



Case series

Localized amyloidosis in usual-type vulvar intraepithelial neoplasia: High-risk HPV association and potential clinical significance. A series of 45 cases

K.M. Sadeq Islam^a, Yiang Hui^b, Kamaljeet Singh^a, Katrina Hansen^a, C. James Sung^a, M. Ruhul Quddus^{a,*}

^a Department of Pathology and Laboratory, Women & Infants Hospital/Alpert Medical School of Brown University, Providence, RI 02905, United States

^b Department of Pathology and Laboratory, Hospital Pathology Associates, PA, Allina Health Laboratories, 2800 10th Avenue South, Minneapolis, MN 55407, United States

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ABSTRACT

Localized cutaneous amyloidosis was reported recently in association with vulvar squamous intraepithelial neoplasia (VIN). High-risk human papillomavirus (hrHPV) type 16 is the most commonly reported subtype found in usual-type VIN. However, it is unknown whether any hrHPV subtype(s) is/are prevalent in simultaneous squamous intraepithelial lesions and localized amyloidosis in the same individual - the subject matter of this report. To observe the potential clinical significance, study cases were followed and compared to usual-type VINs without amyloid deposition. Of 45 patients of usual-type VINs associated with amyloidosis, 33 had detectable hrHPV, and 12 were negative. HPV 16 alone or in combination with HPV 31 accounted for 72%, HPV 51 alone accounted for 2% of the cases, and 26% were negative for hrHPV. Lack of demonstrable hrHPV in a significant proportion of cases (26%) raises the possibility of a novel or presently undetected hrHPV subtype. Five of the total 22 (23%) patients with amyloid had either Squamous cell carcinoma or high-grade VIN on follow-up. In contrast, 14 of 18 (78%) patients exhibiting lesions without amyloid had disease on follow-up. These findings may indicate that amyloid deposition may represent a feature of regression or a potential favorable prognostic indicator.

1. Introduction

Secondary cutaneous amyloidosis is characterized by the deposition of clinically unapparent amyloid proteins in association with a pre-existing skin condition. In secondary localized amyloidosis, these proteins accumulate in the papillary dermis. Secondary systemic amyloidosis with cutaneous involvement typically shows accumulation in the deep dermis, dermal appendages, and blood vessel walls (Kumakiri and Hashimoto, 1979). Amyloid deposits are reported to derive from epithelial keratin in secondary localized cutaneous amyloidosis. It is postulated that keratin monofilaments undergo “filamentous degeneration,” and keratinocytes “drop off” into the dermis, forming amyloid (Eto et al., 1984). One pathogenic theory proposes that apoptotic basal keratinocytes (colloid bodies) release cytokeratins covered with

autoantibodies, phagocytized by macrophages, and enzymatically degraded into keratin-associated amyloid, a key feature of localized cutaneous amyloidosis (Eto et al., 1984; Zemheri et al., 2014).

Secondary cutaneous amyloidosis has been described in association with a spectrum of both malignant and benign cutaneous lesions, including basal cell carcinoma, porokeratosis, solar elastosis, Bowen's disease, mycosis fungoides, discoid lupus erythematosus, and cellular dermatofibromas (Pirog et al., 2014; Hashimoto and King, 1973; Tsuji et al., 1982; Aso et al., 1990; Holzmann and Schott, 1965; Schott and Holzmann, 1965).

The components of the amyloid in localized amyloidosis with VIN described in a recently published report were found to be Cytokeratin 5 (CK5) and Cytokeratin 14 (CK14) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) studies (Quddus et al., 2014; Islam

* Corresponding author at: Department of Pathology & Laboratory Medicine, Women & Infants Hospital/Alpert Medical School of Brown University, 101 Dudley Street, Providence, RI 02905, United States.

E-mail addresses: kislam@lifespan.org (K.M. Sadeq Islam), ksingh@wihri.org (K. Singh), khansen@wihri.org (K. Hansen), jsung@wihri.org (C. James Sung), mquddus@wihri.org (M. Ruhul Quddus).

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et al., 2019).

