Human Papillomavirus–Induced Squamous Intraepithelial Lesions in Vulvar Lichen Planus

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Objectives: Approximately 50% of vulvar cancers arise after transforming infections with human papilloma virus (HPV) via the precursor squamous intraepithelial lesion (SIL). Lichen planus (LP)–associated vulvar cancers are typically HPV negative and arise via the precursor differentiated vulvar intraepithelial neoplasia (d-VIN).

Methods: An index case of vulvar high-grade squamous intraepithelial lesion (H-SIL) in an LP patient prompted this 12-year retrospective analysis about frequency of HPV-induced SIL in 785 biopsies of 584 patients with vulvar LP. All SIL were analyzed for p53 and $p16^{ink4a}$ overexpression and for presence of DNA of 32 HPV subtypes.

Results: Nine (1.6%) of 584 women with papular (3) and mucosal "erosive" LP (6) presented with H-SIL (7) and low-grade SIL (2). All SILs harbored HPV16-DNA and showed $p16^{ink4a}$-overexpression. Concomitant immune suppression included T-suppressor lymphocyte deficit (1), systemic (1), and topical (2) cortisone therapy. H-SILs regressed spontaneously (1) or after imiquimod therapy (3). Three women with erosive LP discontinued imiquimod because of side effects and had laser destruction (1), skinning vulvectomy (1), and surgery (1) for definitive treatment. Two women have recurrent vulvar SILs, and 1 woman progressed to invasive SCC. In the same patient population, 16 of 584 women had a d-VIN, and 9 of 16 with progression to SCC.

Conclusions: H-SILs in vulvar LP are rare and may occur in the setting of risk factors. If clinical suspicion arises, biopsy and histological examination assist in correct etiologic classification of a precancerous lesion and subsequent therapy decisions. The minimal risk for H-SIL development in vulvar LP patients should not preclude therapy of LP.

Key Words: HPV-negative vulvar carcinoma, squamous intraepithelial lesion, vulvar precursors, vulvar dermatosis, vulvar intraepithelial neoplasia, vulvar squamous cell carcinoma, differentiated vulvar intraepithelial neoplasia

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Approximately 50% of vulvar squamous cell carcinomas (SCCs) arise through transforming infections with human papillomavirus (HPV) via the precursor lesion vulvar intraepithelial neoplasia (VIN). The WHO classifies moderate VIN (VIN II) and high-grade VIN (VIN III) synonymously *as* high-grade squamous intraepithelial lesion (H-SIL).[1] Autonomous proliferation in transforming HPV infection occurs after interaction of oncogenes E6 and E7 with host cellular proteins. This leads to nuclear and cellular accumulation of cyclin-dependent kinase inhibitor $p16^{ink4a}$, which can be visualized by immunohistochemistry as $p16^{ink4a}$ overexpression. The remaining vulvar squamous cell carcinomas (SCC) arise independent of HPV. Human papillomavirus–negative SCCs are often associated with lichen sclerosus and lichen planus (LP),[1,2] and they arise through the precursor differentiated VIN (d-VIN) or synonymous differentiated simplex in situ *SCC*.[1,3] Differentiated VINs do not show $p16^{ink4a}$ overexpression but often reveal nuclear p53 staining. Compared with H-SIL, d-VIN is a more rapidly progressing precursor, occasionally with development of invasive cancer in LP patients within 3 to 12 months.[3–6] Correct etiologic classification of a precancerous lesion therefore impacts on therapy options and often requires biopsy and histological workup. Usually, the 2 pathways of carcinogenesis occur independently, although a rare occurrence of HVP-induced cancers in men with penile LP and LS has been described.[7] The observation of an index case of vulvar H-SIL in a LP patient prompted us to investigate the frequency of HPV-induced SIL in 785 biopsies of 584 consecutive patients with vulvar LP diagnosed during the past 12 years. We report about the diagnostic and therapeutic challenges of HPV-induced SIL in patients with vulvar LP.

