Hypoepiluminescence Microscopy of Pigmented Skin Lesions: New Approach to Improve Recognition of Dermoscopic Structures

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BACKGROUND Hypoepiluminescence microscopy (HELM) is a new dermoscopic approach for analysis of pigmented skin lesions (PSLs) after surgical excision.

OBJECTIVES The objective was to verify whether this method could provide additional morphologic information for diagnostic or didactic purposes compared to conventional epiluminescence microscopy (ELM).

PATIENTS AND METHODS Thirty-one PSLs from 30 patients were consecutively evaluated by ELM and, after excision, by HELM. For HELM examination, the lesion was positioned on a glass slide and illuminated from above with a halogen lamp and from underneath with an LED source. All lesions were subsequently examined histopathologically.

RESULTS In 11 of 31 (35.5%) lesions, a typical pigment network, as assessed by ELM, appeared bidimensional with HELM. In 9 lesions (9/31; 29%) ELM showed a gray-blue area, while HELM allowed us to distinguish 5 lesions (5/9, 55.5%) with gray area predominant showing a lichenoid lymphocytic infiltration and few melanophages from the other 4 lesions (4/9, 44,5%) with heavy dermal accumulation of pigmented melanocytes or melanophages where a blue area was clearly visible at HELM. In 9 other cases (29%), ELM analysis revealed a central homogeneous dark brown/black pigmentation that in 7 cases was seen under HELM examination to consist of globules.

CONCLUSIONS HELM is particularly useful in evaluating heavily PSLs or structures located in the reticular dermis.

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In the past two decades dermoscopy [dermatoscopy, epiluminescence microscopy (ELM), skin surface microscopy] has grown enormously from a research tool to a standard technique for in vivo diagnosis of pigmented skin lesions (PSLs).[1–15] Therefore, an important limitation of dermoscopy is that this technique cannot be used to investigate structures in the mid and deep dermis. For example, dermoscopy differentiates poorly between the blue veil of regression structures, histopathologically related to melanophages in the upper dermis, and the pigmentation due to the presence of typical or atypical melanocytes in the dermis. The recognition of the blue structures might be related with dermoscopist's experience. Furthermore, two recent studies[16,17] demonstrated that the presence of

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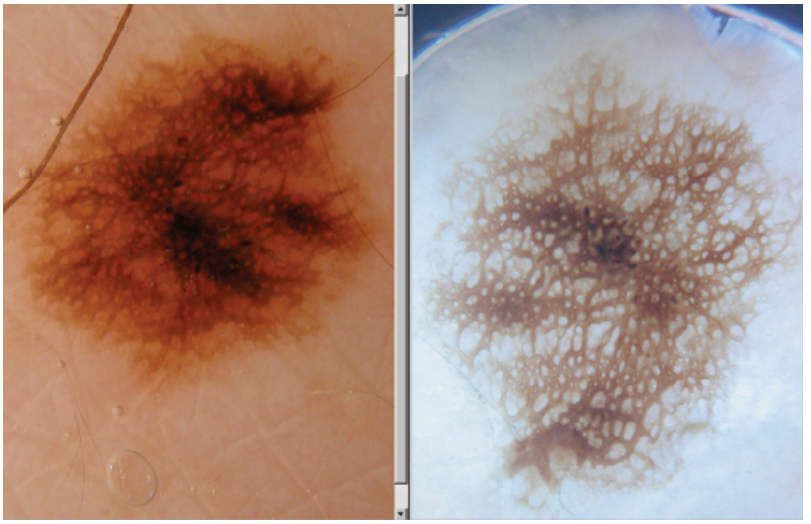

Figure 1. Stereotypical ELM (left) pigment network that appeared bidimensional with HELM (right).

dermoscopic signs of regression may predict histopathologic disagreement.

In 2000, Braun and colleagues[18] introduced a new technique for the study of PSLs known as hypoluminescence microscopy (HLM). This technique involves visualizing the morphologic structures of a surgically excised PSL using illumination from the dermal side.

In our study we combined classic ELM with HLM to create hypoepiluminescence microscopy (HELM). The aim was to determine whether this method could provide additional morphologic information for diagnostic or didactic purposes, compared to conventional ELM.

