International Journal of Dermatology

Clinical trial

In vivo reflectance confocal microscopy for the differential diagnosis between vitiligo and nevus depigmentosus

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Conflicts of interest: The authors state no conflict of interest.

Abstract

Background Nevus depigmentosus (ND) is frequently confused with vitiligo. Differential diagnosis can be difficult. *In vivo* reflectance confocal microscopy (RCM) is a noninvasive technique for real-time en face imaging of the superficial layers of the skin down to the superficial dermis with cellular level resolution close to conventional histopathology. In this study, we tried to use this new technology to study the features of the distribution of pigment cells of these two hypopigmentation disorders and then concluded the differential features.

Methods Sixty vitiligo patients and 62 ND patients were enrolled in the study. Three points in each patient (lesional, margin of the lesions and adjacent non- lesional points) were examined with RCM. The gray value of image was quantified using software, and we calculated the relative gray value.

Results The RCM image feature was different between vitiligo and ND patients. The differential diagnosis was made based on the following four RCM features: complete absence of pigment cells; the distribution of pigment cells; the margins; and the relative gray value.

Conclusion RCM can be used as an auxiliary diagnostic tool for the differential diagnosis between vitiligo and ND.

Introduction

Vitiligo is characterized by a localized and progressive loss of melanocytes. ND is defined as a congenital nonprogressive hypopigmented macule or patch that is stable in its relative size and distribution throughout life.

ND is frequently confused with vitiligo. The distinction between vitiligo and ND is important because these disorders have significantly different prognosis and psychological effects. Differential diagnosis relies on medical history and physical examination. However, differential diagnosis can be difficult even when a biopsy is performed. Vitiligo may not always be devoid of melanocytes, and melanin pigmentation may remain for a period of time after the development of vitiligo.[1] Also, melanocytes and melanin pigment may be observed in the border areas around lesions of vitiligo.

In vivo reflectance confocal microscopy (RCM) is a noninvasive technique for real-time en face imaging of the superficial layers of the skin down to the superficial dermis with cellular level resolution close to conventional histopathology.[2] In a few studies, the correspondence of confocal features with dermatoscopy and histopathology has been evaluated.[3,4] According to the principles of reflectance confocal microscopy, melanin presents a higher reflectance index (1.7) in comparison with the total skin (1.4).[5] Melanocytes and pigmented keratinocytes are seen as bright structures on a dark background. Therefore, pigmentary disorders caused by abnormal amounts of melanin in the skin could be the most suitable candidates for RCM examination. The RCM features of the lesional skin of vitiligo and ND patients have been preliminarily studied, and the applications include the therapeutic monitoring.[6,7] The purpose of this study is to define differential features of vitiligo and ND with the help of RCM.

Material and methods

Subjects

Between June 2008 and October 2009, patients with hypopigmented skin lesions diagnosed as vitiligo or ND were enrolled in the study at Fudan University Huashan Hospital. All patients were examined by a specialized dermatologist to exclude other hypopigmentary disorders. A diagnosis of vitiligo

was based on medical history, physical examination, and a Wood's lamp examination, and the diagnosis of ND was based on the clinical criteria proposed by Coupe.[8] Sixty patients (32 females and 28 males), age ranging from 2 to 69 years, with localized vitiligo and a median duration of six years (range from 10 months to 19 years) entered the study. The disease was stable in all the patients for at least six months (range from six months to 19 years) at the time of enrollment. Sixty-two patients (30 females and 32 males), age ranging from eight months to 16 years with isolated (39 patients) and segmental (23 patients) ND lesions with a median duration of two years (range from three month to eight years) entered the study. The patients presented different skin phototype (SPT) ranging from IV to V.

RCM evaluation

RCM was performed with the commercially available near-infrared RCM Vivascope 1500 (Lucid Inc, Rochester, NY, USA). This system uses a diode laser at a wavelength of 830 nm and a ×30 water immersion objective lens with numeric aperture of 0.9. The laser power is typically 0–10 mW. The RCM objective was attached to the skin via a stainless steel ring, which in turn was attached to the corneal layer of the skin with an adhesive. This RCM steel ring fixture stabilizes the area of interest and helps maintain constant mechanical contact between the skin and the RCM. A small drop of immersion oil was applied to the skin lesion.

Three points in each patient (lesional, margin of the lesions and adjacent non-lesional points) were examined with RCM. In order to conserve the anatomical variability of RCM features, lesional as well as non-lesional skin was taken contralaterally and distant from any other lesions. The skin was scanned layer by layer, obtaining horizontal microscopic images with a lateral resolution of 1–1.5 μm and a maximum depth of 250–300 μm, displaying up to 5 × 5 mm of tissue. In this study, we focused on the dermo–epidermal junction (DEJ) level images.