MATERIALS AND METHODS

During the past 12 years, 584 patients with 785 biopsies were diagnosed with LP at the Institute of Pathology at the Medical University Graz, Austria, a reference center of vulvar pathology for Austria. A total of 511 biopsies of 326 patients were obtained in 3 centers specialized on vulvar diseases. The remaining 274 biopsies of 258 patients were sent by general gynaecologist throughout Austria. A total of 785 formalin-fixed and paraffin-embedded vulvar biopsies were examined using hematoxylin-eosin stain. Was SIL present, further evaluation with immunohistochemistry with antibody to $p16^{ink4a}$ (Roche-mtm Laboratories, Heidelberg, Germany) and p53 (clone DO-7; DAKO, Denmark) and with multiplex PCR and duplex hybridization for detection of DNA of 32 HPV subtypes (CHIPRON GmbH, Berlin, Germany) inclusive HPV high-risk subtypes 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; HPV low-risk subtypes 6 and 11; and potentially carcinogenic HPV subtypes 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, and 97. Overexpression of $p16^{ink4a}$ as "surrogate marker" for transforming infection with HPV high-risk-genotypes was defined as uniform continuous staining of all dysplastic cells. Discontinuous patchy staining of dysplastic cells—independent of percentage of staining—was interpreted as negative. All patients gave consent for photographic documentation. Ethics committee approval has been obtained (Medical University, 20-255ex 08/09).

RESULTS

Only 9 of 584 women with histologically diagnosed vulvar LP (1.6%; median age, 55 years at diagnosis; age range, 23–66 years) had a diagnosis of HPV-induced SIL (Table 1). All patients were in regular follow-up in intervals between 6 months and 1 year. The duration of vulvar LP was between 2 months and 20 years before diagnosis of SIL. At initial diagnosis of SIL, 6 lesions were H-SIL (1.2% of patients), and one of these 6 patients progressed to SCC within 6 months at the side of the last laser destruction. Three lesions were L-SIL. Clinical presentation of SIL ranged from one to multiple papules, red macules (Figure 1), and plaques (Figure 2). All SILs showed "aceto-whitening," a gradual color change of skin/mucosa to white within 2 to 3 minutes

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TABLE 1. Summary of SIL in patients with vulvar LP.

Age at diagnosis of SIL	Type of LP; duration of LP	Squamous intraepithelial lesion	Therapy for SIL	Side effects	Treatment of LP at time of diagnosis of SIL	Concomitant diseases	Recurrence/ persistence	Follow-up since (last) SIL
Patient 1, 23 yr	Erosive; 8 yr	5 mm papule H-SIL/VIN III	Imiquimod	Leukopenia	6 mo topical corticosteroid	Perianal Psoriasis	No recurrence	36 mo
Patient 2, 39 yr	Erosive; 10 yr	1 cm macule; H-SIL/VIN II	Imiquimod	Erosions, pain	Systemic cortisone for 6 yr	Autoimmune hepatitis	No recurrence	42 mo
Patient 3, 50 yr	Papules; 3 yr	1 cm plaque of H-SIL/VIN II	Laser		None	Vitamin D deficiency	No recurrence	60 mo
Patient 4, 52 yr; Fig. 1A	Papules; 2 mo	3 mm papule of L-SIL/VIN I	Imiquimod		None	Vitamin D deficiency; 2 cervical H-SIL 5 and 6 yr before vulvar SIL	No recurrence	60 mo
Patient 5, 54 yr	Papules; 2 mo	5–10 mm H-SIL/ VIN II and VIN III L-SIL/VIN I; condylomata ac.	Excision (1) cidofovir (1) imiquimod (1) laser (5)	Pain, fever, chills, flu-like symptoms after imiquimod	None	Extragenital psoriasis, vitamin D deficiency	10 recurrences in 8 yr	6 mo developed a pT1b HPV 16–induced SCC at the side of last laser-treated H-SIL
Patient 6, 58 yr; Fig. 1E–F	Erosive; 3 yr	Vestibular plaque of L-SIL/VIN I	None		3 yr topical corticosteroids and pimecrolimus		Awaiting spontaneous regression	12 mo
Patient 7, 61 yr	Erosive; 2 yr	1 cm macule of H-SIL/VIN III	Imiquimod followed by surgery	Erosions, pain	None		18 mo persistence despite imiquimod	30 mo
Patient 8, 66 yr; Fig. 2	Erosive; > > 20 yr	H-SIL/VIN III involving entire anterior vulva	Skinning vulvectomy		20 yr intermitant topical corti costeroid th.	Systemic (oral) LP, T-cell deficiency	No recurrence	36 mo
Patient 9, 60 yr; Fig. 1C–D	Erosive with vaginal stenosis; > > 20 yr	4 mm papule H-SIL/VIN II	Laser (2), laser and Imiquimod (1)		18 yr topical corticosteroid maintenance th.	Extragenital psoriasis	2 recurrences in 7 yr	45 mo