Materials and Methods

Thirty-one PSLs observed at the outpatient clinic of the Department of Dermatology, University of L'Aquila, from January 1996 to May 2005 were included in the study. Selection criteria included: (1) equivocal clinical findings (based on ABCDE criteria) and/or (2) equivocal dermoscopic features (irregular pigment network, gray-blue pigmentation, irregular dots, and globules and streaks). Every PSL was consecutively evaluated by ELM and, after excision, by HELM.

After covering the lesion with immersion oil, we performed classic ELM in vivo using a digital

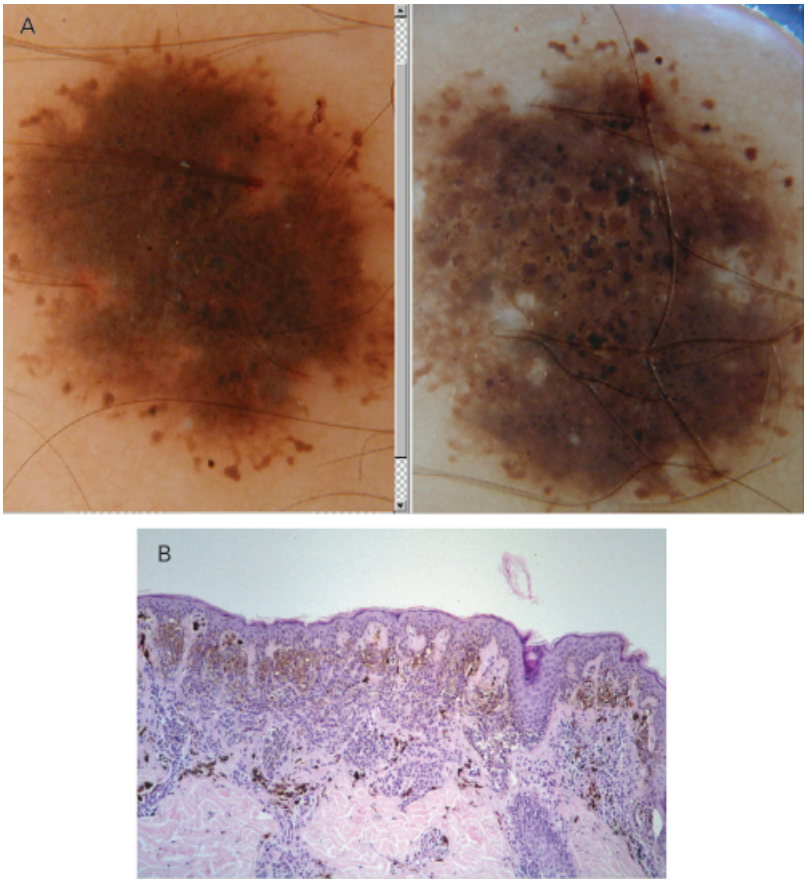

Figure 2. (A) ELM analysis (left) revealed a central homogeneous dark brown/black pigmentation that consists of globules at HELM examination (right). (B) Large and deeply pigmented nests of melanocytes within the junction and/or beneath the epidermis (hematoxylin and eosin; original magnification, × 100).

