Differentiated Vulvar Intraepithelial Neoplasia

A Brief Review of Clinicopathologic Features

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● Differentiated-type vulvar intraepithelial neoplasia (dVIN) is a non–human papilloma virus (HPV)-related precursor lesion to vulvar squamous carcinoma. The terminology has only become recognized clinically and histopathologically in recent years despite being described more than 50 years ago. As opposed to the HPV-related VIN (uVIN), dVIN has different features of histomorphology, risk of progression, and molecular pathogenesis. Notably, dVIN commonly develops in a background of chronic inflammatory dermatoses such as lichen sclerosis and lichen simplex chronicus. The recognition of dVIN remains a challenge owing to lack of accurate and reproducible diagnostic criteria. Morphologically, basal layer atypia, dyskeratosis, and elongation and anastomosis of the rete ridges are regarded as very useful diagnostic features. Ancillary tests can be very helpful to establish a definitive diagnosis in some ambiguous cases. In contrast to uVIN, dVIN is more likely to progress to vulvar squamous carcinoma in a shorter period. The goal of this review is to elaborate on the clinicopathologic characteristics and underline the key histologic features that best facilitate the diagnosis of dVIN.

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There are 2 main pathogenetic pathways for the development of precursors to invasive squamous cell carcinoma of the vulva (VSCC): human papilloma virus (HPV) related (classic or usual-type vulvar intraepithelial neoplasia, uVIN) and non–HPV-related (differentiated or simplex vulvar intraepithelial neoplasia, dVIN). The HPV-related pathway is linked to high-risk HPV 16 and 18, while the non–HPV-related pathway is associated with inflammatory dermatoses including lichen sclerosis (LS) and lichen simplex chronicus (LSC).[1]

The first case of dVIN was described in 1961 by Gosling and colleagues[2] and termed intraepithelial carcinoma, simplex type. They appreciated that this particular type was a histologically unique entity from the previously recognized HPV-related Bowen type, which is currently known as VIN3.[2] In 1977, Hart and Millman[3] introduced the term differentiated to highlight the highly differentiated histologic features of the simplex type. It was not until 1986 that the ISSVD (International Society for the Study of Vulvovaginal Disease) adopted the term VIN III, differentiated type.[4] After almost 50 years of evolution, dVIN has now been recognized as high-grade dysplasia with rapid interval of progression to VSCC without association with HPV infection.[5] We aim to summarize the clinical presentation and pathologic features of this special precursor lesion of vulva and to remark on associated practical issues that should be considered in the diagnosis and prognosis of these lesions.

CLINICAL FEATURES

The incidence of both uVIN and dVIN has increased during the last 30 years. It has been reported as ranging from 0.013 per 100,000 (1985–1988) to 0.121 per 100,000 (1994–1997), based on a population study.[6] Compared to uVIN, dVIN typically occurs in older populations with a mean age of 68 years.[7] Clinically, dVINs overlap with LS and LSC, and are less commonly recognized as discrete lesions. In general, they appear to be unifocal and unicentric in comparison to the multifocal lesions seen in uVIN. The lesions are usually grey-white discolorations with a rough surface, vaguely defined thick white plaques, or elevated nodules.[4] dVIN frequently develops in a background of chronic inflammatory dermatoses including LS and LSC.[4,8] In addition, dVIN is commonly found adjacent to VSCC or in patients with a history of vulvar cancer.[9]