Over 130 human papillomavirus types have been documented in the literature. Approximately 40 of these infect the anogenital region and are considered sexually transmitted. Of the sexually transmitted HPV genotypes, 14 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are designated as high risk as they cause anogenital squamous dysplasia and carcinoma (Quddus et al., 2014). Usual-type VINs are commonly associated with HPV16 and less commonly other subtypes. The role of any hrHPV subtype(s) is/are associated with secondary localized cutaneous amyloidosis has not yet been elucidated. Since only certain cases of usual-type VINs are associated with localized amyloidosis, we investigated the clinical course of these patients, simply comparing them to those without amyloid deposits during the similar time period. We also identified the hrHPV subtype(s) in usual-type VINs associated with localized amyloidosis using a PCR-based hrHPV assay.

2. Materials and methods

This is a retrospective study of forty-five cases selected from the archival files (Group-A) between 2008 and 2014, after approval of the Institutional Review Board. *Consecutive usual-type high-grade VINs with incidentally detected secondary amyloidosis cases were selected for this study. High-grade VINs of a similar age group without secondary amyloidosis and within the study time frame was randomly selected as control. The total number of VINs during 2008–2014 at the author's institution was 810 for usual-type high-grade VIN and 162 dVIN.* All cases were histologically confirmed usual-type vulvar intraepithelial neoplasia (VIN) with incidentally diagnosed localized amyloidosis in papillary dermis. The diagnoses were reconfirmed by re-review of the hematoxylin and eosin (H&E) and Congo red-stained slides by one pathologist (MRQ). The previously published classic morphologic diagnostic features by the same authors on localized amyloidosis were used to diagnose amyloidosis on routine hematoxylin and eosin stain, and when identified Congo-Red was done for these cases to confirm amyloid, if not done previously (10). The available clinical history was reviewed to exclude systemic amyloidosis, any known previous malignancies, or systemic chronic inflammatory conditions that may give rise to secondary amyloidosis. Just for comparison 18 cases of usual-type high-grade vulvar intraepithelial neoplasia (VIN III) were collected and follow-up was recorded (Group-B). The hematoxylin and eosin stained slides of Group-B were also re-reviewed carefully to confirm absence of any amyloid deposits. As there were no morphological evidence of amyloid deposition in Group-B patients, Congo-Red special stain was not done in this group.

The appropriate paraffin tissue blocks were selected for Group-A cases, and DNA was extracted from the tissue blocks after ensuring the presence of adequate diagnostic tissue material in the paraffin block for future use should novel treatment opportunities become available, which may require retesting of previous biopsy materials. Lesional material was cut from the block with precautions to prevent contamination by changing the blades and bleaching the area in between cases.

DNA was extracted using the QIAamp DNA FFPE Tissue Kit (QIAGEN Inc, Valencia, CA 91355, Cat No. 56404). The hrHPV genotyping was performed by using a proprietary GenomeMe GeneNav HPV One qPCR Kit (GenomeMe, Richmond, BC, Canada) which specifically detects HPV 16 & 18 and detects 12 other rare subtypes (HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66, and HPV 68) as a group. Subsequently, all non-16/18 positive samples were further genotyped for confirmation through the GenomeMe GeneNav HPV Genotyping qPCR Kit. All tests were performed on the Bio-rad CFX Touch qPCR instrument. qPCR was not done for usual-type VINs without localized amyloidosis.

Twenty-two cases with amyloid deposition and 18 cases of HG-VIN without localized amyloidosis were followed for at least two consecutive years for regression, persistence, or progression of the disease. The control group cases also had areas of high-grade VINs, squamous cell carcinoma, and warty changes of HPV infection.

3. Results

The median age was 59 years (range 37–93 years) for the amyloid-positive cohort and 53 years for the non-amyloid control group. Of the 45 cases, 31 cases had high-grade (VIN II and VIN III) usual-type VIN alone, while 14 cases showed VIN with areas of invasive squamous cell carcinoma, condyloma, or squamous hyperplasia. In all cases, amyloid deposits were only present in the papillary dermis, mostly underneath usual-type VIN. No amyloid deposits were noted in the dermis directly associated with the concurrent lesions of SCC, condyloma, or squamous hyperplasia. All 45 cases were stained appropriately with Congo red and producing apple-green birefringence under polarized light (Fig. 1).

