

SHORT COMMUNICATION

Dermoscopic and Reflectance Confocal Microscopy Findings in Extra-genital HPV16-associated Pigmented Squamous Cell Carcinoma *in situ*

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Squamous cell carcinoma *in situ* (SCCIS) affecting the ano-genital area (also termed Bowenoid papulosis, BP) is mostly associated with high-risk strains of human papilloma virus (HPV) (1). Isolated HPV-associated SCCIS in the extra-genital area, without concomitant genital HPV infection, has been reported infrequently (2); such cases may present as pigmented papules or plaques, which may be difficult to differentiate from melanoma.

We describe here the clinical and dermoscopic features of isolated HPV-associated pigmented SCCIS (termed extra-genital BP) in a small case series. We also describe the findings of reflectance confocal microscopy (RCM), a bedside imaging technique that is useful for differentiating keratinocytic from melanocytic neoplasms. We use histopathology and immunohistochemistry (IHC) to elucidate the tissue correlates of the RCM findings. Finally, we compare the dermoscopic and RCM findings of the present series with those observed in 2 cases of classic genital BP.

CASE REPORTS

Five pigmented plaques and papules, from 5 patients, presented clinical features that raised suspicion for the diagnosis of melanoma (Table S1¹); one lesion was located in the inguinal area, while 4 lesions were distant from the ano-genital region. On dermoscopy, the lesions presented a mostly-structureless pattern with multiple colours, including brown, grey-blue and black, distributed in asymmetrical fashion; dermoscopic structures included irregularly distributed brown-to-grey dots, radial lines and, at times, scales (Fig. S1¹).

All 5 lesions underwent RCM imaging prior to excisional biopsy (Fig. S1¹). On RCM, all lesions showed surface scale, bright dendritic cells and an irregular and disarranged honeycomb pattern at the spinous-granular layers of the epidermis. Keratinocyte atypia was represented by significant irregularity in size and shape of the cells. In the dermo-epidermal junction (DEJ), a ringed pattern composed of small closely-set edged papillae was seen. In the superficial dermis, plump-bright cells and small bright dots were observed; increased blood flow was also notable on real-time video-mode imaging. The RCM findings were suggestive of pigmented SCCIS.

All lesions underwent excisional biopsy. Specimens were submitted for histopathological analysis and all were initially diagnosed as pigmented SCCIS. Haematoxylin and eosin

(H&E) stained specimens revealed an epithelial neoplasm characterized by loss of the normal epidermal maturation pattern with numerous atypical keratinocytes displaying large nuclei and perinuclear halos (i.e. features of koilocytes); these cytological findings raised the possibility of HPV-related SCCIS (Fig. S2A¹). Numerous atypical mitoses were observed in the upper epidermis, as well as frequent dyskeratotic cells. There was diffuse epidermal hyperpigmentation notable in basal keratinocytes and in dendritic extensions, with melanin spanning the extent of the epidermis up to the stratum corneum. Numerous melanophages could be seen in the papillary dermis. IHC staining for Melan-A and CD1a was performed. CD1a stain revealed increased number of Langerhans cells (Fig. S2D¹), while Melan-A immune-staining showed only normal-appearing basal melanocytes (Fig. S2C¹). Finally, linear array HPV-genotyping test was positive for HPV-16 (Fig. S2B¹) in all cases, establishing the final diagnosis of extra-genital BP. Notably, none of the patients harboured genital lesions, had history of sexually transmitted diseases, or presented with immunosuppressive disease.

In 2 separate cases of classic genital BP, we observed multiple verrucous brown papules and plaques without a surface scale, distributed in the genital area (dorsum of penis). On dermoscopy, the lesions showed a homogenous pattern, brown-to-grey in colour. On RCM, the structures observed were very similar to the ones found in the extra-genital cases; namely, dendritic cells at the suprabasal epidermis, small ringed papillae at the DEJ and plump-bright cells in the dermis.

DISCUSSION

SCCIS in extra-genital location is mostly related to chronic sun exposure (3). It usually presents as an erythematous, scaly patch. Pigmented SCCIS is found at a lower frequency; Cameron et al. (4) found the pigmented variant in only 5.5% of 951 histopathologically verified cases. Interestingly, the pigmented cases were located in anatomical areas known to be associated with HPV, such as the ano-genital and periungual regions. Outside these regions, finding mucosal high-risk strains of HPV in SCCIS is uncommon (5, 6). Zheng et al. (5) studied 41 patients with extra-genital Bowen's disease and found HPV 16 and 33 in only 7% of cases; the HPV positive cases were coincident with lesions near to the genital area (e.g. lower abdomen and buttock). In contrast to the cases described by the authors above, 4 of our 5 cases presented clinically as verrucous, pigmented, non-scaly lesions located far from the genital

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2399>

area. These patients had dark skin phototype and no clinical signs of chronic photo-damage or significant risk factors for developing skin cancer.

Pigmented SCCIS is a known melanoma-mimic, both clinically and dermoscopically (7–10). RCM can be useful in establishing the specific bedside diagnosis of pigmented SCCIS and pigmented actinic keratosis in its various degrees of atypia (11). In the present case series, the RCM features of atypical and sometimes disarranged honeycomb pattern at the spinous-granular layers and small closely-set rings of bright basal cells around the dermal papillae were indeed commensurate with the diagnosis of pigmented SCCIS (12). Of note, RCM showed the presence of numerous bright dendritic cells in the supra-basal epidermis; using IHC staining for CD1A and Melan-A, these dendritic cells proved to be Langerhans cells and not melanocytes in Pagetoid spread (13). Similarly, Moscarella et al. (14) described intraepidermal proliferation of Langerhans cells in pigmented actinic keratosis, represented by dendritic cells seen on RCM.

In addition, the density and cyto-morphology of melanocytes in this series appeared normal. For this reason, we assume that the abundance of melanin within the epidermis is probably due to activation of melanocytes, which can be observed through RCM in pigmented actinic keratosis, resulting in small bright polygonal keratinocytes surrounding the follicular opening (15).

While the cases included in the present study presented very similar RCM features to those observed in 2 cases of classic genital BP, we could not visualize RCM structures suggestive of koilocytes in either the classic and extra-genital BP. Thus, the suspicion of HPV-associated neoplasms relied on the cytological findings of koilocytes on histopathology; indeed, this association was confirmed by the finding of HPV-16 on DNA linear array testing.

In summary, RCM may help to distinguish pigmented SCCIS from melanoma. However, clear visualization of the entire DEJ with its typical characteristics of epidermal hyperplasia (small closely-set rings of bright basal cells around at the dermal papillae) is mandatory in all cases in order to rule out a melanoma.

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The authors declare no conflicts of interest.

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