Pilot-study of photodamaged skin and melasma using reflectance confocal microscopy

Estudo-piloto da pele fotodanificada e do melasma pela microscopia confocal de reflectância

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ABSTRACT

Introduction: Photoaging and melasma are frequent dermatological complaints. Confocal reflectance microscopy (CRM) is a recent technique that can be used for diagnostic evaluation of these dermatoses.

Objectives: To evaluate the characteristics of the epidermis and dermis containing pigmentary alterations caused by photodamage and melasma with the assistance of CRM, and compare the findings linked to these changes with the perilesional region.

Methods: A pilot study was conducted with eight female individuals (aged 38 to 50 years, Fitzpatrick phototypes from II to IV) with clinical diagnoses of photodamage (n = 4) and melasma (n = 4) in the facial malar region. The perilesional and lesional regions were compared regarding the thickness of the stratum corneum and viable epidermis, the depth of the interpapillary crests, and the presence of hyper-refractive structures.

Results: The pigmentary alterations in the photodamaged skin revealed a morphological pattern – such as an increase in the depth of the interpapillary ridges in the lesion region – typical of solar lentigo. In the lesional region of volunteers bearing melasma, it was possible to observe the presence of dendritic cells in the epidermis and melanophages in the dermis. All volunteers had hyper-refractive keratinocytes in the lesional epidermis region.

Conclusions: Considering the number of patients evaluated, it was possible to characterize and compare cutaneous pigmentary alterations caused by photodamage to those cause by melasma.

Keywords: Aging; Microscopy, confocal; Diagnosis

RESUMO

Introdução*: O fotoenvelhecimento e o melasma são queixas dermatológicas frequentes. A microscopia confocal de reflectância (MCR) é técnica recente que pode ser usada para avaliação diagnóstica dessas dermatoses.*

Objetivos: *Avaliar as características da epiderme e derme nas alterações pigmentares da pele fotodanificada e do melasma pela MCR e comparar os achados dessas alterações com a região perilesional.*

Métodos: *Foi realizado estudo-piloto com oito participantes do sexo feminino, com idades variando de 38 a 50 anos, fototipos de II a IV, com diagnóstico clínico de fotodano (n = 4) e melasma (n = 4) na região malar da face. Foram comparadas a espessura do estrato córneo e da epiderme viável, a profundidade das cristas interpapilares e a presença de estruturas hiper-refrativas na região perilesional e lesional.*

Resultados e Discussão: *As alterações pigmentares da pele fotodanificada revelaram padrão morfológico característico do lentigo solar, como aumento na profundidade das cristas interpapilares na região da lesão. Nas voluntárias com melasma, foi possível observar a presença de células dendríticas na epiderme e melanófagos na derme na região da lesão. Todas as voluntárias apresentaram queratinócitos hiper-refrativos na epiderme da região lesional.*

Conclusões*: Considerando o número de pacientes avaliados, foi possível caracterizar e comparar as alterações pigmentares na pele fotodanificada e no melasma.*

Palavras-chave: *Envelhecimento da pele; Microscopia confocal; Diagnóstico*

Original Articles

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INTRODUCTION

Photoaging and melasma are frequent complaints in dermatological practices.[1-3] From a clinical point of view, photodamaged skin has wrinkles, changes in texture and pigmentation, and loss of elasticity and firmness.[3-5] Patients with melasma typically have brown spots with irregular borders and clear delimitation, located in areas exposed to the sun, especially in the face and in women. Both dermatoses can have impacts on the patients' quality of life.[5-9] In Brazil, roughly 8.4% of the population have some pigmentation disorder, while 10% of the Latino population living in the United States have melasma.[7,10]

Solar lentigo, also known as senile lentigo or aging spot, is a benign pigmentary disorder that arises with the process of photoaging. This hyperchromia occurs in areas exposed to the sun, especially in the dorsum of the hands, forearms and face.[11-14] It affects more than 90% of the Caucasian population over 50 years of age.[15] Factors linked to its onset would be related to exposure of the skin to external factors, such as ultraviolet (UV) radiation, polycyclic aromatic hydrocarbons (pollution), and the expression of growth and inflammatory factors.[12,16,17]

