Histol Histopathol (2021) 36: 1209-1218

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

REVIEW **Open Access**

Relationship of human papillomavirus with seborrheic keratosis of the female genital tract - a case-series and literature review

Shatavisha Dasgupta[1]*, Rachel van Eersel[2]*, Beth Morrel[3], Henk A.M. van den Munckhof[4], Vera A. de Geus[5], Nick M.A. van der Hoeven[3,5], Miekel M. van de Sandt[4], Marta Piso-Jozwiak[5], Wim G.V. Quint[4], Irene A.M. van der Avoort[6], Senada Koljenović[1], Patricia C. Ewing-Graham[1] and Folkert J. van Kemenade[1]

[1]Department of Pathology, Erasmus MC, University Medical Center Rotterdam, The Netherlands, [2]Department of Obstetrics and Gynecology, University Medical Center Gent, Belgium, [3]Department of Obstetrics and Gynecology, Erasmus MC, University Medical Center Rotterdam, [4]DDL Diagnostic Laboratory, Rijswijk, [5]Department of Gynecologic Oncology, Erasmus MC Cancer Institute, Rotterdam and [6]Department of Obstetrics and Gynecology, Ikazia Hospital, Rotterdam, The Netherlands

*These authors contributed equally

Summary. Seborrheic keratoses (SKs) are benign lesions of uncertain etiology, which can develop in both genital and extra-genital locations. For genital SKs there has been conjecture about the pathogenic role of human papillomavirus (HPV), in view of the frequent association of this virus with genital lesions.

In light of the potential consequences on patient management, we investigated the relationship between HPV and SKs of the female genital tract (FGT). For this, we evaluated the current evidence on this relationship by performing an in-depth review of the literature. Furthermore, to add to the evidence on this association, we investigated the presence of HPV in a series of vulvar SKs (n=15), using a novel multimodal approach. This involved whole tissue section-polymerase chain reaction (WTS-PCR) using SPF10-DEIA-LiPA25 for HPV detection and genotyping. In addition, immunohistochemistry (IHC) was performed with cellular biomarkers p16 and MIB-1, and viral biomarker E4, to augment HPV-testing. Finally, laser-capture microdissection-PCR (LCM-PCR) was performed to locate HPV to specific lesional cells, and to rule out incidental detection of resident HPV with WTS-PCR.

Our findings from the literature review as well as the case-series are presented.

Corresponding Author: Dr Shatavisha Dasgupta (MBBS, MD), PhD Candidate, Department of Pathology, Erasmus MC, University Medical Centre Rotterdam, Address: Postbus 2040, Be-building, 3000CA Rotterdam, The Netherlands. e-mail: s.dasgupta@erasmusmc.nl

DOI: 10.14670/HH-18-357

Key words: Vulvar neoplasms, Viral skin diseases, Seborrheic keratosis, Human papillomavirus DNA tests, Cyclin-dependent kinase inhibitor p16, Viral oncogene proteins, Laser capture microdissection

Introduction

Seborrheic keratoses (SKs) are benign, wart-like epidermal lesions, prevalent in middle-aged or elderly individuals of both sexes (Chan, 2019). These lesions can develop on any part of the body, but are relatively uncommon in the genital area (Tardio et al., 2012). Although sun-exposure and aging have been postulated to be risk-factors, the exact mechanism of the pathogenesis of SKs remains unknown.

For genital SKs, the role of human papillomavirus (HPV) in the development has been previously investigated (Leonardi et al., 1991). This is in view of the frequent association of HPV-infection with primary epithelial lesions of the genital tract, and the histological similarity of SKs with the low-risk(LR)-HPV-related lesion, condyloma acuminata (Leonardi et al., 1991; Li et al., 1994; Talia and McCluggage, 2017). HPV was detected in genital SKs in multiple studies, however, there has been debate on the significance of this finding. Some researchers argued whether these results could be attributed to incidental detection of resident HPV, which is part of the microbiological flora of normal skin (Forslund et al., 2004). Others commented that these studies could have erroneously included condylomas, instead of SKs, due to the use of non-stringent histological criteria (Li et al., 1994). To date, the

©The Author(s) 2021. **Open Access.** This article is licensed under a Creative Commons CC-BY International License.

association of HPV and genital SKs remains controversial.

Determining the exact relationship of HPV with genital SKs is not merely of academic interest, as the HPV-status of a lesion may have bearing on management decisions (Garland et al., 2009; Adams and Mbatani, 2018). In general, SKs do not warrant treatment, and are usually removed when symptomatic, or for cosmetic reasons (Von Krogh et al., 2000). Where there is an association with HPV, treatment of SKs will need to be directed to addressing the viral cause, in order to prevent lesional recurrence or unnecessary surgical procedures (Garland et al., 2009).

Through this study, we aimed to establish the association between HPV and SKs of the female genital tract (FGT). For this, we evaluated the current evidence on this association by performing an in-depth review of the literature. In addition, to add to the existing evidence, we investigated the presence of HPV in a series of vulvar SKs (VSKs), using a novel combination of direct and surrogate approaches. This involved (i) whole tissue section-polymerase chain reaction (WTS-PCR) for HPV detection and genotyping; (ii) immunohistochemistry (IHC) with cellular biomarkers p16 and MIB-1, and the viral biomarker E4, to augment HPV-testing; and (iii) laser-capture microdissection-PCR (LCM-PCR) to locate HPV to specific lesional cells. Where HPV was detected, we investigated whether the patient had a recent history of other HPV-related vulvar lesions, and performed WTS-PCR and IHC on these for comparison.

Materials and Methods

Literature review

Electronic search strategies combining Medical Subject Headings (MeSH) and free-text words were prepared with the help of medical librarians at Erasmus MC. Biomedical bibliographic databases, namely, Embase.com, MEDLINE (Ovid), Cochrane Central Register for Controlled Trials (Wiley), Web of Science Core Collection (Web of Knowledge), and Google Scholar were searched. The full search strategy is provided in supplement 1. The last search was conducted in December, 2020.

A total of 374 unique references were identified from the databases. These were screened by reading the title and / or abstract by one author (SDG). Original and review articles that met the following criteria were included-(i) abstract available; (ii) written in English language; and (iii) reporting on SKs of the FGT. Case reports, conference abstracts, animal studies, and *in-vitro* studies were excluded.

Thirty-nine references were included after the first round of screening. These references were screened by two authors (SDG and BM) to specifically identify studies that fulfilled the inclusion criteria. Five such references were found and for all of these, full text was available. Five additional studies were included to prepare the narrative synthesis. The process of reference selection is depicted in Fig. 1.