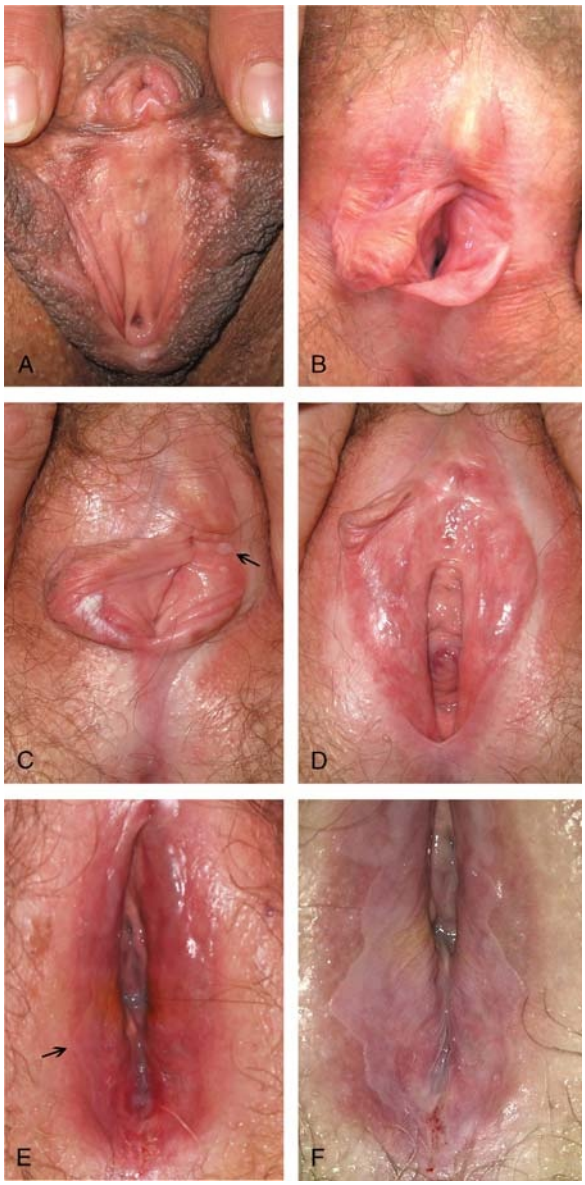

FIGURE 1. SIL in vulvar LP. A, Patient 4 with L-SIL periclitoral LP with postinflammatory changes in the vestibule and a 3-mm (in diameter) whitish, raised, well-circumscribed papule of L-SIL in the midline between the clitoris and urethra. B–D, Patient 9 with H-SIL. B, 2006: Advanced scarred vulvar LP with narrowing of the vaginal introitus with erosive areas. C, 2011: Biopsy of white raised grouped papules (arrow) revealed H-SIL. The other irregular raised white confluent papules were manifestations of a highly active LP. D, 2015: After laser therapy and surgical correction of the introital stenosis, the patient's erosive LP is well controlled under topical corticosteroid therapy. E–F, Patient 6 with L-SIL in mucosal (erosive) LP. E, Mucosal LP with an erythematous vestibule. Arrow indicates ridge of dysplastic epithelium, which (F) appeared as sharply demarcated slightly raised white epithelium within 2 to 3 minutes after application of 3% to 5% acetic acid (aceto-whitening).

after application of 3% to 5% acetic acid (Figure 1, E and F, and Figure 2, E and F). All SIL showed basaloid differentiation with immunohistochemical $p16^{ink4a}$ overexpression (Figure 2, G and H) and were p53 negative. In all lesions (H-SIL and L-SIL), HPV16 DNA was detected.

Concomitant systemic immune suppression included a T-suppressor lymphocyte deficit (n = 1) and systemic corticosteroid therapy for autoimmune hepatitis (n = 1). One woman had 2 previous cervical H-SILs 5 and 6 years earlier. Treatment before development/diagnosis of SIL consisted of systemic cortisone therapy (n = 1), long-term application of topical high-potency corticosteroids (n = 2), cortisone alternating with pimecrolimus (n = 1), or none (n = 5). High-grade SILs regressed spontaneously in 1 woman, and 1 patient with L-SIL presently is awaiting spontaneous regression. Squamous intraepithelial lesions were treated successfully with imiquimod within 16 to 20 weeks in 3 women. Three women with erosive LP began with imiquimod but discontinued because of severe side effects. These SILs finally were treated with laser destruction (n = 1), skinning vulvectomy (n = 1), and excision (n = 1). One woman had 2 recurrences (patient 9), and 1 woman developed an invasive SCC after a 7-year course of multiple recurrent SILs (patient 5).