Melasma is a pigmentary disorder characterized by the presence of macules located mainly in the central, malar and / or mandibular regions of the face.[1,7,16-18] Ultraviolet (UV) radiation, female hormones (pregnancy, endocrine dysfunction, use of oral contraceptives) and inflammatory processes are involved in its pathogenesis as triggering factors, associated with genetic predisposition.[1,7,19,20] This disorder's pathophysiology has not yet been fully elucidated, however some theories suggest that its emergence would be linked to the increase in the expression of melanogenic factors and specific receptors such as estrogen.[1,19] The increase in the number and caliber of blood vessels in the affected region, as well as the increase in the expression of vascular endothelial growth factor, is also involved.[16,20]

The diagnosis of pigmentary changes of the face is predominantly performed by clinical examination and / or histological examination, when there is suspicion of malignancy. A new noninvasive technique, such as confocal reflectance microscopy (CRM), can assist in the clinical diagnosis and also contribute to increase therapeutic efficacy.[21-23] In addition, CRM can be applied in the quantification of epidermal pigmentation, [24] since this methodology's principle consists of the emission of infrared light on the skin and its subsequent selective capture when reflected by cutaneous structures that have different refractive indices, yielding black and white images. Keratin, melanin, and collagen fibers of the dermis are hyperrefractive structures, being visualized in a lighter color. [25-28]

Methods for instrumental evaluation of photodamaged skin and melasma

There are currently a variety of techniques for instrumentally evaluating the skin, assisting clinical diagnosis.[29] Among the methods available for evaluating melasma and photodamage, reflectance spectroscopy and high-resolution image analysis stand out in the investigation of skin color and melanin distribution.[30,31] The Cutometer® device (Courage-Khazaka, Germany) evaluates the mechanical properties (changes in the elasticity and firmness) of the photodamaged skin. In addition, the analysis of epidermis' and dermis' thickness using high frequency ultrasonography substantially contributes to the evaluation of the therapeutic efficacy.[32,33]

Confocal reflectance microscopy (CRM) is an advanced technique that allows examination of the epidermis and papillary dermis with a resolution close to that of histological examination, with the ability of identifying structures and cells with high resolution.[2,34] This technique's basic principle involves beaming infrared light on the skin and the selective capture of this light, after being reflected by cutaneous structures such as keratin, melanin and collagen fibers, whose refractive indexes are diverse.[26] Confocal reflectance microscopy is considered a tool for diagnostic confirmation of pigmentary changes in the photodamaged skin and melasma, in this manner avoiding cutaneous biopsy in the face.[2]

In light of this, the objective of the present study was to evaluate the characteristics of the epidermis and papillary dermis caused by pigmentary alterations linked to melasma and cutaneous photodamage using CRM, and to compare the findings related to these alterations with the characteristics of the perilesional region.

METHODS

Recruitment

This pilot study was performed after approval by the Research Ethics Committee of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto (CEP / FCFRP, Protocol No. 1,418,673 / 2015). The study sample corresponded to 8 female participants aged 38-50 years, Fitzpatrick phototypes II to IV, with alterations of hyperpigmentation in the malar region, diagnosed with pigmentary skin alterations (n = 4) and melasma (n = 4).

Instrumental evaluation

Images of the malar regions were obtained in triplicate (lesional and perilesional) using a confocal reflectance laser microscope VivaScope 1500 (Lucid, USA), having been standardized using the coupled software Vivastack (Lucid, USA). The images were obtained at every 1.5μm, starting from the stratum corneum up until the depth of 37.5μm, and at every 3μm up until the depth of 132.5μm. Based on the obtained images, the thickness of the stratum corneum, the viable epidermis (granulosum, spinosum and basal layers), and the depth of the interpapillary ridges were evaluated quantitatively and objectively. Likewise, the presence and absence of hyperrefractive structures in the perilesional and lesional region were evaluated qualitatively and subjectively. These evaluations were performed all volunteers.