Case-series

Lesions that had been histologically diagnosed as VSKs (2009-2018) were retrospectively identified from Erasmus MC, and Ikazia Hospital, Rotterdam. Hematoxylin-eosin (HE) stained slides were retrieved from the archives and reviewed by two pathologists (SDG and PCEG). Relevant clinical information was gathered from the patient records. All patient data were anonymized.

For WTS-PCR, sections were prepared from the formalin-fixed paraffin embedded (FFPE) tissues using a sandwich method to yield one 4 µm-thick section for HE-staining (HE-before); two 3×8 µm sections for WTS-PCR; three 4 µm-thick sections for IHC; and one 4 µm-thick section for HE-staining (HE-after). The HE-before and HE-after slides were independently reviewed by an experienced pathologist (MvdS) to confirm the histological diagnoses.

WTS-PCR

WTS-PCR was performed using the SPF10-DNA Enzyme Immunoassay (DEIA)-Line Probe Assay (LiPA) 25 system (Labo Bio-Medical Products, The Netherlands), containing probes for 25 different HPV-genotypes (HPV6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74).

IHC

IHC was conducted with p16 using the ready-to-use primary mouse monoclonal antibody (mAb) clone E6H4 (Ventana), with MIB-1 using the clone Ki-67 (Ventana), and with E4 using the PanHPVE4 mAb XR-E4-1 (SILgrade-E4 kit, Labo Bio-Medical Products). IHC was scored as follows:

p16: complete lack of staining=no expression; nuclear and / or cytoplasmic staining in non-contiguous cell clusters extending to less than 1/3rd of the epithelial thickness=non-block-type / patchy expression; nuclear and/or cytoplasmic expression extending from the basal layer through at least 1/3rd of the epithelial thickness (Darragh et al., 2012)=block-type expression.

MIB-1: nuclear staining in a single or double row of cells only in the basal / parabasal layers=negative; at least two moderately to strongly stained nuclei within the same high-power field (400x magnification) in the upper 2/3rd of the epithelium=positive (Pirog et al., 2000; Logani et al., 2003).

E4: no staining=0; focal staining restricted to groups of cells in the upper epithelial layers=1; extensive staining in the upper half of epithelium or more=2. Score ≥1 was interpreted as positive (Leeman et al., 2020).

LCM-PCR

Regions for LCM-PCR were selected on the digital scans (Aperio Inc., CA) prepared from the glass-slides. Areas of these regions ranged from 30,262-74,322 μm^2 (mean=57,936 μm^2). These were excised using Zeiss PALM microbeam UV-laser-microdissection system, and thereafter analyzed using SPF10-DEIA-LiPA25.

Methodology of WTS-PCR, IHC, and LCM-PCR is detailed in supplement 2.

Results

Literature review

Information extracted from the five studies that investigated the association of lesions having the histology of SKs of the FGT and HPV are presented in

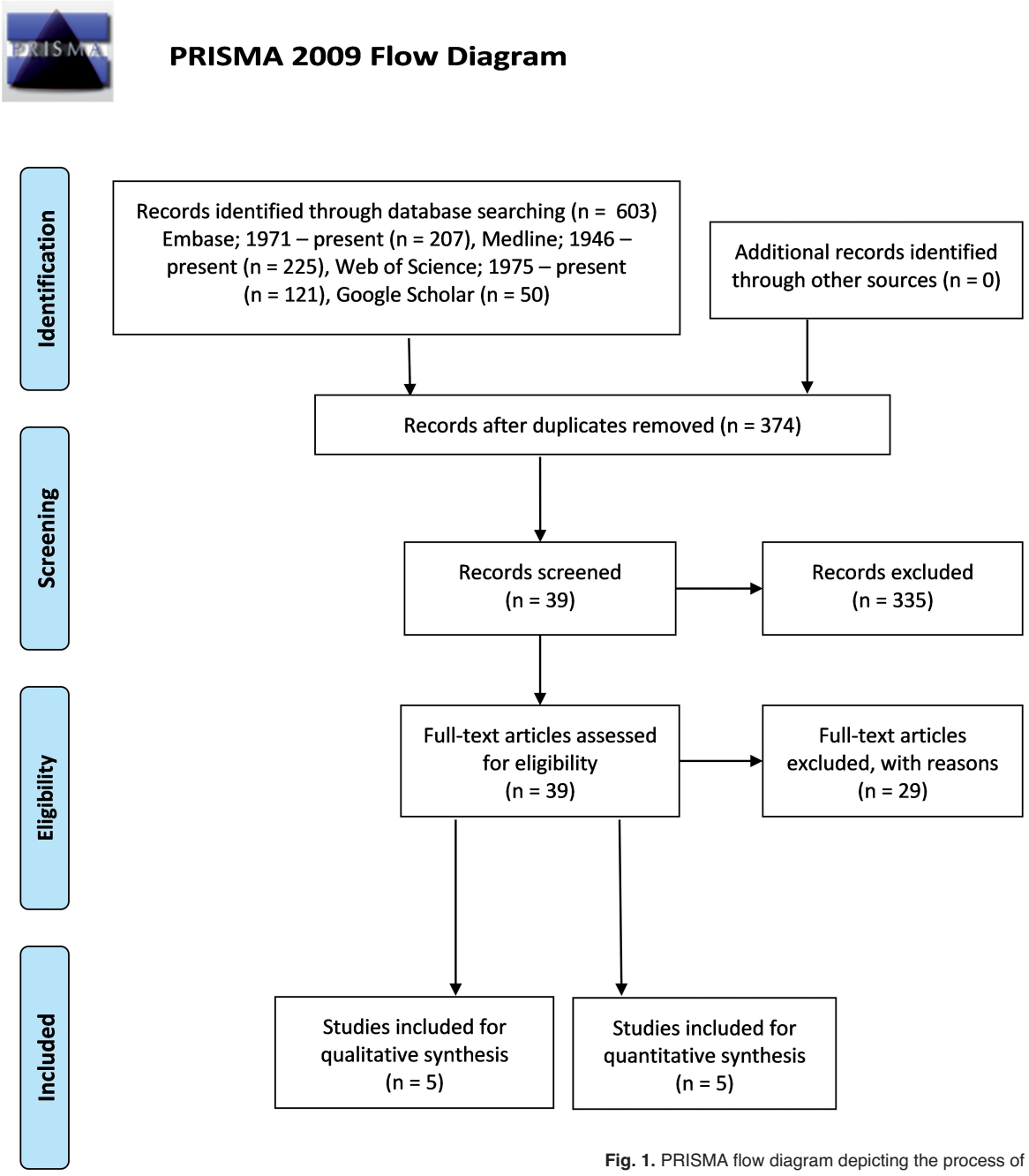

Fig. 1. PRISMA flow diagram depicting the process of literature inclusion.

