Is Ki-67, keratin 16, involucrin, and filaggrin immunostaining sufficient to diagnose inflammatory linear verrucous epidermal nevus? A report of eight cases and a comparison with psoriasis vulgaris*

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Abstract: Inflammatory linear verrucous epidermal nevus and linear psoriasis are sometimes hard to differentiate clinically and pathologically. Although immunohistochemical expression of keratin 10 (K10), K16, Ki-67, and involucrin may be useful for differentiating both entities, these results have been reported in only a few cases. We collected data from 8 patients with inflammatory linear verrucous epidermal nevus, 11 with psoriasis vulgaris, and 8 healthy controls and evaluated immunohistochemical expression of Ki-67, K16, involucrin, and filaggrin among them. Ki-67 and K16 overexpression was similar in inflammatory linear verrucous epidermal nevus and psoriasis vulgaris compared with normal skin. Although staining for involucrin showed discontinuous expression in parakeratotic regions in 4 inflammatory linear verrucous epidermal nevus cases, it was continuous in the other 4 cases and in all psoriasis vulgaris cases. Filaggrin expression was present in hyperkeratotic regions but scarce in parakeratotic areas in both inflammatory linear verrucous epidermal nevus and psoriasis vulgaris. The immunostaining pattern of Ki-67, K16, involucrin, and filaggrin may be insufficient to discriminate inflammatory linear verrucous epidermal nevus from psoriasis vulgaris.

Keywords: Keratinocytes; Keratin-16; Ki-67 Antigen; Psoriasis

INTRODUCTION

Inflammatory linear verrucous epidermal nevus (ILVEN) is a rare variant of verrucous epidermal nevus that usually occurs in infancy and rarely in adulthood. It is characterized clinically by pruritic, erythematous, verrucous plaques following Blaschko lines, and pathologically by alternating parakeratosis with hypogranulosis and orthokeratosis with hypergranulosis.[1] ILVEN may sometimes be hard to distinguish from linear psoriasis because of their clinicopathological overlap and concomitance.[2-5]

Although immunohistochemical expression of keratin 10 (K10), K16, Ki-67 and involucrin may be useful for differentiating ILVEN from linear psoriasis, these results have been reported in only a few ILVEN cases.[2,6-9] We tried to evaluate the expression of Ki-67, K16, involucrin, and filaggrin for the differential diagnosis between ILVEN and psoriasis vulgaris (PV).

MATERIALS AND METHODS

This study collected data from 8 untreated ILVEN patients, 11 PV patients, and 8 healthy controls, but no cases of linear psoriasis. These patients were diagnosed based on clinicopathological features[10] and not associated with systemic disorders. ILVEN patients had no personal and family histories of psoriasis. PV lesional area was <10% of the body surface area.

We performed immunohistochemistry on paraffin-embedded sections. Primary antibodies included goat anti-K16 (SC-49176, 1:200; Santa Cruz Biotechnology, CA, USA), mouse anti-involucrin (SC-21748, 1:200; Santa Cruz), filaggrin (SC-66192, 1:200; Santa Cruz), and rabbit anti-Ki-67 (RMA-0542, prediluted; Zhongshan Jinqiao Biotechnology Co Ltd, Beijing, China). Epidermal Ki-67$^+$ cells were reckoned in 5 different fields at × 200 magnification and expressed in mm of the basement membrane.[11] The data were analyzed using ANOVA with Dunnett T3 test.

RESULTS

Clinical data of 8 ILVEN cases are listed in table 1. Mean age and disease course were 36.6 ± 25.0 and 20.1 ± 13.3 years, respectively. All patients presented with localized or linear verrucous papules, nod-

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Table 1: Clinical features and involucrin expression in 8 patients with ILVEN

Case	Gender	Age/duration	Lesional distribution	Involucrin expression
1	Male	55/30 years	Right cheek, neck, axilla, and inner thigh	Discontinuous
2	Male	5/0.2 years	Perianal area	Discontinuous
3	Male	14/13.7 years	Left lower lip	Discontinuous
4	Female	8/5 years	Left forearm, thigh, and leg	Discontinuous
5	Male	41/20 years	Left inner thigh and upper leg	Continuous
6	Male	42/25 years	Right thigh, leg, and dorsal foot	Continuous
7	Female	73/40 years	Left temple	Continuous
8	Male	55/27 years	Left inner and anterior thigh and posterior leg	Continuous