Statistical analysis

The data had normal distribution, meaning that the t-test was used to compare the morphological alterations between the lesional and perilesional regions. Results were expressed as mean

values and standard deviations. A significance level of $p < 0.05$ was used. The Origin8Pro® software (OriginLab, USA) was employed to evaluate the distribution of the data, while GraphPad Prism 5® software (GraphPad Software, USA) assisted in the statistical analysis.

RESULTS

The quantitative and objective analysis of the data obtained from the volunteers diagnosed with photodamage showed a non-significant increase in the values of the thickness of the stratum corneum and viable epidermis in the lesional region as compared to the perilesional region. Also, a significant increase ($p < 0.05$) in the depth of the interpapillary ridges of the lesional region was observed as compared with the perilesional region. These findings were not observed among volunteers bearers of melasma (Table 1).

Based on the qualitative and subjective analysis of the images, it was possible to observe the presence of hyperrefractive structures in the lesional region of all volunteers (Table 2 and Figures 1L.e, 2L.e and 3L.e).

In all volunteers diagnosed with photodamage, it was possible to observe a disorganized pattern for the interpapillary ridges and accumulation of hyperrefractive keratinocytes in the lesional region when compared to the perilesional region (Table 2 and Figure 1 L. JDE).

Two volunteers diagnosed with melasma presented hyperrefractive keratinocytes in the perilesional region (Table 2). In addition, it was possible to observe the presence of dendritic cells in the lesional region of a volunteer diagnosed with melasma (Table 2 and Figure 2L.e) and of melanophages in the lesional region's dermis of another volunteer who was also diagnosed with melasma (Table 2 and Figure 3 L.d).

In a third volunteer bearer of melasma, it was possible to observe a disorganized pattern for the interpapillary ridges in the perilesional region (Table 2).

Table 1: Comparison of the CRM findings related pigmentary changes caused by photodamage and melasma in the lesional (L) and perilesional (P) regions

Pigmentary disorder	Stratum corneum's thickness (µm)	Viable epidermis' thickness (µm)	Interpapillary ridge's depth (µm)
Melasma			
perilesional	25 +/- 4.5	32.9 +/- 3.5	30.12 +/- 6.8
lesional	24.5 +/- 1.47	33.6 +/- 5.8	35.7 +/- 10.1
Photodamaged skin			
perilesional	21.6 +/- 2.6	30.8 +/- 4	23.5 +/- 6.4
lesional	25.2 +/- 7.6	46.3 +/- 12.2	58.6 +/- 16.2*

* $p < 0.05$ related to the perilesional region. Values are expressed as mean values and standard deviations.

DISCUSSION

The images obtained by CRM evidenced the presence of hyperrefractive keratinocytes in the epidermis of the regions with benign pigmentation disorders. This outcome indicates the accumulation of melanin in keratinocytes, which is one of the

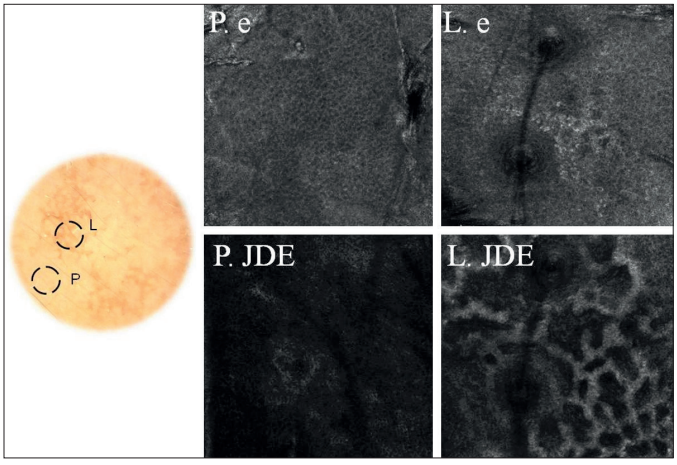

Figure 1: Lentigo solar: P - perilesional region; L - lesional region; Pe - perilesional region's epidermis; P. JDE: perilesional region's dermoepidermal junction; L. e: lesional region's epidermis; L. JDE: lesional region's dermoepidermal junction