Quantification

Ten images were randomly exported from each patient, five from lesional side and five from non-lesional side. The gray value of images was quantified using a software [ImageJ software version 1.43 (National Institute of Mental Health, Bethesda, Maryland, USA)]. An average of the five gray values was recorded. We calculated the relative gray value (RGVs) using,

$$\text{RGV}\ (\%) = \text{average gray value of affected lesion}/\text{average gray value of non} - \text{lesional area} \times 100$$

Statistical analysis

SPSS software (Statistical Program for Social Science version 10.0) (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The vitiligo and ND groups were compared with RCM features using Pearson's correlation test and Fisher exact test. Statistical significance was defined as $P < 0.05$.

Results

Non-lesional skin

The distribution of melanin in DEJ level of the non-lesional skin showed no significant difference between vitiligo patients and ND patients. The DEJ layer was found at an approximate depth of 38–48 μm below the skin surface in this study. Melanin is a major source of contrast of the RCM pictures. These keratinocytes with melanin appeared as bright granules, were uniform in size, and each measured approximately 7–12 μm in dimension. They formed bright ring structures around the dermal papillae. (Fig. 1a)

Lesional skin and margin of the lesions

Vitiligo patients

In 47 of 60 (78.33%) cases, the disappearance of the normal ring structures, corresponding to the complete loss of pigment cells at the basal layer, was seen (Fig. 1b). Moreover, in 11 of 60 (18.33%) cases, pigment cells were absent partially. (Fig. 2a). Pigment cells and ring structures were found in these lesions, but they were not homogeneously distributed. Melanocytes (Fig. 1e) or ring structures (Fig. 2b) were seen around the hair follicles in five cases. The margins were distinct in 37 of 60 (71.15%) cases. The lesional side of the pictures was dark and the non-lesional side was bright; the dividing line was made easily. (Fig. 3b) The relative gray value of vitiligo patients is 63.82 ± 24.30.

ND patients

In 55 of 62 (88.71%) cases, the pigment cells were small, and the brightness was low compared with non-lesional skin (Fig. 1d). Moreover, the density of the pigment cells was low. The pigment cells were distributed homogeneously (43 case, 69.35%). None of the patients showed complete absence of pigment cells. The margins were indistinct (60 cases, 96.77%). The dividing line of the lesion was ambiguous, because the difference between lesional side and non-lesional side was not predominant compared with vitiligo patients. (Fig. 3a) The relative gray value of ND was 81.83 ± 13.40.

The differential diagnosis

The differential diagnosis was made based on the following four RCM features (Table 1): (i) Complete absence of pigment cells; (ii) Distribution of pigment cells; (iii) Margins; (iv) Relative gray value.