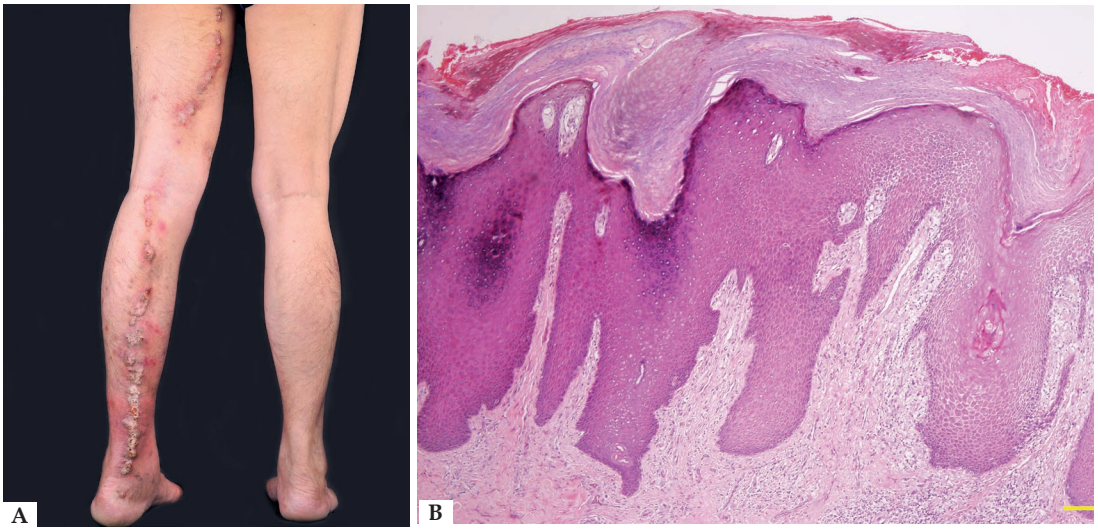

Figure 1: Clinicopathological presentation of ILVEN in case 8. **A.** Linearly arranged brown verrucous nodules and plaques along the left inner thigh and posterior leg. B. Epidermal acanthosis, alternating parakeratosis with agranulosis, orthokeratosis with hypergranulosis, and perivascular lymphocytic infiltration (Hematoxylin & eosin, scale bar = 200µm)

ules, or plaques (Figure 1A). All patients but one had intense pruritus. Typical pathological features were epidermal acanthosis, alternating parakeratosis with hypogranulosis, orthokeratosis with hypergranulosis, and perivascular lymphocytic dermal infiltration (Figure 1B).

Eleven PV cases comprised 8 males and 3 females. The average age and disease course were 34.6 ± 15.8 and 3.4 ± 4.3 years, respectively. Eight controls included 7 males and 1 female, with average age of 39.9 ± 9.8 years.

Ki-67 was expressed in the basal and parabasal keratinocyte layers of the epidermis, pilosebaceous units, and in some eccrine duct cells. Epidermal Ki-67$^+$ cells in ILVEN (6.86 ± 2.34) and PV (5.31 ± 2.54) were higher than those of normal skin (1.78 ± 0.83) ($p < 0.01$), but the difference between ILVEN and PV was not significant ($p > 0.05$).

K16 expression was absent in the epidermis of the normal skin. However, we observed K16 expression from the suprabasal layer to the horny layers of parakeratotic regions, while its expression was absent or present only in the spinous layers of hyperkeratotic regions in ILVEN and PV patients.

Filaggrin was expressed in the horny and granular layers in normal skin. However, its expression was evident in the lower horny and granular layers of hyperkeratotic areas but deficient in parakeratotic areas in ILVEN and PV patients.

We detected involucrin expression in the granular and upper spinous layers in normal skin, but it extended into the middle spinous layer in ILVEN and PV patients. Involucrin showed discontinuous staining (e.g., increased expression in hyperkeratotic regions but absent in parakeratotic regions) in 4 ILVEN cases, but continuous staining in the other ILVEN cases and in all PV cases (Table 1, Figures 2 and 3).