Table 2: Comparison of the CRM findings related to the pigmentary changes caused by photodamage and melasma in the lesional (L) and perilesional (P) regions

Pigmentary disorder	Hyperrefractive keratinocytes		Dendritic cells in the epidermis		Melanophages in the dermis		Disorganized pattern of interpapillary ridges	
Melasma	L	P	L	P	L	P	L	P
1	+	+	-	-	-	-	-	-
2	+	-	-	-	-	-	-	+
3	+	-	-	-	+	-	-	-
4	+	+	+	-	-	-	-	-
Photodamage	L	P	L	P	L	P	L	P
5	+	-	-	-	-	-	+	-
6	+	-	-	-	-	-	+	-
7	+	-	-	-	-	-	+	-
8	+	-	-	-	-	-	+	-

L: hyperpigmented region; P: perilesional region; +: presence; -: absence

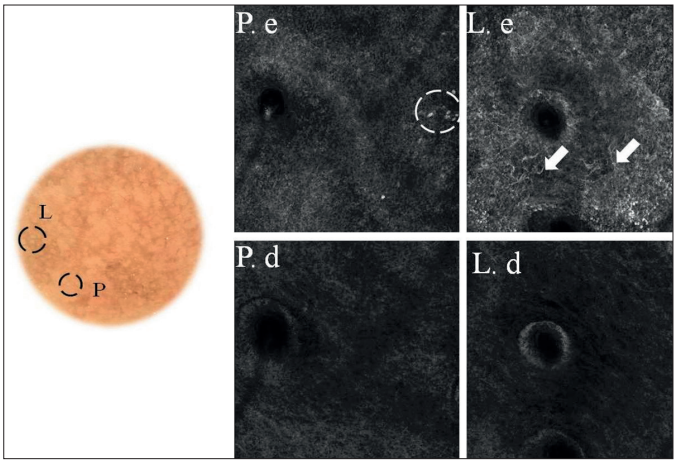

Figure 2: Melasma: P - perilesional region; L - lesional region; P.e - perilesional region' epidermis with ovoid cells suggesting inflammatory infiltrate; P.d: perilesional region's dermis; L.e: lesional region's epidermis with dendritic cells indicated by the arrows; and L.d: lesional region's dermis

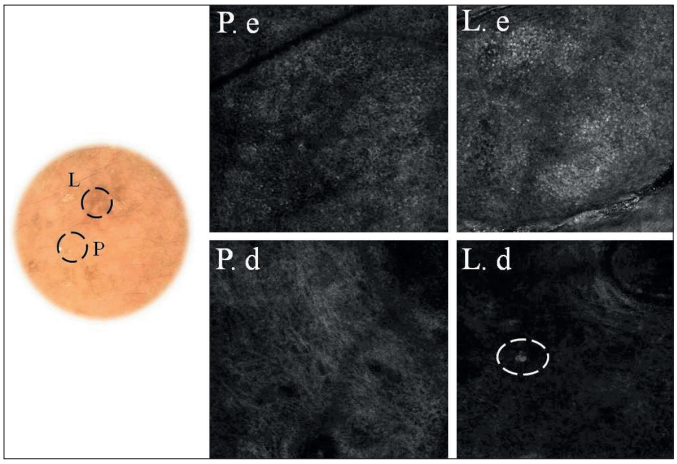

Figure 3: Melasma: P - perilesional region; L - lesional region; Pe - perilesional region' epidermis; P.d - perilesional region' dermis; L.e: lesional region's epidermis; L.d: lesional region's dermis with melanophages identified by the dotted circular region, characteristic appearance of the dermal melasma; Lentigo solar: P - perilesional region; L - lesional region; Pe - perilesional region' epidermis; P. JDE - perilesional region's dermoepidermal junction; Le - lesional region's epidermis; L. JDE - lesional region's dermoepidermal junction

morphological consequences of photoaging.[36] Irregular deposition of melanin in the skin has been reported in CRM studies and is characterized by the observation of bright structures due to the high refractive index of melanin.[21, 36,37]

