCM technique as an objective method for the noninvasive assessment of psoriasis vulgaris that is potentially applicable in clinical studies and in monitoring the evolution of lesions under treatment. In psoriasis lesions, RCM virtual horizontal sections at the level of the dermo-epidermal junction featured numerous and prominent dermal papillae that were not surrounded by bright rings of basal cells. Micromorphological examination of the lesions using this technique revealed that mean values of the section area, the perimeter and the Feret’s diameter of the dermal papillae were significantly higher in psoriatic lesions than in normal skin. An increased number of capillary vessels per lesional dermal papilla as compared to healthy skin was observed. Furthermore, micromorphological parameters of dermal capillaries were increased in psoriatic skin. These observations point to the utility of in vivo RCM as a promising technique for the non-invasive diagnosis of psoriasis vulgaris, for monitoring the evolution of lesions at a micromorphological level under various treatments and for gaining a better understanding of the pathophysiological processes that occur in the evolution of this disease.

Introduction

Psoriasis is a worldwide recurrent inflammatory skin disorder with a high prevalence in the general population (1.2) and a major negative impact on the quality of life of affected patients (3). The pathophysiological mechanisms involved in psoriasis are complex, including genetic predisposition, the influence of immunologic factors and environmental triggers (4,5). Effective treatment of psoriasis is often challenging (6,7). Despite its characteristic clinical aspects, histological examination remains the gold standard in psoriasis diagnosis (8,9). However, skin biopsy is invasive and may be associated with various complications, including infection and scarring. Histological examination only provides information on one specific tissue area and on the status of the tissue at the time it is excised. In addition, in early stages of the disease, the histopathological changes may be modest and the results are inconclusive. Skin biopsy is not useful for dynamic monitoring of skin changes during treatment, as it is not possible to evaluate the same area over time (9-11).

In vivo reflectance confocal microscopy (RCM) is a novel imaging technique that offers noninvasive, real-time visualization of the superficial layers of the skin down to the papillary dermis, with a resolution that allows for examination of cellular details. The images are obtained and processed rapidly and in real-time, and the area may be reviewed whenever necessary to assess the dynamic changes of the cutaneous tissue during various pathophysiological processes or the response to treatment. In addition, in contrast to conventional skin histology images, RCM optical sections are in the horizontal plane,
enabling a detailed examination of a wide skin area at a given depth (10-18).

In the present study, in vivo RCM was used for the objective assessment of dermal papillary and microvascular parameters in skin lesions of psoriasis vulgaris patients. RCM is a method potentially applicable in clinical studies for monitoring the evolution of the lesions under treatment, which may be useful for gaining a better understanding of the pathophysiological processes involved in this disease and to determine noninvasive diagnostic criteria that may replace classic histopathology.

Materials and methods

Patients. The present study was performed at the Department of Dermatology, Nicolae Paulescu National Institute of Diabetes, Nutrition and Metabolic Disease (Bucharest, Romania) in accordance with the guidelines of the local ethics committee. A total of 23 subjects (12 male and 11 female; 35.22±2.736 years old) were enrolled in the study, written informed consent was obtained from all participants prior to their inclusion in the study.

Of these patients, 9 were treated for plaque psoriasis. The median age of this group of patients was 38.44±4.664 years, and the male-to-female ratio was 5:4. The lesions were characteristic erythematosquamous plaques and were located on the trunk and limbs. The diagnosis was established by clinical observation and further confirmed by histological examination. None of the patients was under systemic or topical treatment at the time of the first clinical examination.

The other 14 subjects were healthy volunteers who were age- and gender-matched with the Psoriasis group. In this group, there was an even number of males and females and the median age was 33.14±3.371 years.

Study protocol. Patients were examined under medical supervision, under standard temperature (22±1˚C) and humidity (50±5%), and in a comfortable position. On the area of the skin to be analyzed, cleaned and dried in advance, a few drops of immersion oil (crodamol) were applied to engage the optical disc and reduce refractive irregularities.

In the Psoriasis group, a clinically significant lesion that was easily accessible for examination was selected for each subject. Lesions covered by thick scales or located in hairy areas were avoided. In the Control group, the RCM images were taken from the healthy skin of the dorsal non-dominant hand.

RCM images were obtained using a VivaScope 1500 confocal microscope (Lucid Inc., Rochester, NY, USA) with a 785 nm wavelength and a lighting power below 30 mW.

The RCM images were examined using Image J 1.45 image analysis software (National Institutes of Health, Bethesda, MD, USA). The percentage of dermal tissue area from the total examined cutaneous tissue at the level of the dermo-epidermal junction was also identified on the reflectance confocal microscopy images. Values are expressed as the mean ± standard deviation. **P=0.0002.

Results

Assessment of dermal papillae of psoriasis patients by RCM. In psoriasis lesions, RCM virtual horizontal sections at the level of the dermo-epidermal junction exibited numerous and prominent dermal papillae, with polygonal shapes, separated by thin epidermal ridges; they were well defined, but unlike dermal papillae of healthy skin, they were not surrounded by any bright ring of basal cells. In psoriasis lesions, the area of papillary dermal tissue accounted for 27.90±4.577% of the total examined cutaneous tissue area, which was significantly different from the percentage (18.69±4.864%) found in healthy skin. The statistical analysis showed a significant difference between the two groups (P<0.001).