Adequate DNA material was extracted from formalin-fixed paraffin-embedded blocks of all 45 cases for qPCR genotyping. Of 45 cases of VIN associated with amyloidosis, 33 cases were positive for hrHPV. Twelve cases were negative for the hrHPV subtypes tested. Of the 33 positive cases, 31 were positive for HPV type 16 alone, and 1 for HPV 16 and HPV 31. One was positive for HPV 51 (Fig. 2). None were positive for HPV 18. HPV genotyping was not pursued for the non-amyloid group, as the association and subtypes are well-documented in the literature already.

Twenty-two cases of VIN/SCC with incidentally detected localized amyloid deposition were followed for at least two years, including 4 (18%) high-grade VIN with concurrent invasive squamous cell carcinoma (SCC) and 18 (82%) with high-grade VIN alone. Simultaneously, 18 cases of VIN/SCC without amyloid, including 2 SCC (11%) and 16 high-grade VIN (89%) diagnosed during the same time frame, were also followed for comparison.

Five of the 22 (23%) amyloid group patients had either SCC or high-grade VIN on follow-up. In contrast, 14 of 18 (78%) cases without amyloid had disease on follow-up ($p = 0.001$; Table 1).

Of the 18 cases of VIN with the amyloid group, two cases progressed to SCC, while two showed recurrent VIN over the follow-up period. One patient initially diagnosed with amyloid-associated SCC (1/4, 25%) showed recurrent high-grade VIN on subsequent biopsy. Fourteen (14/18, 78%) of these patients demonstrated recurrent disease after resection. Among non-amyloid cases, 8 of 16 had recurrent high-grade VIN and four progressed to SCC, and both the SCC cases had recurrent high-grade VIN.

All cases of high-grade VINs were initially treated by wide-local excision. And cases with associated invasive squamous cell carcinoma, depending on the depth of stromal invasion and various other risk-factors, were managed by wide local excision with/without sentinel lymph node or groin lymph node dissection.

Interestingly, three patients with high-grade VIN in the amyloid group (3/22, 14%) showed suspicious symptomatic lesions on follow-up. However, biopsies of these lesions demonstrated only residual dermal amyloid deposition with the resolution of the corresponding high-grade VIN.

4. Discussion

Secondary localized cutaneous amyloidosis constitutes a heterogeneous subgroup of diseases. These include cytokeratin-derived amyloid deposits seen in cutaneous tumors, polypeptide-derived amyloidosis in endocrine tumors, and inflammation-associated amyloid (AA protein) often seen in Hodgkin disease and renal cell carcinoma (Northcutt and Vanover, 1985). Localized amyloid deposits in the vulvar skin were found to consist mainly of cytokeratin (Quddus et al., 2014). Amyloid deposition in classic VIN is typically found in the papillary dermis and varies from subtle to extensive (Quddus et al., 2014). The components of amyloid in usual VIN were found to be cytokeratin 5 (CK5) and cytokeratin 14 (CK14) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) studies (10–11). Usual VIN is uniformly associated with hrHPV type 16. Other sexually transmitted hrHPV genotypes causing VIN includes HPV type 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. We examined the hrHPVs present in usual-type VIN associated with