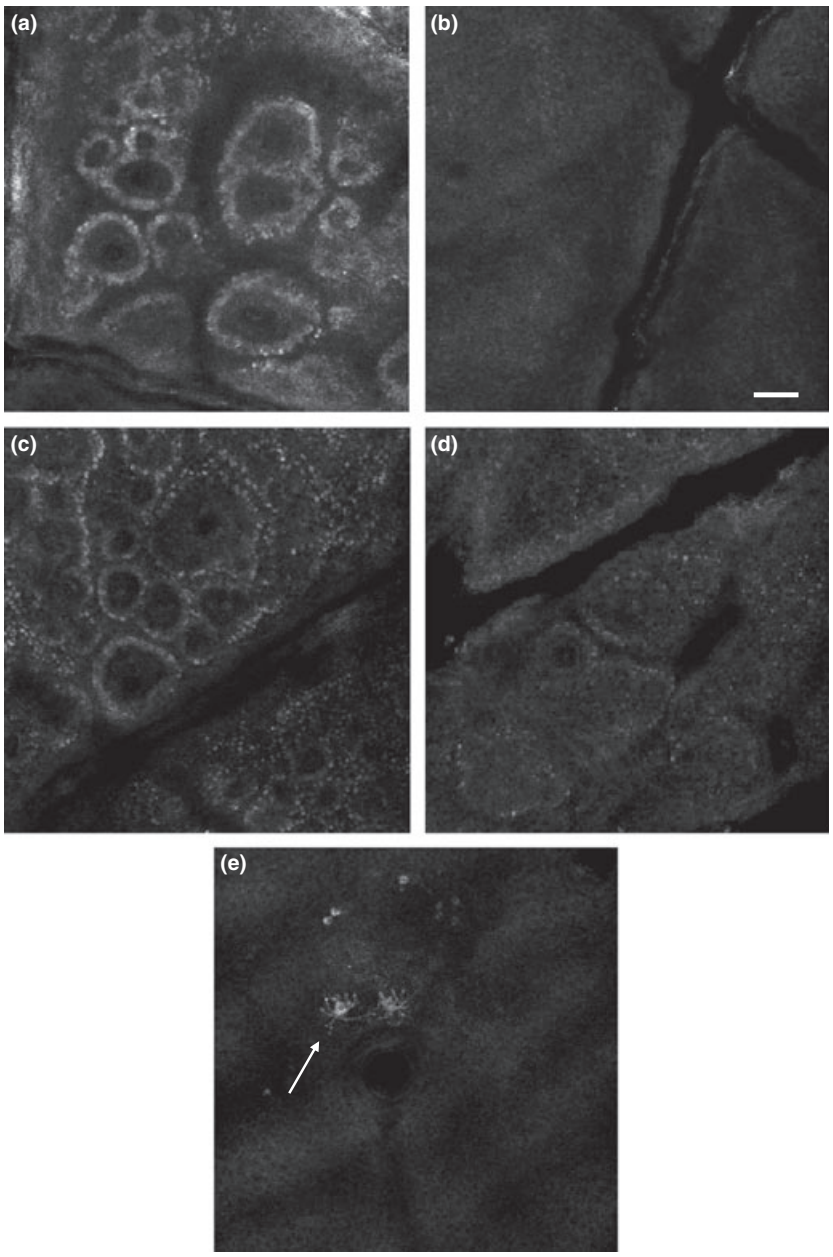

Figure 1 RCM images of vitiligo and ND. (a) (c) Non-lesional skin of vitiligo and ND. Brightly refractile pigment cells are seen, and they are arranged in the ring structures. (b) Lesional skin of vitiligo. Absence of the pigment cells and ring structures. (d) Lesional skin of ND. The pigment cells are small, low brightness. The density of the pigment cells is low. (e) Lesional skin of vitiligo. The dendritic cells (arrow) correspond to melanocytes around the hair follicle. Scale bar 50 μm

Discussion

RCM has proven to be a promising, noninvasive, high-resolution imaging tool for histologic evaluation of the skin *in vivo*. It is confirmed that the distribution of melanin on non-invasive optical sections obtained by RCM corresponded well with the distribution of melanin pigment determined on histological sections.[5,9] Because the RCM en-face visualization of skin layers can display up to 5×5 mm of tissue, it provides far more information of the distribution of melanin than the traditional histological vertical sections. In this study, we tried to use this new technology to study the feature of the distribution of pigment cells of these two hypopigmentation disorders and then concluded the differential features.

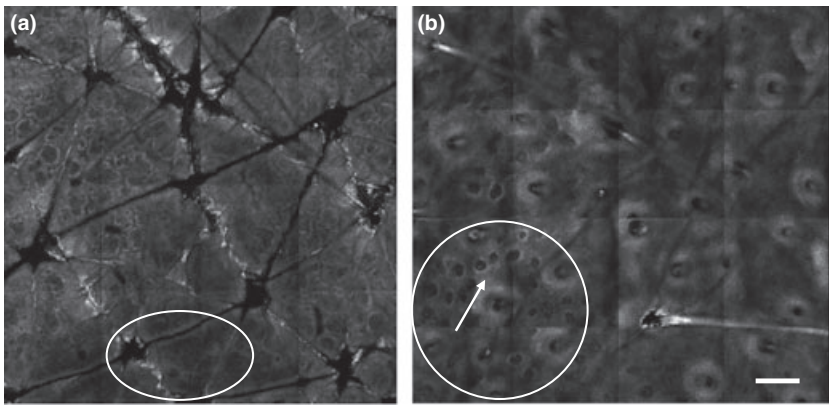

Figure 2 Vitiligo. RCM Mosaic images (2 × 2 mm). (a) Lesional skin pigment cells were absent in partial region. Absence of ring structures in dark area (circle). (b) Lesional skin. Note the ring structures (circle) were seen around the hair follicle (arrow). Scale bar 250 μm

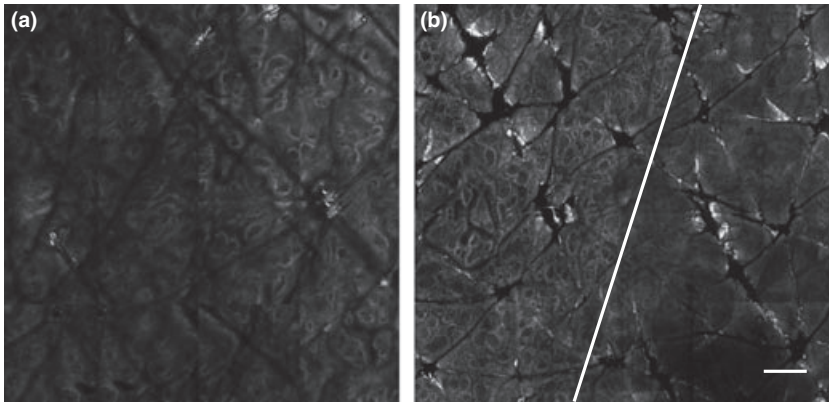

Figure 3 Margin of the lesion. RCM Mosaic images (2 × 2 mm). (a) Margin of the ND lesion. The margins were indistinct. (b) Margin of the vitiligo lesion. The margin was distinct. The dividing line was clear. Scale bar 250 μm