In the same patient population, 16 (3%) of 584 patients were diagnosed with d-VIN. Nine of these 16 (56%) patients progressed to invasive SCC. One of these 9 patients with LP who was in 6-month control intervals developed a plaque

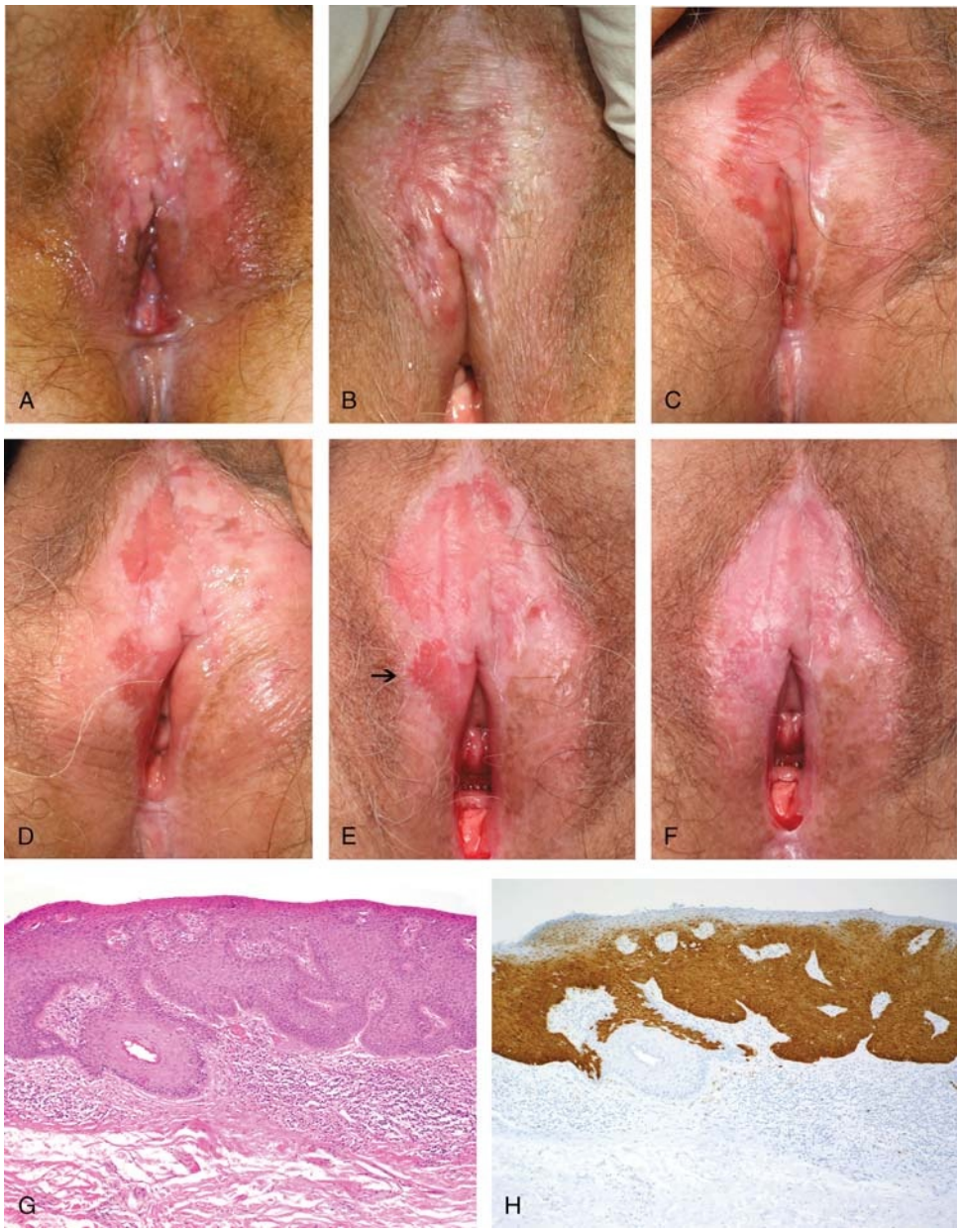

FIGURE 2. Patient 8 with extensive H-SIL after a 20-year history of LP. A, 2003: The LP involved periclitoral area, residual labia minora, and vestibule. Hypertrophic areas alternated with erosive areas. B, 2006: Scarring is more prominent, and LP appears atrophic. C, June 2011: A large red sharply circumscribed area is surrounded by white mucosa. At this stage, the patient refused biopsy and topical as well as systemic therapy offers. D, August 2011: The erythematous macules (right) were smaller, and the surrounding mucosa appeared hypertrophic. Numerous small erosions were present on the left side. Again, she refused biopsy and therapy. E, January 2013: Preoperative pictures before skinning vulvectomy 4 weeks after a biopsy revealed HSIL and show several large irregular but sharply circumscribed erythematous macules and patches with follicular accentuation (arrow). F, "Aceto-whitening" 3 minutes after application of 5% acetic acid. All erythematous areas turned white. G, Histological workup confirmed extensive H-SIL corresponding to the aceto-white areas. Hematoxylin-eosin stains show an HSIL in the area indicated by arrow in E.

5 months after the last uneventful control visit. Biopsy showed a pT1b SCC with adjacent d-VIN.

DISCUSSION

We report on the rare occurrence of HPV-induced vulvar precancers in patients with vulvar LP. Overall, this was a less frequent event (1.2%) than development of d-VIN or HPV-negative SCC in LP (3%). A similar proportion of HPV-induced and HPV-negative precursors has been described for 27 patients with lichen sclerosus, where 8 patients had H-SIL and 18 patients had HPV-negative precursors.[8]

Newly arising papules were biopsied with the differential diagnosis of d-VIN, and persisting erythematous macules and plaques were biopsied for confirmation of erosive LP. "Aceto-whitening" (appearance of a sharply demarcated and raised white vulvar epithelium 2–3 minutes after application of acetic acid) may hint toward HSIL[9] and is considered an abnormal finding