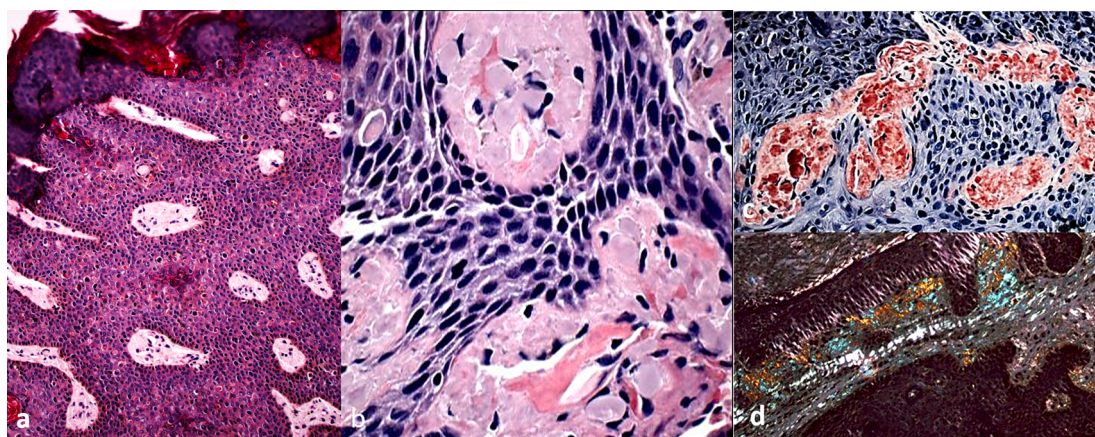


Fig. 1. (a & b) Low, and high magnification view of usual-type vulvar intraepithelial neoplasia (VIN III) with classic appearance of Amyloid deposits in papillary dermis (H&E 4x and 40x respectively) (c) amyloid deposit in papillary dermis immediately beneath the dermo-epidermal junction (Congo-Red 10x), (d) Apple-green birefringence of amyloid deposits under polarized light (Congo-Red, polarized light 10x).

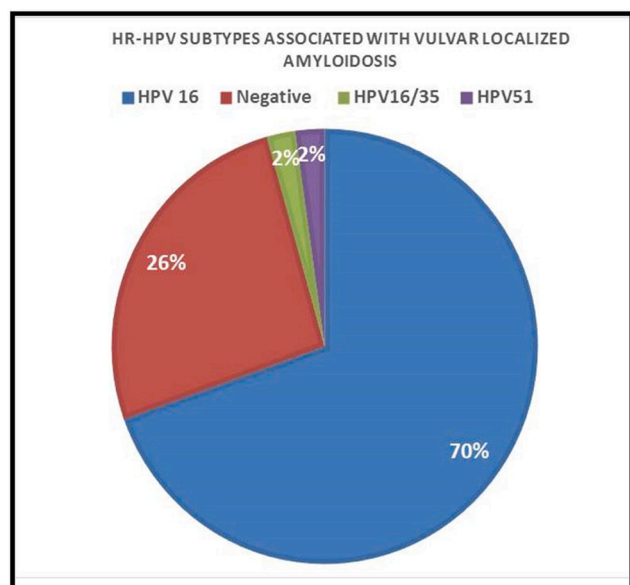


Fig. 2. Pie Chart showing hrHPV subtypes localized vulvar amyloidosis-associated usual-type VINs (n = 45),

localized amyloidosis. Importantly, all cases of amyloidosis were incidentally detected on biopsies or excisions in patients with VIN. The patients presented to the clinicians for gynecologic care or lesions on the vulva.

Overall 33 of 45 cases (74%) cases were positive for one or more types of hrHPV. The majority of the cases (31 out of 45 cases) were positive for the HPV16, accounting for 70% of the cases. This is

concordant with the recognized association between HPV16 and classic-type VIN. One case was positive for both HPV16 and HPV31, lending support that co-infection of HPV16 and 31 may enhance the risk of disease progression. HPV51 was identified in one case, affirming that types other than HPV16 can be associated with VIN. Though common, none of the cases were positive for hrHPV 18 in our series. Our series identified only three subtypes of hrHPV in VIN associated with localized amyloidosis. Whether the localized deposition of the amyloid is specific to these three hrHPVs remains unclear. It also raises the question of whether the amyloid deposition is directly induced by the activity of the virus or indirectly by inducing transformation in the squamous epithelium.

In our series, 12 cases (26%) were negative for HPV. The distribution pattern high-risk HPV is similar to what is known in vulvar lesions. However, a yet unknown HPV subtype cannot be ruled out through this assay. The use of newer E6/E7 assays applied in future investigations may help clarify the nature of such cases. Furthermore, HPV infections are self-limiting and often spontaneously regress within 1–2 years due to cell-mediated immunity or represent a phenomenon of the “hit and run” theory of viral carcinogenesis. In one study, two-thirds of adolescents infected with low-risk HPV types spontaneously cleared their infections by 12 months, as did over 50% of those infected with high-risk HPV types (Brown et al., 2005; Stephenson and Denehy, 2012). The absence of detectable HPV in some cases in our series could be due to spontaneous resolution of the hrHPV infection.

