VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2020, vol. 91, no. 4, 181–184

ISSN 0017–0011

DOI: 10.5603/GP.a2020.0035

Presence of human papilloma virus in Caucasian women living in the central Europe diagnosed with vulvar intraepithelial neoplasia

Marta Robenek, Anita Olejek, Lukasz Witek, Aleksandra Augusciak-Duma, Bogna Drozdzowska, Iwona Gabriel

Medical University of Silesia, Katowice, Poland

ABSTRACT

Objectives: The role of human papilloma virus (HPV) in the development of cancerous states of female reproductive tract has been widely debated. However, the information about presence of HPV in the Caucasian women living in the central Europe diagnosed with vulvar intraepithelial neoplasia (VIN) is missing. So far, no recommendation was made to complete HPV detection in time of vulvar biopsy or after the results of positive VIN are obtained. We aimed to assess the presence of HPV in women with vulvar intraepithelial neoplasia diagnosed at the Department of Gynecology, Obstetrics and Oncological Gynecology in Bytom, Poland.

Material and methods: The retrospective examination of 120 consecutive vulvar biopsies obtained from women with persistent vulvar itching was done. Only patients with diagnosis of VIN were included in the further analysis. HPV DNA was detected using HPV Linear Array Genotyping Test including 14 HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

Results: Out of 120 vulvar samples retrieved, 18 women were positive for VIN, including15 usual VIN (uVIN) and three differentiated type (dVIN). 10 samples were eligible for DNA detection. HPV DNA was found in two women with uVIN (HPV 16 and 51).

Conclusions: It is advisable to recommend HPV genotyping in women with VIN, regardless of their age and histologic type. The incidence of HPV infection in Caucasian women from the central Europe with VIN should be further studied.

Key words: vulvar intraepithelial neoplasia; human papilloma virus; vulvar itching

Ginekologia Polska 2020; 91, 4: 181–184

INTRODUCTION

Human Papilloma Virus (HPV) infection has been identified as an important factor for subsequent carcinogenesis in women of all ages [1, 2]. In recent decades, the incidence of vulvar intraepithelial neoplasia (VIN) increased with concurrent decrease in the age of women suffering from this disease [3]. It is a premalignant vulvar skin disorder that often causes severe and long-lasting pruritus, pain and psychosexual dysfunction. HPV– associated VIN is the most common in women from 30 to 40 years old [4]. Previous data from Poland show 15% prevalence of HPV in vulvar cancer tissue with a predominant type 16 (71%), which was recently underlined in report by Nowakowski et al. [5, 6]. In the Estonian study, high-risk HPV (16, 18, 31, 33, 45, 52, 58) was present in 90% of vulvar cases [7]. In Czech, as many as 37% of randomly enrolled women were detected positive for HPV during the observational vaccination study [8]. Recent Austrian study showed 23% of vulvar cancers had been HPV positive, with more than 90% of them being a single-type infection [9].

Objectives

There is a gap in reporting of HPV detection among Caucasian women from central Europe with vulvar intraepithelial neoplasia. Currently, it is not a standard procedure to evaluate HPV status in time of vulvar biopsy. Thus, we retrospectively evaluated vulvar samples obtained during diagnosis of persistent vulvar itching, with special focus on VIN patients.

Corresponding author:
Iwona Gabriel
Medical University of Silesia, Katowice, Poland
e-mail: ginsiar@interia.pl

MATERIAL AND METHODS

Material

The study included 120 subsequent vulvar biopsies obtained from women undergoing vulvar sampling due to persistent vulvar itching (more than six months). The Ethics Committee of Medical University of Silesia granted the permission for this study. Only patients with positive diagnosis of VIN were included in further HPV genotyping.

Two paraffin blocks were used for further processing and one was used as back-up sample. At least, two paraffin sections were systemically obtained for each paraffin block.

Methods

Pathologic diagnosis

Pathology review was done by an experienced pathologist (B.D.) and confirmed by immunohistochemistry.