Table 1, and summarized in the following sections.

Prevalence of HPV in SK of female genital tract (FGT)

Of the 5 previous studies, 2 were on VSKs (Bai et al., 2003; Reutter et al., 2014), 2 were on VSKs, pubic and perianal SKs (Leonardi et al., 1991; Tardio et al., 2012), and 1 was on cervico-vaginal SK-like lesions (Talia and McCluggage, 2017). In 3 of these studies, non-SK genital lesions, comprising benign (85%), pre-malignant (11%), and malignant lesions (4%) constituted the control group.

Mean age of patients with SKs (n=88) was 47.8 years (range: 5-80 years). For all SKs, the median prevalence of HPV was 57.5% (range: 14.2-75%; mean: 51%), while for VSKs, this was 46% (range: 14.2-100%; mean: 51.5%). Low-risk (LR)-HPV6 was detected most frequently (72%), followed by rarer untypable HPV-genotypes (12%), LR-HPV11 (3%), and high-risk (HR)-HPV16 (3%).

Mean age of the patients with non-SK genital lesions (n=67) was 47.9 years (range: 9-70 years). In 1 study, condyloma acuminata was studied as the non-SK lesion, and HPV was detected in all of these (genotyping not performed). For the rest of the non-SK genital lesions, median prevalence of HPV was 0% (range: 0-9.1%; mean: 3.3%), and the detected HPVs comprised LR-HPV6 and HR-HPV16.

Histology

A summary of the histological description of the SKs extracted from the included references is presented below.

The lesions are circumscribed or polypoid, having a stuck-on configuration, composed of broad, coalescing sheets, islands, and interconnecting trabeculae of cells. Lesional cell nests are surrounded by scant fibrovascular stroma with prominent hyaline basement membrane-like material and ectatic, thin-walled vessels. The lesional cells have a bland, basaloid appearance. Nuclei are uniform, ovoid to spindle-shaped, with occasional nuclear grooves. Mitotic count is generally low. Areas of peripheral palisading, hyperkeratosis, basket-weave or laminated orthokeratosis, papillomatosis, acanthosis, variable melanin pigmentation, horn pseudo-cysts, and squamous eddies are characteristic features. Clusters of cells with clear cytoplasm can be occasionally present. Viral cytopathic changes e.g. koilocytes, parakeratosis, compact orthokeratosis, or hypergranulosis are typically absent.

Reutter et al. reported parakeratosis to be a specific predictor of the presence of HPV in VSKs (Reutter et al., 2014). In contrast, Bai et al. and Tardio et al. observed that HPV-status of SKs could not be reliably predicted from any particular histological feature (Bai et al., 2003; Tardio et al., 2012).

IHC

p16: Two studies studies (Bai et al., 2003; Talia and McCluggage, 2017) investigated p16-expression in SKs. In the series of Bai et al., 11% (2/18) of the HPV-positive VSKs showed non-block-type / patchy p16-expression, while the non-SK genital lesions did not show p16-expression. In the series of Talia et al., none of the HPV-positive SK-like lesions (n=3) showed p16-expression, whereas, 75% (3/4) of the HPV-negative SK-like lesions showed a mosaic pattern of p16-expression.

Table 1. Studies that investigated the relationship between HPV and SKs of the female genital tract.

References	Genital SKs				Genital non-SK lesions		
	Method of HPV-detection	Location (number)	Mean age (Range)	HPV prevalence; genotypes (frequency)	Lesion (number)	Mean age (Range)	HPV prevalence; genotypes (frequency)
Leonardi et al., 1991	HPV DNA PCR-Dot-blot and/or Southern blot	vulva (1); pubis (1); groin (1); buttock (1)	27.5 (5-44)	75%; HPV6 (2), HPV11 (1), HPV16 (1)	SF (6); BD (5); SD (4); LP (3); BCC (2); BH (1); CD (1); SCC (1); EA (1); Len. (1); DN (1); EN (1)	No data	0%; NA
Bai et al., 2003	HPV-DNA PCR-RFLP	vulva (25)	46 (16-80)	72%; HPV6 (15), novel HPV (3)	NCA (10); FEP (12)	44.7 (25-70)	9.1%; HPV6 (1), HPV16 (1)
Tardio et al., 2012	HPV DNA PCR-hybridization	vulva (5); pubis (9); perianal (1)	42.7 (5-80)	33.3%; HPV6 (5)	AK (5); FEP (3); EC (2); Hem. (1); MN (1)	38.5 (9-54)	0%; NA
Reutter et al., 2014	HPV DNA PCR-RFLP	vulva (21)	67.5 (no data)	14.3%; HPV6 (2), novel HPV (1)	Cond. A (6)	60.7 (no data)	100%; genotyping not performed
Talia et al., 2017	HPV DNA PCR-hybridization	cervix (4); upper vagina (3); un-determined (1)	55.4 (41-70)	42.9%; HPV42 (3)	not studied		
Present series, 2021	HPV DNA PCR-reverse hybridization	vulva (15)	50.4 (25-70)	73.3%; HPV44 (5), HPV6 (4), HPV42 (2), HPV53 (1)	not studied		

SK: seborrheic keratosis; PCR: polymerase chain reaction; AK: angiokeratoma; EC: epidermal cyst; FEP: fibroepithelial polyp; hem.: hemangioma; MN: melanocytic nevus; SF: soft fibroma; BD: Bowen's disease; SD: superficial dermatitis; LP: lichen planus; BCC: basal cell carcinoma; BH: benign hyperplasia; CD: chronic dermatitis; SCC: squamous cell carcinoma; EA: epidermolytic acanthoma; Len.: lentigo; DN: dysplastic nevus; EN: epidermal nevus; NA: not applicable; RFLP: restriction fragment length polymorphism; NCA: non-condylomatous acanthoses; FEP: fibroepithelial polyp; AK: angiokeratoma; Hem.: hemangioma; MN: melanocytic nevus; cond. A: condyloma acuminatum.