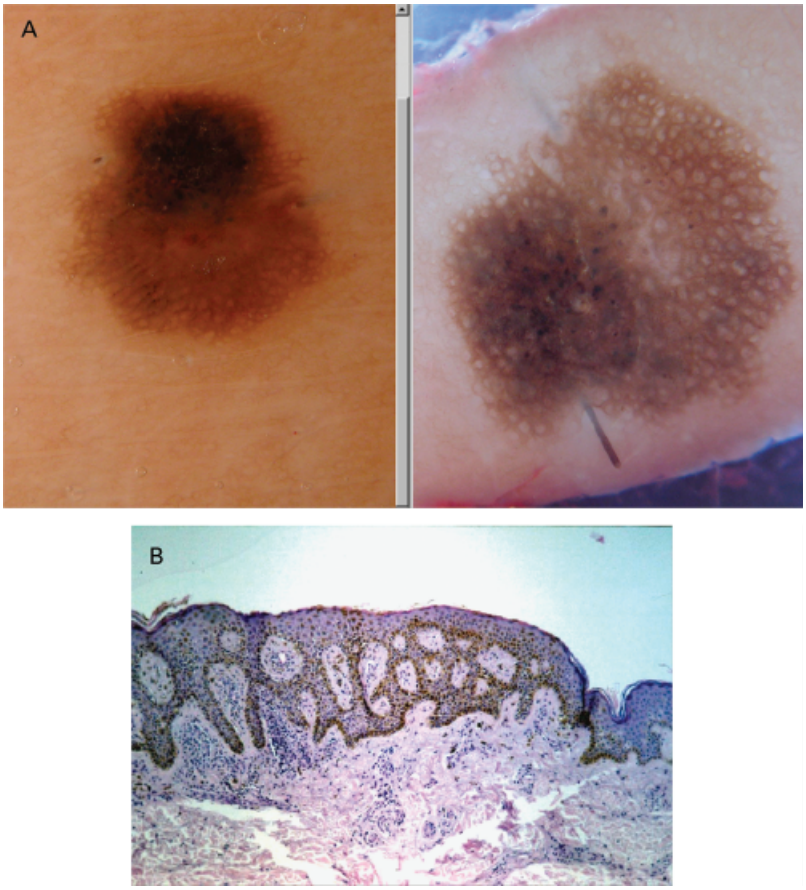

Figure 3. (A) Presence of peripheral black lamella at ELM examination (left) that corresponded to network and black dots with HELM (right). (B) Pigmentation of the basal layer of the epidermis and melanin in the stratum corneum (hematoxylin and eosin; original magnification, × 100).

camera (Coolpix 990, Nikon Corporation, Tokyo, Japan) with a special dermoscopy objective containing a halogen lamp (Nevuscreen, Arké s.a.s., Avezzano, Italy). The lesion was visualized on a 15-in. high-resolution monitor, and the image was captured in JPEG format under high-quality compression (2048 × 1536 resolution) and stored in our computer database (Nevuscreen). The magnification of the images varied from 22 to 66 × . The excised lesion was placed in a drop of alcohol on a glass slide, with the digital camera of the ELM system located above the slide. The lesion was illuminated from the dermal side using a dermatoscope (Dermlite, 3Gen LLC, Dana Point, CA) with an LED source. We used the same ELM system for visualization and storage of the HELM images, reproducing similar magnification and orientation of ELM images to permit their comparison.

Results

Thirty-one PSLs were excised from 18 women and 12 men, ranging in age from 18 to 76 years (mean, 39 years). Eighteen of 31 PSLs (58.1%) were located on the trunk, 5 (16.2%) on the lower extremities, 3 (9.7%) on the buttocks, 2 (6.4%) on the upper extremities, 2 (6.4%) on the acral sites, and 1 (3.2%) on the face. Histopathologic examination identified 25 melanocytic nevi (23 Clark nevi—14 dysplastic, 5 compound, and 4 junctional nevi —and 2 Spitz/Reed nevi), 3 melanomas, 1 lentigo simplex, 1 irritated seborrhoeic keratosis, and 1 actinic keratosis.

HELM revealed additional morphologic features that were not apparent with ELM analysis, particularly regarding pigment network, gray-blue and dark brown/black pigmentation. Eleven of 31 lesions (35.5%) showed a typical ELM pigment network that appeared bidimensional with HELM (Figure 1). ELM analysis of 9 other lesions (29%) revealed a central homogeneous dark brown/black pigmentation that in 7 cases was seen under HELM examination to consist of globules (Figure 2A) corresponding histopathologically to large and deeply pigmented nests of melanocytes within the dermal-epidermal junction (Figure 2B) and/or beneath the epidermis. In the remaining 2 cases (32.3%), HELM showed pigment network and black dots (Figure 3A) that corresponded to pigmentation of the basal layer of the epidermis and melanin in the stratum corneum (Figure 3B).