Sections (4 µm) from formalin-fixed, paraffin-embedded tissues were cut and placed on salinized slides. Following deparaffinization in xylene, slides were rehydrated in graded alcohol and washed in deionized water. Antigen retrieval was performed by cooking slides for 20 minutes at 95°C in Heat-Induced Epitope Retrieval Buffer (Thermo Scientific, Fremont, USA): for p16 — pH6, for p53 — pH9. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase. To block the possibility of non-specific binding of antibodies to other proteins, sections were incubated with Protein Block (Thermo Scientific) for 5 minutes. Then, slides were incubated in humidified chamber with primary antibodies: p16 INK4A (MX007 clone, dilution 1:200, Immunologic, Netherlands) for 40 minutes at room temperature, p53 (D07 clone, dilution 1:300, Cell Marque, USA) for 30 minutes at room temperature. After washing with Tris-buffered saline 3 times for 3 minutes, the sections were treated with Primary Antibody Amplifier Quanto and then HRP Polymer Quanto (Thermo Scientific). For the visualization was used 3-3´-diaminobenzidine DAB Quanto (Thermo Scientific). Finally, tissue sections were counterstained with hematoxylin, dehydrated and covered with coverslips.

Based on extent of immunolabeling, p16 (nuclear and cytoplasmic staining) and p53 (nuclear staining) immunoexpressions were recorded as positive (+), focal positive or negative (–).

DNA extraction

DNA from FFPE samples was isolated according to the protocol of the supplier of AllPrep DNA/RNA FFPE Kit (Qiagen, Germany). Briefly, three sections of the paraffin sample block were deparaffinized with xylene, and then lysed with PKD buffer and proteinase K for 30 min. After isolation of total RNA, DNA was extracted with additional lysis step with ATL Buffer and proteinase K. Elution of DNA was done with supplier buffer ATE. DNA concentrations were determined using spectrophotometer (Nanodrop2000, ThermoScientific, USA). The amount and quality of the obtained DNA was poor.

Subsequently, for removal of all organic solvents, DNA was purified with Agencourt AMPure XP solution (Beckman Coulter, USA).

HPV genotyping

Multiple, simultaneous, detection of 37 HPV genotypes on archival tissues was conducted with the use of Linear Array HPV genotyping test (Roche Diagnostics, Germany). For diagnostic purposes test is validated only on specimens collected in cobas® PCR Cell Collection Media or PreservCyt Solution. The sensitivity of this test was also confirmed previously on frozen tissues, when compared with conventional PCR/sequencing [10]. Here, we attempt to use the test on archival FFPE samples. From 50 ng to 500 ng of DNA were tested for high- and low-risk HPV DNA according to the manufacturer's protocol with exemption of specimen preparation, which was done as already described above. The rest of the procedure was conducted exactly as recommended by the manufacturer of Linear Array HPV Genotyping and Detection kit. Briefly, 50 µL of DNA was mixed with 50 µL od Master Mix comprising, inter alia, AmpliTaq Gold DNA Polymerase and pool of biotinylated primers to polymorphic L1 region of the HPV genome that are designed to amplify 13 high risk and 24 low risk HPV genotypes. The amplification reaction was set on 96-Well GeneAmp PCR System 9700 (Applied Biosystems, USA). After amplification denaturing solution (DN) was added to PCR products. Denatured amplicons were hybridized on to the strips containing specific probes for HPV genotypes and β-globin reference genes and detected with the colorimetric reaction with Streptavidin-horseradish peroxidase. Positive reactions were visualized as blue bands on the strip.

RESULTS

Pathology review revealed 18 (18/120; 15%) patients who were diagnosed with vulvar intraepithelial neoplasia. After DNA extraction from embedded tissue in paraffin, only 10 samples (10/18; 55%) were eligible for further HPV genotyping. Table 1 presents procedures that were undertaken in cases of primarily failed DNA detection for 8 patients.

Table 2 presents detailed analysis of each patient status including their age, p16 and p53 staining, HPV presence. Out of ten samples, two (20%) were positive for high-risk HPV: 16 and 51. Figure 1 presents example of positive genotyping for HPV 51.