MIB-1: One study (Bai et al., 2003) investigated MIB-1-expression in VSKs. MIB-1 positivity, i.e. nuclear staining in upper 2/3rd of epithelial thickness, was observed in 72% (18/25) of HPV-positive VSKs, and showed good concordance with the HPV-status (kappa=0.42). In the same study, 22.7% (5/22) of non-SK genital lesions also showed MIB-1-positivity, although data on the concordance of MIB-1-expression and the HPV-status for these lesions were not presented.

Cyclin E: One study (Bai et al., 2003) investigated cyclin E-expression in VSKs. Cyclin E-positivity was observed in 76% of VSKs, and in 68.2% of non-SK genital lesions, and did not show any correlation with the HPV-status of the lesions.

Bai et al. reported that among p16, MIB-1, and cyclin E, the only biomarker that could augment HPV-testing for VSKs was MIB-1 (Bai et al., 2003).

Case-series

Fifteen lesions were identified from 13 patients of ages between 25-70 years (median=52 years) (Table 2). None of the patients were immunocompromised.

Histology

The lesions were acanthotic, composed of monomorphic keratinocytes having low nuclear-to-cytoplasmic ratio, arranged in coalescing sheets or in interconnected trabeculae, with areas of peripheral palisading (Fig. 2). Laminated and/or basket-weave orthokeratosis, horn pseudo-cysts, dilated capillaries within thickened papillary dermis, melanin pigment in the basaloid cells, and squamous eddies were frequently present. Mitotic figures were rare. Histological hallmarks of HPV-infection were absent.

WTS-PCR

HPV-DNA was detected in 11 (73%) VSKs (patients 5-13). The genotypes were LR-HPV6 (n=4), LR-HPV44 (n=4), LR-HPV42 (n=2), probably-carcinogenic HPV53 (n=1), and untypable (n=1) (Table 3).

Patients 5, 6, and 10 had a history of vulvar high grade squamous intraepithelial lesion (HSIL), and patient 11 had a co-existing HSIL. HR-HPV16 was detected in HSILs from patients 5 and 10, and both LR-HPV6 and HR-HPV16 were detected in the HSIL from patient 11. Tissue for HPV-testing was not available for the HSIL from patient 6. Patient 5 developed a condyloma acuminatum on follow-up, in which LR-HPV6 was detected.

IHC

p16: Nine (82%) of the 11 HPV-positive VSKs showed extensive, non-block-type / patchy expression (Table 3, Fig. 3). All three HSILs showed block-type expression, and the condyloma showed extensive, non-block-type expression, similar to the VSKs.

MIB-1: All of the 11 HPV-positive VSKs were positive for MIB-1 (Table 3, Fig. 3). In 2 of these lesions, nuclear staining in the upper 2/3rd of the epithelium was present in fewer foci. In 1 lesion, nuclear staining was mostly present in the upper 2/3rd of the epithelium, with minimal staining in the basal layer. Of

Table 2. Clinical details.

Pt.	Age* (y)	Specimen	H/O vulvar lesions	Macroscopic description	Co-existing vulvar lesions	Site	Follow-up (months)
1	67	Biopsy	SCC; dVIN	Pigmented, raised	None	Not specified	LS (26); thereafter NED (61)
2	53	Biopsy	None	Pigmented, raised	SCC	Lab. majus (R)	dVIN (5); anal hemorrhoids (16); radio-necrotic anal ulcers (34); thereafter NED (15)
3	66	Biopsy	None	Verrucous	SCC	Lab. majus (R)	Granulation tissue - vulva (5); thereafter NED (57)
4	52	Biopsy	SCC; HSIL	Skin tag-like	None	Lab. majus (L)	NED (12)
5	46	Biopsy	HSIL	Papillomatous, pigmented	None	Lab. minus (R)	Vulvar condyloma acuminatum (13); chronic inflammatory changes in vulva (28, 40); thereafter NED (17)
6	66	Excision	HSIL	Verrucous, pigmented	None	Lab. majus (R)	No FU
7	40	Biopsy	None	Papillomatous, pigmented	None	Lab. minus (R)	No FU
8	25	Excision	None	Papillomatous	None	Lab. majus (B/L)	CIN2 (1); Pap smear: NILM, HR-HPV+ve (9); Pap smear: NILM, HR-HPV-ve (19); thereafter NED (19)
9	52	Excision	None	Pigmented, raised	None	Lab. minus (L)	No FU
10	70	Biopsy	HSIL	Pigmented, plaque-like	None	Clitoris	VSK (19); VSK (23); thereafter NED (24)
11	33	Biopsy	None	None	HSIL	Lab. minus (L)	NED (84)
12	37	Biopsy	None	None	None	Mons pubis	NED (84)
13	48	Biopsy	None	None	Squamous hyperplasia	Lab. majus (R)	No FU

Pt.: patient; *age at diagnosis; y: years; H/O: History of; SCC: squamous cell carcinoma; dVIN: differentiated vulvar intraepithelial neoplasia; LS: lichen sclerosus; lab.: labium; NED: no evidence of (vulvar) disease; R: right; L: left; B/L: bilateral; FU: follow-up; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia; NILM: negative for intraepithelial lesion or malignancy.

the 4 HPV-negative VSKs, 2 were MIB-1 positive. All three HSILs and the condyloma were MIB-1-positive.

E4: Four (36%) of the 11 HPV-positive VSKs showed focal expression in upper epithelial layers (score=1) (Table 3, Fig. 3). One HSIL showed extensive expression in the upper half of the epithelium (score=2).

LCM-PCR

A total of 19 lesional areas and one area of adjacent normal epithelium were tested from the 11 HPV-positive VSKs. For 10 VSKs (14 lesional areas), the results of WTS-PCR could be validated on LCM-PCR (Table 4, Fig. 3). For 1 VSK, HPV could not be detected on LCM-PCR although 6 lesional areas were tested. This lesion was E4-positive, which signifies a productive HPV-infection. Therefore, for this lesion, E4-positivity was considered sufficient to rule out incidental detection of HPV from contamination. HPV was also not detected in the area of normal epithelium.

Discussion

Previous studies reported a higher prevalence of HPV in SKs compared to non-SK lesions of the FGT

There has been debate on whether the presence of HPV in SKs could be attributed to the incidental detection of resident HPV (Forslund et al., 2004). Our literature review provides interesting insights in this regard. We observed that some of the studies on SKs had also investigated the presence of HPV in non-SK genital lesions, and both groups of lesions were from women of comparable ages. Interestingly, in these studies, prevalence of HPV was much higher in SKs than in the non-SK lesions. If the detection of HPV in SKs was attributable to contamination, similar prevalence of HPV would be expected in both SKs and non-SK lesions.