The disorganized pattern of the interpapillary ridges – represented by a modification in the shape of the papillae, which become polygonal – associated with irregular alignment, was observed at the dermoepidermal junction in the lesional region of the volunteers with pigmentary alterations due to photodamage. These changes are characteristic of solar lentigo, as described in the literature.[21,37,38] A significant increase ($p < 0.05$) in the depth of the interpapillary ridges of the lesional region and a non-significant increase in the thickness of the viable epidermis were observed in the volunteers who presented photodamaged skin with solar lentigo. To date, according to histological studies, the increase in the keratinocytes' thickness in solar lentigo might be related to hypertrophy or increased cell proliferation.[39,40]

According to the literature, the presence of dendritic cells, commonly observed in melasma, might correspond to active melanocytes.[19] In another volunteer diagnosed with melasma, the presence of ovoid contrast cells located in the dermis was observed, which, according to reports in the literature, could be melanophages.[41,42] The outcomes obtained are aligned to those observed in previous investigations, suggesting a possible morphological difference between these pigmentary disorders, that are detectable by CRM.[19,21,36-24]

CONCLUSION

Considering the number of patients evaluated, it was possible to characterize the pigmentary alterations in the photodamaged skin and in the melasma. Based on the analyses performed with assistance of CRM, it was possible to identify differences between pigmentary alterations and the perilesional areas in the malar region, both in melasma and in photodamaged skin. ●

REFERENCES

1. Sofen B, Prado G, Emer J. Melasma and Post Inflammatory Hyperpigmentation: Management Update and Expert Opinion. Skin Therapy Lett. 2016;21(1):1-7.
2. Martini APM, Mercurio DG, Maia Campos PMBG. Assessment of skin pigmentation by confocal microscopy: Influence of solar exposure and protection habits on cutaneous hyperchromias. J Cosmet Dermatol. 2017;16(3):364-9.
3. Bouloc A, Vergnanini AL, Issa MC. A double-blind randomized study comparing the association of Retinol and LR2412 with tretinoin 0.025% in photoaged skin. J Cosmet Dermatol. 2015;14(1):40-6.
4. Kim HM, An HS, Bae JS, Kim JY, Choi CH, Kim JY, et al. Effects of palmitoyl-KVK-L-ascorbic acid on skin wrinkles and pigmentation. Arch Dermatol Res. 2017;309(5):397-402.
5. Issa MCA, Fassini A, Boechat M, Ferolla ACJ. Photodynamic therapy in photoaging: literature review. Surg Cosmet Dermatol. 2016;8(4):10-6.
6. Rendon MI, Barkovic S. Clinical Evaluation of a 4% Hydroquinone + 1% Retinol Treatment Regimen for Improving Melasma and Photodamage in Fitzpatrick Skin Types III-VI. J Drugs Dermatol. 2016;15(11):1435-44
7. Cestari T, Arellano I, Hexsel D, Ortonne JP. Melasma in Latin America: options for therapy and treatment algorithm. J Eur Acad Dermatol Venereol. 2009;23(7):760-72.
8. Gold MH, Gallagher C. An Evaluation of the Benefits of a Topical Treatment in the Improvement of Photodamaged Hands With Age Spots, Freckles, and/or Discolorations. J Drugs Dermatol. 2013;12(12):1468-72.
9. Kang WH, Yoon KH, Lee ES, Kim J, Lee KB, Yim H, et al. Melasma: histopathological characteristics in 56 Korean patients. British Journal of Dermatology 2002;146(2):228-37
10. Agostinho KM, Karenine MH, Cavalcante PP, Cavalcanti DL. Doenças dermatológicas frequentes em unidade básica de saúde. Cogitare Enfermagem. 2013;18(4):715-21.