DISCUSSION

Expression patterns for Ki-67 and K16 are upregulated in PV cases, and their expression is documented in a few ILVEN cases. Incremental Ki-67$^+$ cells and local K16 staining were seen in 4 ILVEN cases.[6,11,12] Ki-67 expression tended to be lower in 3 ILVEN cases than in 4 psoriatic cases but with a remarkable overlap.[7] In a study, a female patient initially considered to have linear psoriasis was finally diagnosed as ILVEN based on a lower Ki-67 expression.[2] However, our study uncovered an analogous overexpression of Ki-67 and K16 in ILVEN and PV patients. Diffuse and intense K16 staining was observed from the suprabasal layer to the horny layers of parakeratotic regions, while its expression was absent or weak in the spinous layers of hyperkeratotic regions in both ILVEN and PV cases. Hence, our results reveal that epidermal hyperproliferation is

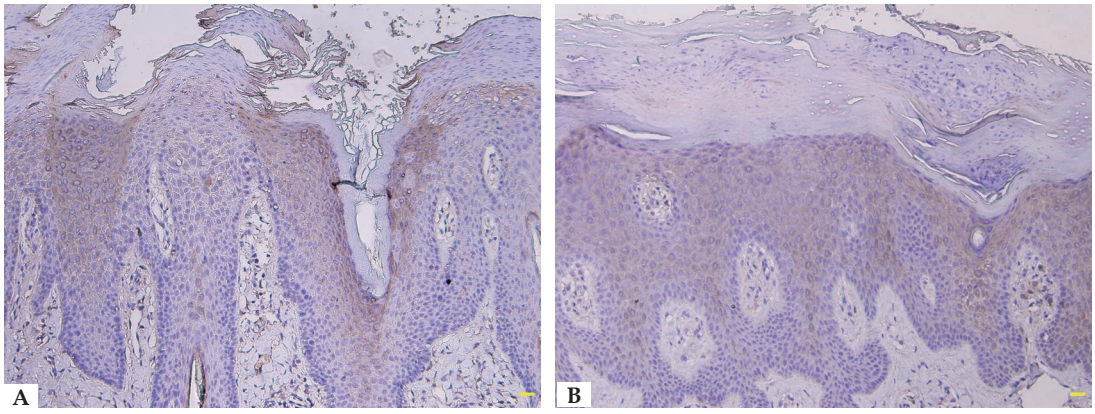

Figure 2: **A.** Discontinuous involucrin immunostaining in ILVEN (immunostaining, scale bar = 100 µm). **B.** Continuous involucrin immunostaining in ILVEN (immunostaining, scale bar = 100 µm)

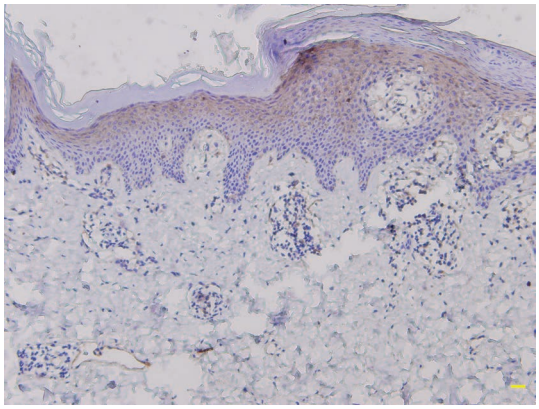

Figure 3: Continuous involucrin immunostaining in psoriasis vulgaris (immunostaining, scale bar = 100µm)

comparable in both disorders and Ki-67 is not a potential marker to distinguish them. Since K16-related inflammatory response could be involved in epidermal hyperproliferation,[13] disparate K16 distribution and intensity between parakeratotic and hyperkeratotic areas might suggest distinct alterations of keratinocytic activation and proliferation.

Involucrin overexpression and filaggrin down-expression are existent in psoriatic lesions.[14-16] Involucrin expression was evident in orthokeratotic regions, but scanty in parakeratotic regions in 4 ILVEN cases, while suprabasal keratinocytes expressed involucrin in parakeratotic areas of psoriasis.[8,9] Some authors considered that involucrin immunostaining might be valuable to distinguish between ILVEN and psoriasis.[8,9,17] However, we observed that involucrin staining was discontinuous in parakeratotic regions in 4 ILVEN cases, while its staining was continuous in the other ILVEN cases and in all PV cases, suggesting that involucrin immunostaining may be unreliable for differentiating both entities. Furthermore, filaggrin expression was present in hyperkeratotic regions but scarce in parakeratotic areas in ILVEN and PV. These results demonstrate that both ILVEN and PV might have an immature epidermal cornification.[14] In parakeratotic areas, lack of filaggrin expression can result from an absent granular layer because its expression is restricted to horny and granular layers in normal skin; the absence of involucrin staining, however, might be due to insufficient production of cytokines since some cytokines can reinforce involucrin expression in both normal and psoriatic keratinocytes.[14]

In summary, our results demonstrate that immunostaining patterns for Ki-67, K16, involucrin, and filaggrin may be insufficient to differentiate between ILVEN and PV. ❑

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