Notably, all cases of incidental localized amyloidosis were seen in the usual-type warty-type VIN. No dVINs were included in this study because no amyloidosis was detected in them. No lichen sclerosis et atrophicus cases, often seen in association with dVIN, were not identified either in this series. The underlying pathogenesis of these two entities, usual-type VIN and dVIN is different. dVIN carries an association with lichen sclerosis and TP53 mutations at the molecular level. NOTCH1 and HRAS mutations have also been implicated in some lesions

Table 1
Follow-up data of Vulvar intraepithelial neoplasia (VIN) with and without amyloidosis.

	VIN alone		VIN with SCC		
	N	Progression to SCC on f/up	N	Recur VIN on f/up	Recurrence/progression on f/up
Amyloid	N = 18 (78%)	2 (11%)	N = 4 (22%)	1 (5.5%)	5/22 (22%)
Non-Amyloid	N = 16 (89%)	8 (50%)	N = 2 (11%)	2	14/18 (78%)
	N = 18	4 (25%)		0	

(Trietsch et al., 2015). Alteration/mutation of NOTCH1, a tumor suppressor, and HRAS, an oncogene, with possible contributions from some somatic mutations, leads to cell proliferation (Trietsch et al., 2015; Nooij et al., 2017). Contrastingly, usual-type VIN is accepted to be high-risk HPV mediated.

The pathogenesis of amyloid deposition in usual-type VIN cannot be solely attributed to inflammatory or chronicity-related processes as differentiated VIN (dVIN) is a chronic process associated with lichen sclerosis and inflammation. Many of the patients of dVIN exhibits stromal elastosis, a form of a degenerative process. However, no amyloid deposition was identified in any of dVIN cases during the same study period. This also suggests that the presence of amyloid in a VIN lesion militates against a diagnosis of dVIN.

The microscopic appearances of usual-type VIN and dVINs are easily recognized by practicing the gynecologic pathologists in most cases, thus no attempt was made to eliminate dVIN from Group-A cases. Of note, no universally accepted and easily reproducible diagnostic criteria are currently available for the diagnosis of dVIN; microscopic appearance is still the hallmark of diagnosis. Mutated p53 above the basal layer of epithelium, when present, is helpful but that is often not the norm.

The etiology and clinical significance of amyloid deposition in usual type VIN is unknown. Spontaneous regression of VIN can occur as reported (Brown et al., 2005), but the presence of localized amyloidosis in those cases have not been documented. After resolution of a usual-type VINs, in this series, amyloid deposits persisted in three patients, clinically mimicking recurrent lesions. However, biopsy of the lesion proved otherwise. This finding certainly raise a legitimate question whether amyloid deposition represents a sign of regression of the lesion. Furthermore, localized amyloid deposition may portend a lower likelihood of progression to invasive disease based on our limited study cases. The disease progression or persistence rate in the amyloid group was lower compared to the non-amyloid group. Although the difference is statistically significant, interrogation of a larger number of cases is essential to ratify this finding.

The limitations of this reports are two folds. The authors would like to emphasize here that this is an observational study and not a case control study. No Congo-Red was done in Group B cases, as in our experience, it was felt that amyloid deposits are recognized on H&E stain by careful scrutiny. qPCR to identify the hrHPV subtypes was not done on the Group-B patients, as abundant literature is available on that subject. Only half of the cases with amyloidosis were followed as only those cases has a minimum of two-year follow-up at our institution. Follow-up of other half of the cases of the study group was not available.

In summary, we observed that among cases of usual-type VIN with amyloid deposition, 26% lack demonstrable hrHPV. Even after resolution of VIN lesions, localized amyloid deposits may persist, mimicking recurrent lesions. It appears that patients with high-grade usual-type VIN with localized amyloid are possibly less likely to develop recurrent vulvar dysplasia, suggesting a possible regressive phenomena or a favorable prognostic indicator. A case control, preferably multi-institutional, prospective study is essential to ratify these observations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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