There has also been debate on whether studies reporting on the presence of HPV in SKs used appropriate histological criteria for case inclusion. In

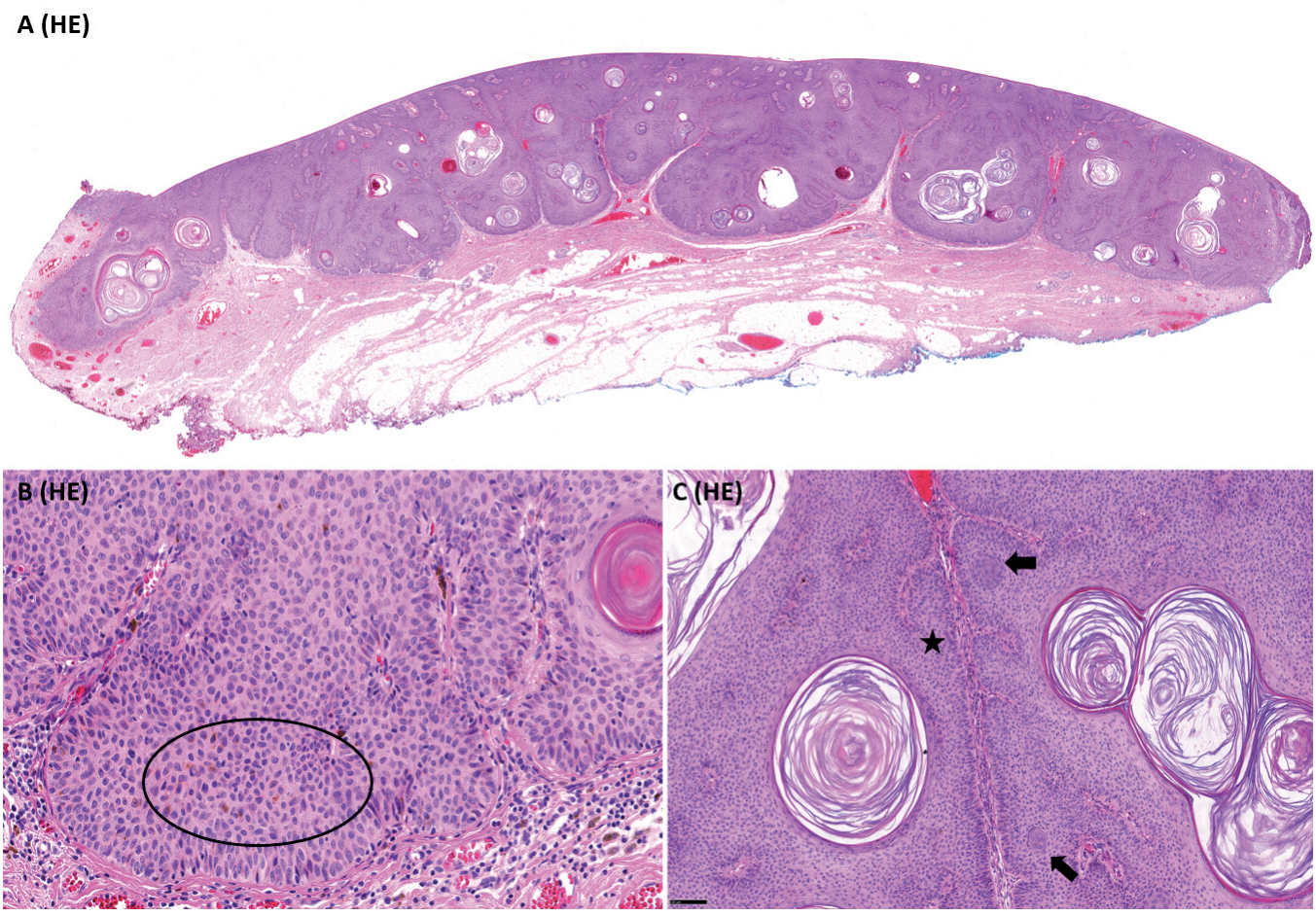

Fig. 2. Histological appearance of the VSK from patient 9, in which HPV42 was detected on WTS-PCR (HE-stain); low **(A)** and high-magnification **(B, C)** images. **A.** Epithelial acanthosis and abundant horn pseudo-cysts can be appreciated. **B.** The lesion is composed of monomorphic basaloid cells with low nuclear-to-cytoplasmic ratio; circled area shows presence of melanin in the basaloid cells. **C.** Peripheral palisading (asterisk) and squamous eddies (arrows) are present within the lesion. A, x 2; B, x 20; C, x 10.

their seminal article, Li and Ackerman opined that histological criteria used in some of these studies were insufficient for discriminating SKs from condylomas (Li et al., 1994). To address this issue, subsequent studies, including our current study, followed stringent histological criteria for case selection and excluded any lesions showing histological changes associated with active HPV-infection. Nevertheless, HPV was still detected in lesions that were judged as SK on histology (Tardio et al., 2012; Reutter et al., 2014). Bai et al. and Tardio et al. therefore concluded that histology was not a reliable predictor of the HPV-status, and the results from our current series support this view.

LR-HPV can produce lesions having the histology of VSK

In our series, HPV was detected in 73% (11/15) of

Table 3. Results of WTS-PCR, IHC, and LCM-PCR.