PATHOGENESIS

The pathogenic role of HPV in uVIN has been well established.[1] However, the pathogenesis of dVIN is poorly understood and most of the evidence of dVIN as a precursor lesion to VSCC has been circumstantial until recently. There are no definitive risk factors responsible for the occurrence by strict Hill criteria.[10] However, dVINs are frequently associated with LS and LSC.[4] At the molecular level, it has been suggested that dVIN is more frequently associated with TP53 mutations than uVIN.[11] In addition, a recent target sequencing study found several other mutations such
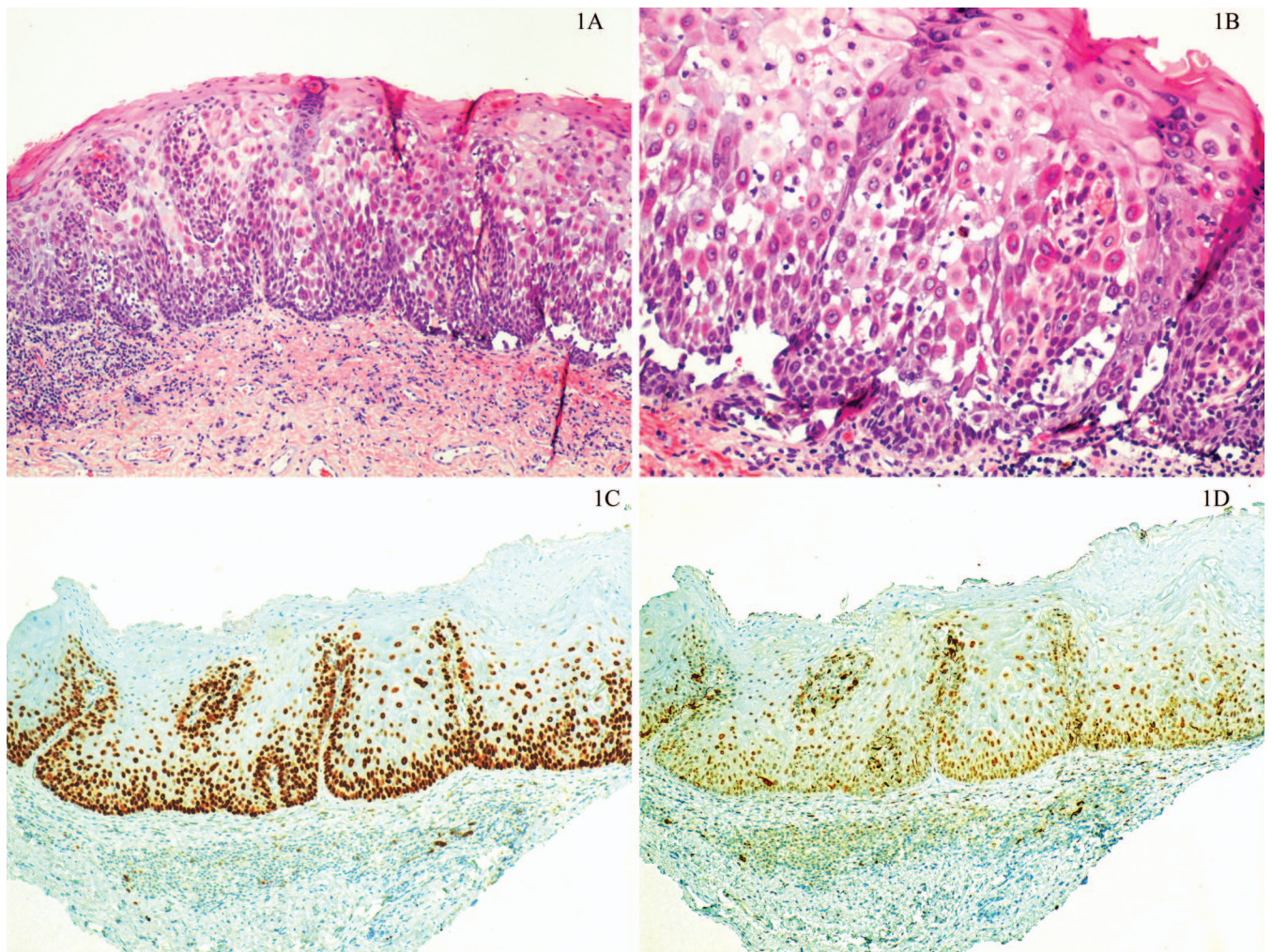
Vulvar intraepithelial neoplasia, differentiated type (dVIN). A, dVIN with marked dysplasia limited to basal and parabasal layers; prekeratinization and hypereosinophilia are present in the upper epidermis. B, dVIN with basal layer atypia, pleomorphic nuclei, and intercellular bridges. C, p53 strongly expressed in basal and parabasal layers. D, Ki-67 shows mild positivity in the basal layer and a thin parabasal layer (hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]; original magnification ×100 [C and D]).