in vulvoscopic examination by the International Federation for Cervical Pathology and Colposcopy.[10] In vulvar LP or LS, the first thought of a suspicious (newly arising) plaque should always be a d-VIN, a biologically more aggressive precursor than HPV-induced precursors with occasional advancement to invasive cancer within several months.[1,2]In the setting of concomitant immune suppression or autoimmune diseases, however, HPV should be suspected as causative agent. Unequivocal distinction of HPV-induced H-SIL from HPV-negative d-VIN is essential for therapy decisions. Because of d-VIN's potential of rapid progression, it should be treated without undue delay.[2] The typically slow progression of HPV-induced precancers, however, allows for time intense treatment option such as (off label) treatment with imiquimod for 20 or more weeks. Erosions, burning, rawness, and pain are side effects that are particularly dramatic in erosive LP, which is painful from the outset. Furthermore, imiquimod may exacerbate or (re)activate LS, LP, and psoriasis[11,12] and cause neutropenia. Other therapy options of H-SIL include laser destruction and surgical excisions.

The rare occurrence of H-SIL in LP may be owed to immunologic control of HPV by the dense lymphocytic infiltrate of LP. A concomitant psoriasis, another T-cell–mediated disease, which is overrepresented in patients with lichenoid dermatoses,[13] may potentiate this effect. On the other hand, proliferating T-lymphocyte clones with monoclonally rearranged T-cell receptor may reduce the T-cell diversity, thus creating a local immune dysregulation[1,14,15] with ineffective tumor-infiltrating lymphocytes. Topical high-potency corticosteroid therapy, the gold standard for vulvar LP, has been recognized in HPV reactivation in vulvar dermatoses previously.[16] In such permissive environments, H-SIL can develop either after a newly acquired HPV infection or via reactivation of a previously immunologically controlled (dormant) HPV with negative HPV detection tests/HPV levels below detection thresholds.[17]

In general, HPV-associated precancerous lesions are rare findings in vulvar LP, but must still be considered, in particularly in the setting of predisposing risk factors. Awareness of such a coexistence and biopsy with immunohistochemical workup can assist in the correct identification of HPV-induced SIL versus HPV-negative d-VIN. The small risk for development of HPV-induced SIL in patients with vulvar LP should not influence treatment decisions for LP,[18] as cortisone and off-label use of immune modulators[19] reduce long-term morbidity and possibly the risk for development of HPV negative vulvar cancer.[20] These benefits outweigh the small risk of reactivation of HPV. Treatment of HPV-induced HSIL in papular vulvar LP in cornified skin and mucosa can be similar to HSIL in patients without LP, but HSIL in erosive LP remains a clinical challenge.

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