Pt.	Age (y)	Lesions	Site	WTS-PCR (SPF10-LiPA25)	IHC			LCM-PCR
					p16	E4	MIB-1	
1	67	VSK	Not specified	neg.	no expression	0	pos.	N/A
2	53	VSK	Lab. majus	neg.	no expression	0	pos.	N/A
3	66	VSK	Lab. majus	neg.	no expression	0	neg.	N/A
4	52	VSK	Lab. majus	neg.	no expression	0	neg.	N/A
5	46	VSK‡	Lab. minus	HPV6, 53	ext., patchy	1	pos.	HPV6, 53
		HSIL (3 yrs before‡)	Lab. majus	HPV16	block-type	0	pos.	N/A
		Condyloma (1yr after‡)	Lab. majus	HPV6	ext., patchy	0	pos.	N/A
6	66	VSK	Lab. majus	HPV44	ext., patchy	1	pos.[1]	HPV44
7	40	VSK	Lab. minus	HPV42	no expression	1	pos.[2]	neg.
8	25	VSK	Lab. majus	HPV6	ext., patchy	0	pos.	HPV6
9	52	VSK	Lab. minus	HPV42	ext., patchy	1	pos.	HPV42
10	70	VSK§	Clitoris	HPV44	ext., patchy	0	pos.	HPV44
		HSIL (1 y. before§)	Lab. minus	HPV16	block-positive	2	pos.	N/A
		VSK (19 mons. after§)	Clitoris	HPV44	no expression	0	pos.	HPV44
		VSK (23 mons. after§)	Lab. majus	HPV44	ext., patchy	0	pos.	HPV44
11	33	VSK	Lab. minus	HPV6	ext., patchy	0	pos.	HPV6
		HSIL (synchronous)	Lab. minus	HPV6, 16	block-positive	0	pos.	N/A
12	37	VSK	Mons pubis	HPV untypable	ext., patchy	0	pos.[1]	HPV44*
13	48	VSK	Lab. majus	HPV6	ext., patchy	0	pos.	HPV6

Pt.: patient; y: years; WTS-PCR: whole tissue section; PCR: polymerase chain reaction; IHC: immunohistochemistry; neg.: negative; pos.: positive; N/A: not applicable; lab.: labium; ext.: extensive; HSIL: high grade squamous intraepithelial lesion; [1]few high power fields with nuclear staining in the upper 2/3rd of the epithelium; [2]nuclear staining mostly in the upper 2/3rd of the epithelium; *HPV55 detected with the LiPA-25+ panel, which has been re-classified as HPV44

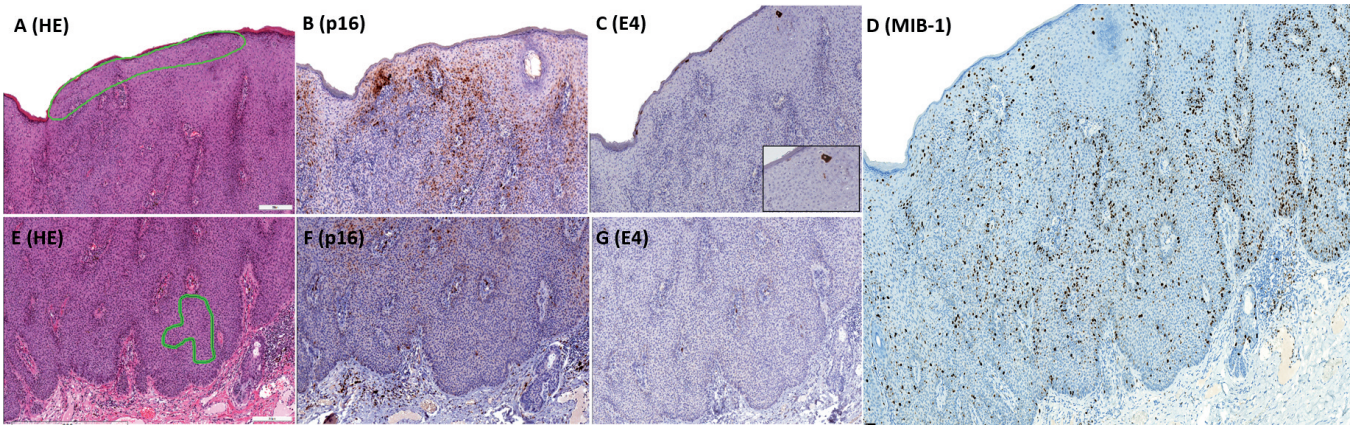

Fig. 3. VSK from patient 9, in which HPV42 was detected on WTS-PCR. **A.** HE-stained lesional area; the area marked by the green line was analyzed using LCM-PCR and HPV42 was detected. **B.** p16-IHC: patchy expression. **C.** E4-IHC: focal expression in the superficial epithelial layer (score=1); inset shows the E4-positive cells under higher magnification. **D.** MIB-1-IHC: increased expression in the upper 2/3rd of the epithelial layer. **E.** HE-stained lesional area; the area marked by the green line was analyzed using LCM-PCR and HPV42 was detected. **F.** p16-IHC: patchy expression. **G.** E4-IHC is negative. Scale bars: 200 μm.

the lesions having the histology of VSKs. The genotypes detected in these VSKs, i.e. LR-HPV6, LR-HPV44, LR-HPV42 and possibly-carcinogenic HPV53, have been previously detected in genital neoplasms in both sexes (Leonardi et al., 1991; Bai et al., 2003; Tardio et al., 2012; Reutter et al., 2014).

To augment HPV-testing, we performed IHC with the cellular biomarkers p16 and MIB-1, and the viral biomarker, E4. p16 is a tumor-suppressor protein that is overexpressed in HPV-infection as a consequence of cell-cycle deregulation by E6/E7 HPV-onco-proteins (Molina et al., 2020). In HR-HPV-infection, the deregulation caused by E6/E7 is more pronounced, and this results in a block-type p16-expression (Singh et al., 2018). In contrast, in transforming infections of LR-HPV, there is limited deregulation, and this may result in an extensive, non-block-type / patchy p16-expression. This pattern of p16-expression was observed in 82% (9/11) of the HPV-positive VSKs in our series (Singh et al., 2018; Leeman et al., 2020). However, patchy p16-expression, albeit weak and focal, may also occur in normal vulvar tissue (Singh et al., 2018). Currently, no reliable cut-offs have been identified that allow accurate distinction of the patchy p16-expression patterns of normal vulvar tissue from that of LR-HPV-infection, and this limits the usefulness of p16-IHC as a surrogate marker for LR-HPV-infection.

MIB-1 is a proliferation marker that is expressed predominantly in the parabasal layers in normal cervical or vulvar tissue. Pirog et al. observed that MIB-1 positivity, i.e. increased expression in the upper 2/3rd of the epithelium correlates strongly with the presence of HPV in vulvar lesions (Pirog et al., 2000). For VSKs, Bai et al. reported good concordance of MIB-1-expression and the HPV-status (Bai et al., 2003). In our series, MIB-1 was positive in all the lesions (VSKs, HSILs, and condyloma) where HPV was detected on WTS-PCR. However, 2 (50%) of the HPV-negative VSKs also showed MIB-1 positivity. This suggests that MIB-1 has a higher sensitivity than specificity for predicting the presence of HPV in genital lesions.