Statistical analysis. Statistical analysis of the data was performed using GraphPad Prism 6 software (GraphPad Inc., La Jolla, CA, USA). The unpaired t-test was used to determine the statistical significance of differences between groups. P<0.05 was considered to indicate a statistically significant difference. Values are expressed as the mean ± standard error of the mean.

Figure 1. Micromorphological parameters of dermal papillae and dermal capillaries. The parameters of the dermal papillae (green contour lines) and of capillary vessels (yellow circles) were measured using Image J 1.45 analysis software. The parameters investigated were area, Perim and Feret. Perim, perimeter; Feret, Feret's diameter.

Figure 2. Difference between the percentage of dermal tissue from the total examined cutaneous tissue at the level of the dermo-epidermal junction. Due to the papillomatosis present in the lesional skin, a larger area of dermal tissue was also identified on the reflectance confocal microscopy images. Values are expressed as the mean ± standard deviation. **P=0.0002.
higher than the area determined for healthy skin (10.70±1.163%; P=0.0002; Fig. 2). Micromorphological examination using the RCM technique revealed that the mean values of the section area, the perimeter and the Feret’s diameter of the dermal papillae were significantly higher in psoriatic lesions than in normal skin. The mean value of the section area of dermal papillae was significantly increased to 5405±749.0 µm in the Psoriasis group as compared with 2243±139.0 µm in the Control group (P<0.0001). In addition, a significant difference between the two groups was identified regarding the papillae's perimeter, with a mean value of 255.6±20.02 µm in the skin affected by psoriasis compared with 169.4±5.127 µm in the skin of the healthy volunteers (P=0.0002). The Feret's diameter of the dermal papillae in the Psoriasis group was also increased to 91.94±7.201 µm compared with 61.03±1.913 µm in the Control group (P=0.0002).

Assessment of dermal capillaries of psoriasis patients by RCM. Dermal capillaries may be visualized on RCM virtual sections as dark, round structures in the center of dermal papillae and blood flow may be observed via real-time imaging. The pathophysiology of psoriasis vulgaris includes changes of the capillary vessels to become dilated and tortuous, describing multiple loops in their trajectory, which was successfully identified in the RCM images (Fig. 4). An increased number of capillary sections per lesional dermal papilla was present as compared with that in healthy skin, with 2.691±0.199 capillary sections per papilla in the psoriasis group as compared with 1.451±0.0447 in the control group; the difference was statistically significant (P<0.0001; Fig. 5). The micromorphological parameters of dermal capillaries were also increased in psoriatic skin. Regarding the section area of
Confocal microscopy also allows for assessing RCM allows for RCM has the advantage of real-time RCM technique, which is potentially applicable in ability of recurrence following treatment compared with the skin. Tracking of histological parameters during therapy provides valuable information on the therapeutic response, the mechanism of action of therapeutic agents and the pathological mechanisms involved in the occurrence of the cutaneous lesions. The method suggested by the present study may be utilized to assess these parameters in an objective manner.

Considering that the dermal microvasculature is affected in a broad spectrum of dermatological disorders, the possibility of studying microvascular changes may be helpful in understanding the underlying pathophysiological mechanisms. In vivo RCM has the advantage of real-time examination of blood flow and dynamic evaluation of morphological changes, while preserving the integrity of the skin. In addition, the cellular resolution of in vivo RCM allows for evaluation of neutrophil migration and accumulation in active psoriatic lesions (33,34). Neutrophils have an established role in the pathophysiology of psoriasis and pathognomonic collections of neutrophils are frequently observed as clusters of highly refractile round to polygonal cells at the level of the stratum spinosum and stratum corneum, where they form micropustules of Kogoj and microabscesses of Munro, respectively (22,33,34).

In vivo confocal microscopy also allows for assessing dermal reactions at the microvascular level induced by exposure to ultraviolet light (35), smoking (36), topical capsaicin (16) or temperature changes (37,38). In addition, this imaging technique was successfully used to verify the viability of skin grafts (39) and musculoscutaneous flaps (40). Dilated and tortuous dermal capillaries represent a histological feature of psoriasis vulgaris (11,12,41), which has been successfully evaluated by RCM. Vessels that run horizontally and perpendicularly to the surface are seen as canalicular or round dark spaces. It has been observed that, compared with the skin of healthy subjects, capillaries examined in lesions of psoriasis have higher mean values of micromorphological parameters.
parameters (area, perimeter and Feret’s diameter) and that for each papilla, the number of capillary sections is higher. It should be noted that in the pathogenesis of psoriasis, dermal capillaries become tortuous, describing multiple loops in their path (11,12,41,42), so the horizontal sections made by RCM examination are likely to display several sections of the same capillary vessel.

In the context of major challenges for healthcare systems and the pharmaceutical industry (43), it is currently of interest to use non-invasive tools for the diagnosis of the skin pathologies, which are more readily accepted by the patient and preserve the integrity of examined skin. In the future, RCM may serve as a method for non-invasive and definitive diagnosis of psoriasis vulgaris, for monitoring the evolution of the lesions at the micromorphological level under various treatments and for better understanding the pathophysiological processes that occur in the evolution of this disease. Until then, further study is required in order to establish the precise diagnostic criteria that may be applied in everyday practice.

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