Table 1 Comparison of RCM features between ND and vitiligo

Feature	Vitiligo patients number (%)	ND patients number (%)	*P*-value
Complete absence of pigment cells in lesional skin	47 (78.33)	0	0.000
Homogeneous distribution of pigment cells in lesional skin	0	43 (69.35)	0.000
Melanocytes or ring structures around the hair follicles in lesional skin	5 (8.33)	0	0.026
The distinct margins	37 (71.15)	2 (3.23)	0.000
The relative gray value under 75	52 (86.67)	11 (17.74)	0.000

ND is a congenital disorder. The commonly used clinical diagnostic criteria for ND are proposed by Coupe.[8] These clinical criteria have been widely used for the differential diagnosis between vitiligo and ND. However, a multicenter study performed in Korea showed that 20.2% of NDs were first detected after the age of 3. Furthermore, the clinical features of ND may initially appear similar to those of vitiligo, especially focal or segmental vitiligo characterized by isolated or a few macules.[10] Segmental vitiligo tends to have an earlier onset and to be more stable than generalized vitiligo. In the end, melanin pigmentation may remain in the depigmented epidermis five years or more after the development of vitiligo.[1] All these make the differential diagnosis difficult.

A previous histopathological study showed that both the amount of melanin and the number of melanocytes were decreased in patients with ND.[11] Electron microscopic finding indicated the reduction of melanosomes inside the melanocytes and type of melanosomes was abnormal.[12] We found the RCM features of ND include the small and low brightness pigment cells and low density of the pigment cells, which agree with the previous studies.[7] Because of the character of en-face imaging, RCM images display the distribution of the pigment cells up to 5×5 mm areas. The distribution of the pigment cells was relative even compared with vitiligo patients. There was no complete loss of the pigment cells. The difference between the lesional and non-lesional skin was in the density and shape of the pigment cells, therefore the dividing line was not so easy to recognize.

Histopathological and histochemical examinations showed the loss of melanocytes and melanin pigment in vitiligo lesions.[13] Our study confirmed that the disappearance of the bright ring structures, corresponding to the complete loss of pigment cells, is the main feature of the vitiligo lesions. However, there are still some pigment cells in some vitiligo patients (18.33%). Although the remaining pigment cells make the differential diagnosis difficult, the distribution of these cells was different from ND lesion. We observed the ring structures and melanocytes around the hair follicles, indicating repigmentation. The clear dividing line of the RCM image is caused by the complete loss of pigment cells in the lesional side, which form a dark area in the picture. Ardigo *et al.*[6] described some changes in non-lesional skin of the vitiligo patients, but we observed half-rings or scalloped border-like features of the rings in only two patients. This could be explained by the fact that most of our patients had stable vitiligo.

The measurement of melanin pigment is useful for the differential diagnosis of hypopigmentary disorders.[14] The principle of RCM is based on different indexes of refraction. Contrast of image depends on: (i) level of imaging; (ii) image saturation; (iii) nature of the lesion.[15] As melanin is the strongest endogenous contrast in human skin, the contrast of the RCM image is related to the amount of melanin containing cells. In order to eliminate the factors of the level of imaging and the image saturation, we used the same laser energy around 2 mW and same level in each patient. We used the ImageJ software to calculate gray values of the image, and introduced RGVs, which represent the ratio of pigmentation at a lesion site relative to that of a non-lesion site. As we expected, the mean RGVs of vitiligo patients was significantly lower than ND patients.

In conclusion, RCM can be used as an auxiliary diagnostic tool for the differential diagnosis between vitiligo and ND. The most important feature for the differential diagnosis was the complete loss of pigment cells or not. The distribution of pigment cells was also different between vitiligo and ND. Moreover, the dividing line was another feature to discriminate these two disorders. Finally we could calculate the RGV. It should be noted that this study has examined only stable phase vitiligo and isolated or segmental ND patients. The value of the RGV has not been fully studied. More extensive study is necessary to confirm the results. In this study, we mainly focused on the distribution of melanin pigment. The application of RCM in the other hypopigmentation disorders, such as post-inflammatory and pityriasis alba, should be explored.

Acknowledgments

The work was supported by the National Science Foundation of China Research project No. 30771935.

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