11. Goorochurn R. Viennet C, Granger C, Fanian F, Varin-Blank N, Le Roy C, et al. Biological processes in solar lentigo: insights brought by experimental models. Exp Dermatol. 2015;25(3):174-7.
12. Choi W, Yin L, Smuda C, Batzer J, Hearing VJ, Kolbe L. Molecular and histological characterization of age spots. Exp Dermatol. 2017;26(3):242-8.
13. Warrick E, Duval C, Nouveau S, Bastien P, Piffaut V, Chalmond B, et al. Morphological and molecular characterization of actinic lentigos reveals alterations of the dermal extracellular matrix. Br J Dermatol. 2017;177(6):1619-32.
14. Choi W, Yin L, Smuda L, Batze J, Hearing VJ, Kolbe K. Molecular and histological characterization of age spots. Experimental Dermatology. 2017;26(6):242-8.
15. Mashiko T, Oka A, Osawa E, Koshima I. A Deceptively Simple Solution for Refractory Melasma: Glycolic Acid Peels and Hydroquinone at Home. Plast Reconstr Surg Glob Open. 2017;5(5):335.
16. Cameli N, Abril E, Agozzino M, Mariano M. Clinical and Instrumental Evaluation of the Efficacy of a New Depigmenting Agent Containing a Combination of a Retinoid, a Phenolic Agent and an Antioxidant for the Treatment of Solar Lentigines. Dermatology. 2015;230(4):360-6.
17. Nakamura M, Morita A, Seit S, Haarmann-Stemmann T, Grether-Beck S, Krutmann J. Environment-induced lentigines: formation of solar lentigines beyond ultraviolet radiation. Exp Dermatol. 2015;24(6):407-11.
18. Perper M, Eber AE, Fayne R, Verne SH, Magno RJ, Cervantes J, et al. Tranexamic Acid in the Treatment of Melasma: A Review of the Literature. Am J Clin Dermatol. 2017;18(3):371-81.
19. Costa MC, Eljaiek HV, Abraham LS, Azulay-Abulafia L, Ardigo M. In vivo reflectance confocal microscopy in a typical case of melasma. An Bras Dermatol. 2012; 87(5):782-4.
20. Cohen PR. Melasma treatment: A novel approach using a topical agent that contains an anti-estrogen and a vascular endothelial growth factor inhibitor. Med Hypotheses. 2017;101:1-5.
21. Longo C, Zalaudek I, Argenziano G, Pellacani G. New Directions in Dermatopathology In Vivo Confocal Microscopy in Clinical Practice. Dermatol Clin. 2012;30(4):799-814
22. Moscarella E, Rabinovitz HI. Zalaudek I, Piana SI, Stanganelli I, Oliviero MC, et al. Dermoscopy and reflectance confocal microscopy of pigmented actinic keratoses: a morphological study. J Eur Acad Dermatol Venereol. 2015;29(2):307-14.
23. Longo C, Casari A, Beretti F, Cesinaro AM, Pellacani G. Skin aging: In vivo microscopic assessment of epidermal and dermal changes by means of confocal microscopy. J Am Acad Dermatol. 2013;68(3):e73-82.
24. Lagarrigue SG, George J, Questel E, Lauze C, Meyer N, Lagarde JM, et al. In vivo quantification of epidermis pigmentation and dermis papilla density with reflectance confocal microscopy: variations with age and skin phototype. Exp Dermatol. 2012; 21(4):281-6.
25. Bielfendt S, Bohling A, Wilherlm KP. Bioengeneering method to acesses aging parameters in the deep of the skin. SOFW Journal. 2011;137:2-9
26. Kang HY, Bahadoran P, Ortonne JP. Reflectance confocal microscopy for pigmentary disorders. Exp Dermatol. 2010;19(3): 233-9.