as *NOTCH1* and *HRAS* in dVIN, indicating that multiple pathways can contribute to the development of dVIN apart from the classical TP53 pathway. It was found that *NOTCH1* was present in 20% of dVINs, and *HRAS* was present in 10% of dVINs. In HPV-negative vulvar cancer cases, 33.3% have *NOTCH1* mutations and 27.8% carry *HRAS* mutations, which suggests a role for NOTCH1 and HRAS in progression of HPV-negative precursors to cancers. The proposed biology of the mutations in *NOTCH1* is due to loss of tumor suppression function, while *HRAS* is an oncogene involved in the RTK/RAS/PI(3)K pathway, and somatic mutations lead to cell proliferation.[11,12] Some dVINs share an identical *TP53* mutation[11] and a similar allelic imbalance with gains of 3q26 with their associated VSCC.[13] When in progression to VSCC, it appears that several additional mutations such as *CDKN2A*, *PIK3CA*, and *PPP2R1A* are involved, since they are not detected or investigated in dVIN but only in VSCC cases.[11]

HISTOPATHOLOGY

The recognition of dVIN can be a challenge, even for experienced gynecologic pathologists. Previous studies have suggested that the interobserver agreement between pathologists, measured by κ statistics, in the diagnosis of dVIN is low (κ, 0.54). However, a substantial agreement value (κ, 0.75) was achieved among gynecologic pathologists after they were educated in applying 5 major criteria (discussed below).[14,15] As the name implies, dVIN is so called because of the paradoxical maturation or high differentiation of abnormal squamous cells above basal layers[1,5] (Figure 1, A and B). In contrast to uVIN, dVIN often lacks full-thickness atypia, and the moderate to marked cellular atypia is only confined to the basal and parabasal cells of the epidermis (lower 2–3 layers of the epithelium). As mentioned above, the following 5 histologic features used to improve agreement value are considered most useful in the diagnosis of dVIN: atypical mitosis in the basal layer, basal cell atypia, prominent nucleoli, dyskeratosis, and elongation and anastomosis of the rete ridges.[15] The major problem with the diagnosis of dVIN is that the full spectrum of histologic changes is not yet well defined. According to a survey, only basal layer atypia can be considered diagnostically essential by consensus, while the additional criteria listed can strongly support the diagnosis.[14] The evidence for basal layer atypia should include nuclei enlargement, irregular

nuclear contour (angulation), basal cell proliferation, coarse chromatin or open vesicular nuclei, variable prominent nucleoli, and scattered mitoses, especially atypical mitoses. Dyskeratosis is characterized by individual cells showing abnormal cell maturation often with abnormal keratinization in the deeper layers of the epidermis.[15] Prominent intercellular bridges (spongiosis or acantholysis) and superficial premature cells with eosinophilic cytoplasm can also be appreciated. For practical purposes, most dVINs will show 1 of 4 general histologic patterns, which may occur alone or in combination: (1) prominent basal atypia associated with LS or LSC; (2) basal cell proliferation and expansion associated with LSC; (3) defects in cell maturation (individual cells with increased cytoplasmic volume and abnormal cytoplasmic keratinization); and (4) spongiosis or acantholysis in the lower third of the epithelium.[16] Being familiar with these different patterns will help to recognize dVIN.