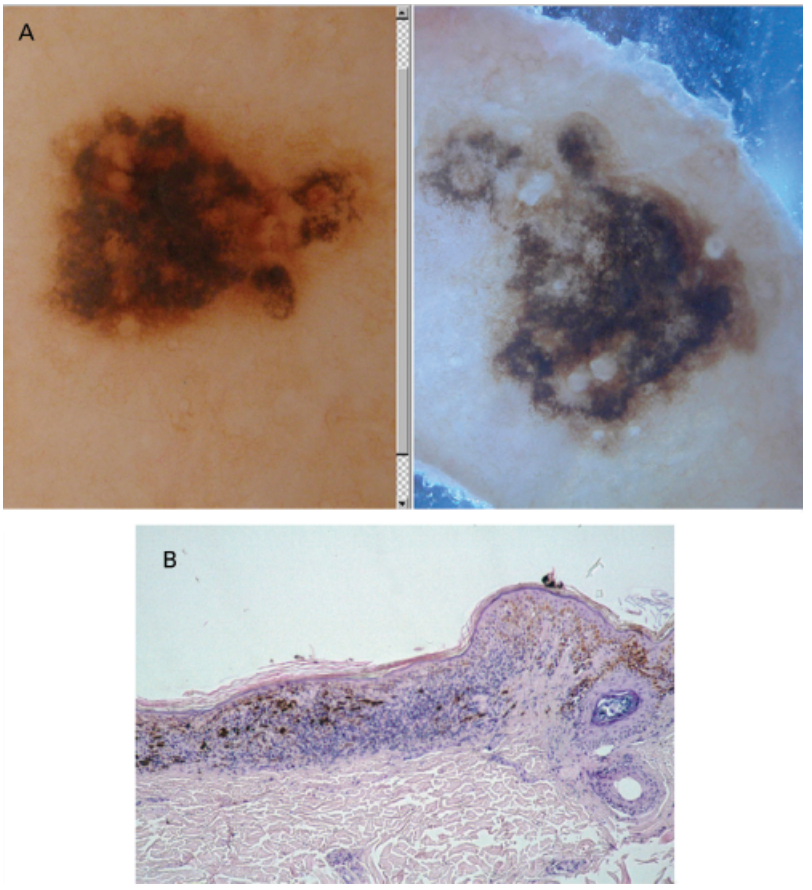

Figure 4. (A) Gray-blue pigmentation with ELM (left) that appeared as a gray area with HELM (right). (B) Heavy lichenoid lymphocytic infiltration together with few melanophages in the upper dermis, suggestive of regression in an early inflammatory phase (hematoxylin and eosin; original magnification, ×100).

Visualization with ELM showed a gray-blue pigmentation in nine other cases (29%) evaluated with both ELM and HELM. In five of these nine cases (55.5%) HELM examination showed a gray area (Figure 4A) corresponding histopathologically to a heavy lichenoid lymphocytic infiltration together with few melanophages in the upper dermis, suggestive of regression in an early inflammatory phase (Figure 4B). In the other four cases (44.5%), HELM disclosed a blue-whitish area (Figure 5A) that corresponded histopathologically to the presence of melanophages or pigmented melanocytes in the dermis (Figure 5B).

In the remaining two cases (6.4%) ELM showed only a globular pattern (Figure 6A), whereas HELM revealed the presence of a pigmented network combined with globules (Figure 6B). Histopathologic examination supported the HELM analysis, showing pigmentation of the epidermal basal layer and nests of melanocytes in the superficial dermis (Figure 6C).

Discussion

At present, dermoscopy is considered fundamental for the study of PSLs. Some clinically equivocal benign PSLs (particularly atypical Spitz and Clark nevi) that can be difficult to distinguish clinically from melanoma, however, remain difficult to differentiate from melanoma also dermoscopically. These problems might be due to the intrinsic difficulties of evaluating these types of lesions, which have dermoscopic features similar to those of melanoma, as well as to technical limitations.

Heavily pigmented or nodular lesions often represent the "gray zone" of dermoscopy. In this study we used a new technique, named HELM, to evaluate "gray-zone" PSLs after surgical excision. Two different sources of illumination (a halogen lamp above and an LED source underneath the excision specimen) were specifically chosen to better differentiate between epidermal and dermal dermoscopic structures. Images obtained with this technique showed better contrast than images obtained using a single type of illumination (i.e., halogen-halogen, LED-LED), and the dermoscopic features became more visible.