The HPV-protein E4 helps disrupt the cytoskeletal structure of the squamous cells to allow viral release and transmission (Doorbar, 2013; Molina et al., 2020). Therefore, accumulation of E4 in the upper layers of the epithelium indicates a productive infection (Doorbar, 2013; Molina et al., 2020). E4-expression was seen in 4 (36%) of the HPV-positive VSKs in our series, and the expression pattern was similar to that previously reported for low-grade cervical intraepithelial neoplasia (Leeman et al., 2020).

Using LCM-PCR, we further confirmed the presence of HPV in lesional cells expressing p16 and/or E4, as well as in lesional cells in the basal/intermediate epithelial layers, where HPV typically sets up infection. In contrast, an area of normal epithelium adjacent to the lesion was negative for HPV. These findings strongly argue against false-positivity due to surface contamination from resident HPV.

The latest edition of the WHO classification of FGT

Table 4. Results of LCM-PCR.

Pt.	WTS-PCR (SPF10-LiPA25)	IHC-scores		LCM-PCR	
		p16	E4	LCM region	SPF10-PCR
5	HPV6, 53	patchy	0	Lesional area-superficial / intermediate epithelial layer	HPV6
		patchy	1	Lesional area-superficial / intermediate epithelial layer	HPV6, 53
6	HPV44	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV44
		neg.	0	Adjacent normal epithelium	neg.
7	HPV42	neg.	0	Lesional area-basal / intermediate epithelial layer	neg.
		neg.	0	Lesional area-intermediate epithelial layer	neg.
		neg.	1	Lesional area-superficial epithelial layer	neg.
		neg.	0	Lesional area-intermediate epithelial layer	neg.
		neg.	1	Lesional area-superficial / intermediate epithelial layer	neg.
		neg.	0	Lesional area-intermediate epithelial layer	neg.
8	HPV6	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV6
9	HPV42	patchy	1	Lesional area-superficial epithelial layer	HPV42
		patchy	0	Lesional area-intermediate epithelial layer	HPV42
10§	HPV44	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV44
	HPV44	neg.	0	Lesional area-basal / intermediate epithelial layer	HPV44
	HPV44	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV44
11	HPV6	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV6
		patchy	0	Lesional area-basal / intermediate epithelial layer	HPV6
12	HPV untypable	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV44*
13	HPV6	patchy	0	Lesional area-basal / intermediate epithelial layer	HPV6

Pt.: patient; LCM: Laser capture microdissection; PCR: polymerase chain reaction; WTS: whole tissue section; IHC: immunohistochemistry; §three different lesions, *HPV55 detected with the LiPA-25+ panel, which has been re-classified as HPV44.

tumors suggests that HPV is more likely to be present in VSKs from younger women, than in those from older women (Focchi et al., 2020). In our series, however, 6 (55%) of the HPV-positive VSKs were from women > 40 years of age. Further investigations at a molecular level may help determine whether the HPV-positive VSKs, HPV-negative VSKs, and condylomas are a part of the same spectrum.

Our results suggest that LR-HPVs can produce lesions that are histologically identical to VSKs. Therefore, pathologists need to be aware that HPV-status of a lesion may not be accurately determined on histology alone. When diagnosing a lesion having the histology of SK, particularly in women with a history of HPV-related genital lesions, IHC with p16 and MIB-1 may be considered. Where p16 shows extensive, patchy expression, and MIB-1 shows increased expression in upper 2/3rd of the epithelium, a PCR may be performed to rule out HPV-infection.

Presence of LR-HPVs in VSKs may influence the clinical management

HPV-infection is known to generate a 'field-effect', which predisposes to co-infection with other LR/HR-HPV genotypes (Adams and Mbatani, 2018). This may result in synchronous or metachronous lesions in contiguous anatomical sites. In our series, 4 patients with HPV-positive VSKs had a previous or co-existent vulvar HSIL. Therefore, thorough examination of the genital skin and follow-up can be important for patients with HPV-positive lesions.

LR-HPV-infection has also been reported to unbalance the vaginal microbiota by facilitating over-proliferation of anaerobic bacteria, such as Gardnerella (Zhou et al., 2019). Therefore, to avoid a bacterial or fungal co-infection, treatment strategies for HPV-positive VSKs should be formulated to address the viral cause.

Determining the prevalence of HPV in lesions having the histology of VSKs will also help to correctly estimate the burden of LR-HPV-related disease, which is vital for informing and updating preventive strategies, such as HPV-vaccination.

Strengths and limitations

We provide a comprehensive review of the literature on a contentious area in diagnostic pathology. To the best of our knowledge, this is the first study to investigate the association of HPV with lesions having the histology of VSKs, using both WTS-PCR and LCM-PCR. The sample size of this study was small, but was comparable to previous studies and is attributable to the rarity of the disease. For HPV-detection, we used the analytically sensitive SPF10-DEIA-LiPA25-system, which can detect HPV-DNA optimally from FFPE-tissues, despite the formalin-induced degradation of the genetic material. Due to the higher sensitivity, DNA-PCR is preferred over DNA-in-situ hybridization (ISH) for detecting HPV from benign lesions having a low viral load (Nuovo, 2002). The novel RNA-ISH-based RNAscope© may offer a higher sensitivity than DNA-ISH, although further research is required to delineate cut-offs for signal interpretation in lesions with low HPV-transcript numbers (Henley-Smith et al., 2021).

Our study, however, may suffer from a selection bias, as some of the patients in our series were under follow-up for other vulvar diseases. This limits the generalizability of our results. Furthermore, due to the retrospective nature of our study, paired normal vulvar tissue could not be tested as controls, and this is another limitation.

Conclusion

Our results, based on the literature review and our case-series, suggest that LR-HPV-infection can produce lesions in the FGT that have the histology of SKs and do not exhibit features associated with HPV-infection. While diagnosing lesions having the histology of SKs, a potential association with HPV should be borne in mind by pathologists, even for SKs from older women. To facilitate appropriate management, HPV-testing should be performed for lesions having the histology of SKs, particularly for women with previous or concurrent HPV-associated genital lesions.

Acknowledgements. The authors are grateful to Ms. Elise Krabbendam (MSc), Biomedical Information Specialist at Medical Library, Erasmus MC, for the help in preparing electronic search strategies, and to Dr Karien E. Hamoen (MD), Pathologist at Maasstad Hospital, Rotterdam, for enabling the use of tissues from Ikazia Hospital.

Conflicts of Interest. There are no conflicts of interest and no external funding was received for this study.