Notably, occasionally dVIN can present with basaloid features including nuclear atypia that extends beyond the basal layer, architectural disorganization, and homogeneous populations of basaloid undifferentiated keratinocytes. These cases can mimic uVIN. However, they are negative for p16 and HPV, and positive for p53.[1,17]

Nevertheless, if dVIN is highly suspected clinically, the case should be reviewed by an experienced gynecologic pathologist.[15]

ANCILLARY STUDIES

Use of biomarkers for diagnosing uVIN and excluding its mimics has been very successful, as p16 is an extremely good surrogate marker for high-risk HPV infection status (>90%)[18,19] and is immunoreative in almost 100% of uVINs. The staining pattern should be diffuse, strong, and continuous (nuclear and/or cytoplasmic), referred to as a *blocklike pattern*.[1] Staining should be present in the basal layer with extension upwards to involve at least one-third of the epithelial thickness.[20] In comparison, less than 17% of dVINs show weak, non-blocklike pattern p16 staining, which is limited to the lower half of the epithelium.[21,22]

Use of biomarkers for recognizing dVIN, however, has some limitations and is considered a work in progress. As mentioned before, p53 immunostaining is positive in more than 80% of dVINs owing to *TP53* mutation (Figure 1, C), as opposed to the HPV-related uVINs, which usually show negative staining.[1,22,23] This makes p53 a good marker for dVIN. The p53 staining in dVIN is strong in the basal layer (in >90% of basal cells) with suprabasilar extension, while patchy in less than 10% of basal cells with no suprabasilar extension in the adjacent normal epidermis.[22] Another p53 staining pattern is complete lack of nuclear staining (null-pattern). In a survey by Singh et al,[24] 6 of 22 dVINs (27%) had null-pattern staining. Since normal epithelium can demonstrate no or very weak p53 staining, the distinction of p53-null dVIN from a normal area can be very difficult. Another caveat is that increased p53 staining can be seen in 5% to 61% of LS and LSC cases, and up to 40% of squamous cell hyperplasia cases. These benign lesions usually have some morphologic overlap with dVIN.[25,26] Owing to these limitations, the reproducibility of p53 interpretation is relatively low, and benign inflammatory conditions could be overdiagnosed as dVIN. Therefore, strict adherence to the staining patterns of p16 and p53, in conjunction with morphologic features and clinical impres-

sion, will help to improve reproducibility and reduce diagnostic error. We should keep in mind that the concept of p53 mutation in the vulva is still evolving, as some "normal"-appearing vulvar skin can demonstrate p53 positivity (like p53 signature in other gynecologic organs such as fallopian tube and endometrium), the significance of which in clinical management is yet to be determined.

Ki-67 can be another helpful marker to distinguish dVIN from reactive changes, normal epithelium, and uVIN. Ki-67 shows negativity in the basal layer or weak positivity in parabasal layers in normal epithelium and reactive changes. In dVINs, Ki-67 staining is mildly positive in the basal layer and thin parabasal layer (Figure 1, D) as opposed to increased full-thickness expression seen in uVIN and localized expression observed in LS or LSC.[18,26]

Use of several other diagnostic markers for dVINs such as SOX2, phosphorylated-S6, and cyclin-D1 is evolving, but further studies with larger numbers of cases are needed to confirm their utility.[1] Recently, cytokeratin (CK) 17 was investigated in differential diagnosis of dVIN, uVIN, LS, and LSC. It was found that 93% showed intermediate to strong and diffuse reactivity in dVIN cases, with 70% showing full-thickness or suprabasilar pattern. No cases of uVIN displayed diffuse CK17 expression, whereas 63% of LS and 29% of LSC cases displayed intermediate to strong diffuse immunoreactivity, but was confined to the upper half of the epithelium. Therefore, CK17 may serve as an adjunct marker for diagnosis of dVIN especially for small biopsy samples.[27]