27. Majdzadeh A, Lee A, Wang H, Lui H, McLean D I, Crawford RI, et al. Real time visualization of melanin granules in normal human skin using combined multiphoton and reflectance confocal microscopy. Photodermatol Photoimmunol Photomed. 2015; 31(3):141-8.
28. Malvehy J, Pellacani G. Dermoscopy, Confocal Microscopy and other Non-invasive Tools for the Diagnosis of Non-Melanoma Skin Cancers and Other Skin Conditions. Acta Derm Venereol. Forthcoming 2017.
29. Mercurio DG, Segura JH, Demets MB, Maia Campos PM. Clinical scoring and instrumental analysis to evaluate skin types. Clin Exp Dermatol. 2013;38(3):302-8.
30. Pandya A, Berneburg M, Ortonne JP, Picardo M. Guidelines for clinical trials in melasma. Br J Dermatol. 2006;156(suppl 1):21-8.
31. Brenner AV, Lubin JH, Calista D, Landi MT. Instrumental Measurements of Skin Color and Skin Ultraviolet Light Sensitivity and Risk of Cutaneous Malignant Melanoma: A Case-Control Study in an Italian Population. Am J Epidemiol. 2002; 156(4):353-62.
32. Ulrich J, Schwürzer-Voit M, Jenderka KV, Voit C. Sonographic diagnostics in dermatology. J Dtsch Dermatol Ges. 2014;12(12):1083-98.
33. Unholzer A, Korting HC. High-frequency ultrasound in the evaluation of pharmacological effects on the skin. Skin Pharmacol Appl Skin Physiol. 2002; 15(2):71-84.
34. Pellacani G, Cesinaro AM, Seidenari S. In vivo assessment of melanocytic nests in nevi and melanomas by reflectance confocal microscopy. Mod Pathol Italy. 2005;18(4):469-74.
35. Mercurio DG, Jdid R, Morizot F, Masson P, Maia Campos PMBG. Morphological, structural and biophysical properties of French and Brazilian photoaged skin. Br. J. Dermatol. 2016;174(3):553-61.
36. Longo C, Casari A, Pace B, Simonazzi S, Mazzaglia G, Pellacani G. Proposal for an in vivo histopathologic scoring system for skin aging by means of confocal microscopy. Skin Res Technol. 2013;19(1):e167-73
37. Pollefliet C, Corstjens H, González S, Hellemans L, Declercq L, Yarosh D. Morphological characterization of solar lentigines by in vivo reflectance confocal microscopy: a longitudinal approach. Int J Cosmet Sci. 2013;35(2):149-55.
38. Kang HY, Bahadoran P, Suzuki I, Zugaj D, Khemis A, Passeron T, et al. In vivo reflectance confocal microscopy detects pigmentary changes in melasma at a cellular level resolution. Exp. Dermatol. 2010;19(8): e228–e233
39. Shin J, Park JY, Kim SJ, Kang HY. Characteristics of keratinocytes in facial solar lentigo with flattened rete ridges: comparison with melasma. Clin Exp Dermatol. 2015;40(5):489-94.
40. Carvalho N, Farnetani F, Ciardo S, Ruini C, Witkowski AM, Longo C, et al. Reflectance confocal microscopy correlates of dermoscopic patterns of facial lesions help to discriminate lentigo maligna from pigmented nonmelanocytic macules. Br J Dermatol. 2015;173(1):128-33.
41. Guitera P, Li LI, Scolyer RA, Menzies SW. Morphologic Features of Melanophages Under In Vivo Reflectance Confocal Microscopy. Arch Dermatol. 2010;146(5):492-98.
42. Liu H, Lin Y, Nie X, Chen S, Chen X, Shi B, et al. Histological classification of melasma with reflectance confocal microscopy: a pilot study in Chinese patients. Skin Res Technol. 2011;17(4):398-403.

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