Braun and coworkers[18] demonstrated earlier that HLM involves

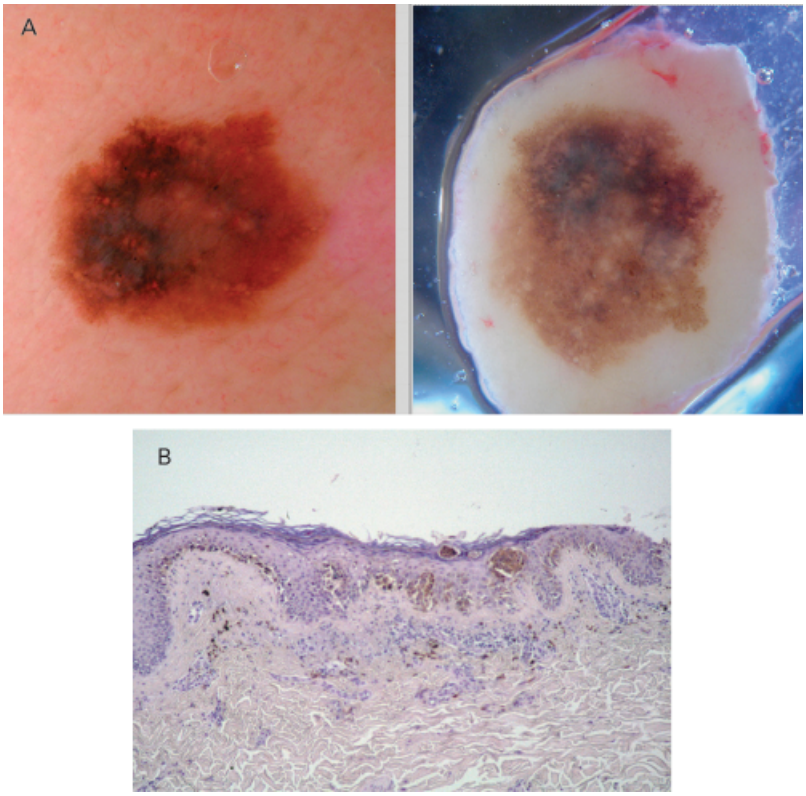

Figure 5. (A) The gray-blue pigmentation of the ELM images (left) was visualized as blue-whitish at HELM examination (right). (B) Presence of melanophages or pigmented melanocytes in the dermis (hematoxylin and eosin; original magnification, × 100).

visualizing the morphologic structures, such as globules, of a surgically excised PSL using illumination from the dermal side to make the dermal structures more visible comparing with ELM. In our study, in 9 of 31 cases (29%), HELM analysis showed that black-brown pigmentation that appeared homogenous by ELM was actually composed of globules (7 cases) or network and black dots (2 cases). In our estimation, the combination of both types of illumination probably creates a special filter that overcomes some of the limitations arising from the use of classic ELM illumination or HLM alone.

The most confounding dermoscopic pattern is the so-called gray-blue area, also known as blue-whitish veil, blue veil, gray-blue pigmentation, peppering, and regression structures. Zalaudek and associates[17] demonstrated that this pattern reflects multiple aspects of regression. Invasive melanoma can also have the same dermoscopic features, however, particularly the blue-whitish veil, with blue areas arising not from regression but from the presence of atypical melanocytes in the mid and deep dermis. Argenziano and colleagues[19] showed that agreement among experts using classic pattern analysis was only fair for the regression structures ($k = 0.44$) and poor for the blue-whitish veil ($k = 0.32$). Although dermoscopically similar, these structures need to be distinguished for proper management of the lesions.

In this study we observed a gray-blue pigmentation in 9 of 31 (29%) cases with ELM, but only 5 of these 9 cases (55.5%) showed a gray area with HELM. Histopathologic examination of these 5 cases revealed a lichenoid infiltrate with few melanophages in the upper dermis (suggestive of regression in an early inflammatory phase). In the remaining 4 cases (44.5%), a blue-whitish area was clearly visible with HELM and was histopathologically related to the presence of sheets of deeply pigmented melanocytes and/or melanophages in the dermis. Therefore, from a practical point of view, HELM allows better differentiation of "blue-gray areas" from "blue-whitish veil," with the former mainly composed of lymphocytes (or, possibly, representing fibrosis) and the latter mainly composed of sheets of pigmented melanocytes and/or melanophages. This finding helps to predict if a suspicious melanoma according to ELM could be a thick (blue-whitish veil) or a thin (blue-gray areas) melanoma using HELM.