DIFFERENTIAL DIAGNOSIS

The histologic findings of dVIN can be subtle and misdiagnosed. Several benign processes and uVIN can enter the differential diagnosis. The major difficulty lies in differentiating dVIN from inflammatory or reactive dermatologic lesions with prominent acanthosis and epithelial atypia such as LS and LSC. The major accepted diagnostic feature of basal nuclear atypia should always be determined when considering a dVIN diagnosis. Some other features favoring dVIN include prominent parakeratosis, a thickened epidermis with elongated and branching rete ridges, abnormal keratinocytes with squamous whorls or keratin pearls, and strong continuous p53 staining of the basal layer.[1,5] LS can have basal cell proliferation and nuclear hyperchromasia; however, nuclear pleomorphism will not be present even though LS cases can occasionally express weak p53 stainings. Lichen simplex chronicus is the most common chronic inflammatory disorder affecting the vulva. It manifests as acanthosis, hyperkeratosis, and inflammation. However, the cells have open chromatin, and LSC does not show basal atypia and epithelial dysmaturation.[1] Squamous cell hyperplasia is usually considered a diagnosis of exclusion because it is frequently seen adjacent to LS, dVIN, and invasive squamous cell carcinoma. As opposed to dVIN, it shows organized maturation of squamous epithelial cells with overlying hyperkeratosis. The nuclei may be slightly enlarged but are not atypical. No mitotic figures are identified above the basal or parabasal layers. The nucleoli are inconspicuous to absent in contrast to dVIN. More importantly, squamous cell hyperplasia does not exhibit features of premature keratinization, expanded rete ridges, or parakeratosis.[4,6] Psoriasis with prominent acanthosis should also be considered. Psoriasis shows uniform acanthosis or hyperplasia of the epidermis with prominent

"test-tube" rete ridges. It is characteristically associated with neutrophils within the parakeratotic plaque forming intra-epithelial microabscess. It does not exhibit basal atypia or abnormal keratinization.

Verruciform dVIN should be differentiated from other verruciform lesions, including uVIN with superimposed LSC, verruciform LSC, vulvar acanthosis with altered differentiation (VAAD), and verrucous carcinoma. uVIN superimposed with LSC changes (prominent hyperkerato-sis, parakeratosis, and hypergranulosis) can mimic dVIN. However, it has greater apoptosis and lacks prominent abnormal keratinization in the lower layers. Also, strong p16 staining can be helpful in their distinction. Verruciform LSC lacks basal atypia, but it has been associated with vulvar cancers and verrucous carcinoma, thus appropriate follow-up for these patients is recommended. VAAD is defined by verruciforn acanthosis, plaquelike layers of parakeratosis, alteration in cytoplasmic differentiation with loss of the granular layer and superficial epithelial pallor, and minimal to no atypia.[28] Verrucous carcinoma is defined by well-differentiated verrucopapillary proliferation with blunt epithelial-stromal interphase and minimal or no nuclear atypia in the basal or superficial layers. It is not associated with HPV and lacks p53 mutation.

MANAGEMENT

The goals of treatment of VIN are to prevent development of vulvar squamous carcinoma and relieve symptoms, while preserving normal vulvar anatomy and function.[1,5] As most dVINs are not clinically evident or cannot be distinguished from background chronic inflammatory changes, clinical management largely relies on careful monitoring with biopsy of any suspicious lesions. If biopsy proves dVIN, conservative excision is recommended as the initial treatment rather than ablation or pharmacologic therapy, followed by continuous follow-up. Another consideration is the assessment of coexistent VSCC in evaluating dVIN where 17% to 78% of the cases are adjacent to cancers. Therefore, thorough sampling of dVIN lesions is important.[1,29]

PROGNOSIS

It has been suggested that dVIN has a higher risk of progression to VSCC (32.8% versus 5.7%) than uVIN, with a shortened timeframe (22.8 months versus 41.4 months) as compared to uVIN.[30] However, given the relatively high misdiagnosis rate of dVIN, the real progression rate might be higher.[1,9] In addition, dVIN is more likely to give rise to keratinizing SCCs as opposed to the basaloid or warty SCCs associated with uVIN. However, there are exceptions in that 37.5% of HPV-positive tumors are keratinizing VSCC and 9.2% of HPV-negative carcinomas can have basaloid or warty features.[1,5,9]

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