Author contributions. Concept and design: S.D.G., R.V.E., B.M., H.A.M.V.D.M., W.G.V.Q., I.A.M.V.D.A., P.C.E.G., S.K., F.J.V.K.; Data acquisition: all authors; Data analysis / interpretation: S.D.G., R.V.E., B.M., H.A.M.V.D.M., M.M.V.D.S., W.G.V.Q., I.A.M.V.D.A., P.C.E.G., S.K., F.J.V.K.; Writing-first draft: S.D.G.; Writing-review and editing all authors; Supervision: P.C.E.G., S.K., F.J.V.K.; Final approval: all authors.

Funding sources: No external funding was received for this study.

References

Adams T.S. and Mbatani N.H. (2018). Clinical management of women presenting with field effect of HPV and intraepithelial disease. Best. Pract. Res. Clin. Obstet. Gynaecol. 47, 86-94.

Bai H., Cviko A., Granter S., Yuan L., Betensky R.A. and Crum C.P. (2003). Immunophenotypic and viral (human papillomavirus) correlates of vulvar seborrheic keratosis. Hum. Pathol. 34, 559-564.

Chan M.P. (2019). Verruciform and condyloma-like squamous proliferations in the anogenital region. Arch. Pathol. Lab. Med. 143, 821-831.

Darragh T.M., Colgan T.J., Cox J.T., Heller D.S., Henry M.R., Luff R.D., McCalmont T., Nayar R., Palefsky J.M., Stoler M.H., Wilkinson E.J.,

Zaino R.J., Wilbur D.C. and Members of L.P.W.G. (2012). The lower anogenital squamous terminology standardization project for hpv-associated lesions: Background and consensus recommendations from the college of american pathologists and the american society for colposcopy and cervical pathology. Arch. Pathol. Lab. Med. 136, 1266-1297.

Doorbar J. (2013). The E4 protein; structure, function and patterns of expression. Virology 445, 80-98.

Focchi G.R.A., Bosse T., Saco A., Regauer S., Rakislova N. (2020). Tumours of the vulva: Benign squamous lesions. In: WHO classification of tumours. Female genital tumours. 5 ed. International Agency for Research on Cancer. Lyon (France). Available from: https://tumourclassification.iarc.who.int/chapters/34

Forslund O., Lindelof B., Hradil E., Nordin P., Stenquist B., Kirnbauer R., Slupetzky K. and Dillner J. (2004). High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in "stripped" biopsies from the same tumors. J. Invest. Dermatol. 123, 388-394.

Garland S.M., Steben M., Sings H.L., James M., Lu S., Railkar R., Barr E., Haupt R.M. and Joura E.A. (2009). Natural history of genital warts: Analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. J. Infect. Dis. 199, 805-814.

Henley-Smith R., Santambrogio A., Andoniadou C.L., Odell E. and Thavaraj S. (2021). RNA in situ hybridization for human papillomavirus testing in oropharyngeal squamous cell carcinoma on a routine clinical diagnostic platform. J. Oral. Pathol. Med. 50, 68-75.

Leeman A., Jenkins D., Marra E., van Zummeren M., Pirog E.C., van de Sandt M.M., van Eeden A., Schim van der Loeff M.F., Doorbar J., de Vries H.J.C., van Kemenade F.J., Meijer C. and Quint W.G.V. (2020). Grading immunohistochemical markers p16(ink4a) and HPV E4 identifies productive and transforming lesions caused by low- and high-risk HPV within high-grade anal squamous intraepithelial lesions. Br. J. Dermatol. 182, 1026-1033.

Leonardi C.L., Zhu W.Y., Kinsey W.H. and Penneys N.S. (1991). Seborrheic keratoses from the genital region may contain human papillomavirus DNA. Arch. Dermatol. 127, 1203-1206.

Li J., Ackerman A.B., Jacyk W.K., Leonardi C.L. and Penneys N.S. (1994). 'Seborrheic keratoses' that contain human papillomavirus are condylomata acuminata. Am. J. Dermatopathol. 16, 398-408.

Logani S., Lu D., Quint W.G., Ellenson L.H. and Pirog E.C. (2003). Low-grade vulvar and vaginal intraepithelial neoplasia: Correlation of histologic features with human papillomavirus DNA detection and MIB-1 immunostaining. Mod. Pathol. 16, 735-741.

Molina M.A., Carosi Diatricch L., Castany Quintana M., Melchers W.J.G. and Andralojc K.M. (2020). Cervical cancer risk profiling: Molecular biomarkers predicting the outcome of hr-HPV infection. Expert Rev. Mol. Diagn. 20, 1099-1120.

Nuovo G.J. (2002). Diagnosis of human papillomavirus using in situ hybridization and in situ polymerase chain reaction. Methods Mol. Biol. 179, 113-136.

Pirog E.C., Chen Y.T. and Isacson C. (2000). MIB-1 immunostaining is a beneficial adjunct test for accurate diagnosis of vulvar condyloma acuminatum. Am. J. Surg. Pathol. 24, 1393-1399.

Reutter J.C., Geisinger K.R. and Laudadio J. (2014). Vulvar seborrheic keratosis: Is there a relationship to human papillomavirus?. J. Low. Genit. Tract. Dis. 18, 190-194.

Singh N., Gilks C.B., Wing-Cheuk Wong R., McCluggage W.G. and Herrington C.S. (2018). Interpretation of p16 immunohistochemistry in lower anogenital tract neoplasia. British Association Gynecol. Pathol.

Talia K.L. and McCluggage W.G. (2017). Seborrheic keratosis-like lesions of the cervix and vagina: Report of a new entity possibly related to low-risk human papillomavirus infection. Am. J. Surg. Pathol. 41, 517-524.

Tardío J.C., Bancalari E., Moreno A. and Martín-Fragueiro L.M. (2012). Genital seborrheic keratoses are human papillomavirus-related lesions. A linear array genotyping test study. APMIS 120, 477-483.

von Krogh G., Lacey C.J., Gross G., Barrasso R. and Schneider A. (2000). European course on HPV associated pathology: guidelines for primary care physicians for the diagnosis and management of anogenital warts. Sex Transm. Infect. 76, 162-168.

Zhou Y., Wang L., Pei F., Ji M., Zhang F., Sun Y., Zhao Q., Hong Y., Wang X., Tian J. and Wang Y. (2019). Patients with LR-HPV infection have a distinct vaginal microbiota in comparison with healthy controls. Front. Cell Infect. Microbiol. 9, 294.

Accepted June 25, 2021