These promising results, although preliminary and based on a small number of samples, should inspire further HELM studies,

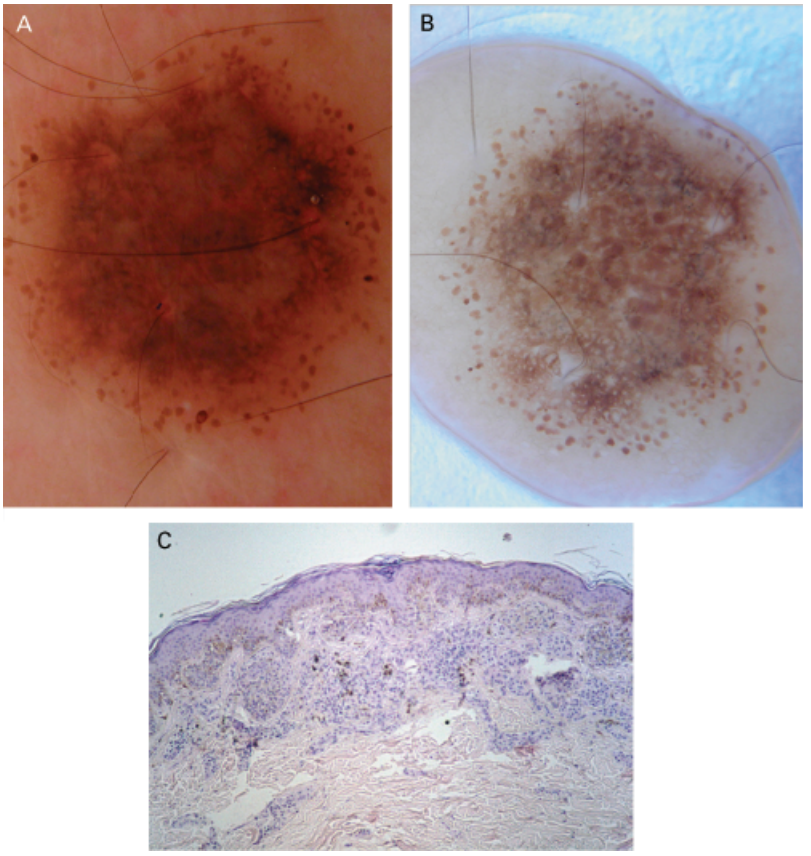

Figure 6. (A) ELM image showed a globular pattern. (B) HELM examination revealed the presence of a pigmented network combined with globules. (C) Pigmentation of the epidermal basal layer and nests of melanocytes in the superficial dermis (hematoxylin and eosin; original magnification, × 100).

above all on clinically or dermoscopically equivocal PSLs, to evaluate the utility of this method in distinguishing the blue veil of regression structures from that of deep melanophages–melanocytes in the dermis.

In summary, HELM provides additional information compared to ELM allowing to distinguish the dermoscopic structures located at different layers of the epidermis. A diffuse pigmentation at ELM can demonstrate the presence of a pigment network and/or dots and globules using HELM. Moreover, HELM permits us to differentiate the bluish pigmentation of regression from that of melanophages or sheets of melanocytes in the dermis.

The appearance of dermoscopic structures under HELM is also important for didactic purposes. Visualization of some PSLs using double illumination brought out a pigment network that was not evident with classic ELM and further showed a bidimensional aspect to the network, which appeared to be raised. In addition, the shape, size, and colors (black or brown) of the black dots and the brown globules were clearly visible, and they appeared to be located in two different vertical plans, with the black dots more superficial than the brown globules. These observations were confirmed by the histopathologic correlates.

Using HELM we might better relate dermoscopic features to their histopathologic correlates. For example, differentiating between black dots and brown globules or blue structures can often be difficult or impossible in routine practice, but in many cases this problem can be solved with HELM. On the basis of our results, we can recommend HELM as a useful bridge between classic ELM and histopathology.

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