DISCUSSION

Results of this study indicate the need of assessment of HPV status at time of VIN diagnosis. The reported incidence of HPV in our group is slightly higher than in the previous Polish report (20% vs 15%) [5], but remains comparable to those presented by Austrian research (20% vs 23%) [9]. It is

Table 1. Procedural modifications in patients with primary failed DNA detection

Patient	Identification	Remarks for procedure protocol	DNA isolation ng/uL	280/260	260/230	NO RESULT
1	1A	According to protocol	432.7	1.92	2.24	No result
	1B	First lysis — 30 min	316.9	1.79	2.31	No result
2	2A	According to protocol	–7.9	–23.59	1.77	No result
	2B	First lysis — 30 min Second lysis — 140 uL proteinase K	1.7	2.66	–0.38	No result
3	3A	According to protocol	40.5	1.44	2.06	No result
4	4A	According to protocol; after deparaffinization — gel	–2	–14	0.38	No result
	4B	First lysis — 30 min	–15.2	–9.8	3.75	No result
5	5A	According to protocol	16.7	1.58	4.42	No result
	5B	According to protocol	8.8	2.75	–1.04	No result
6	6A	According to protocol	14.5	1.52	1.7	No result
	6B	First lysis — 30 min	–14.4	–12.36	1.47	No result
7	7A	According to protocol	–0.6	–0.066	0.78	No result
	7B	First lysis — 30 min	–4.7	–0.66	2.8	
8	8A	First lysis — 60 min	1	2.02	–0.022	No result
	8B	First lysis — 30 min Second lysis —140 uL proteinase K	1.7	3.07	–0.57	

Table 2. Detailed analysis of retrieved samples (VIN status, p53 status, p16 status)

Patient n^0	VIN	VIN type	Age	p16	p53	HPV
1	VIN1	Usual	75	focal +	–	
2	VIN1	Usual	58	focal +	–	
3	VIN1	Usual	54	+	–	
4	VIN3	Usual	69	+	–	
5	VIN1	Usual	59	–	–	HPV 16
6	VIN1	Usual	51	+	–	
7	VIN1	Usual	59	focal +	–	
8	VIN3	Usual	53	+	–	
9	VIN2	Usual	37	–	focal +	HPV 51
10	VIN1	Usual	66	+	–	

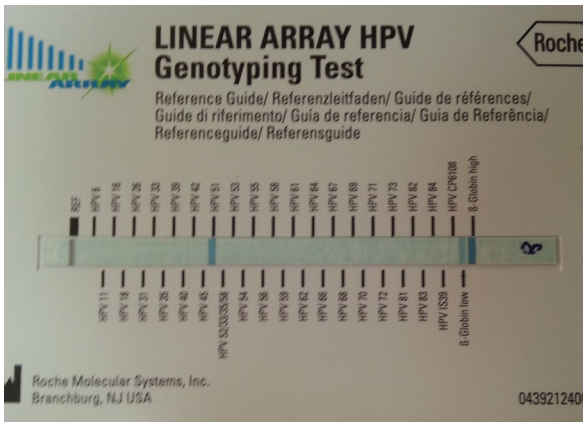

Figure 1. HPV 51 positive detection on HPV Linear Assay

below another world-wide report, which showed that 86% of VIN2/3 cases had been HPV positive or 87% in all VIN [11, 12]. It is noteworthy, that 7 of 10 patients in our group had VIN1 according to the previous classification, so this may explain existing differences.

What is very important is we avoided previous limitation to use separate biopsies for pathology and HPV analysis and we simultaneously controlled for p16 and p53 staining [3]. The increase in p16 protein production is linked to elevated transcription mediated by high-risk HPV-encoded oncoprotein E7 which inactivates Rb protein, releasing p16 from negative feedback control [13]. Yet, in our analysis both women with positive high-risk HPV had negative staining for p16.

Another strength of this study is that we did not limit our inclusion criteria to one histological type of VIN [14].

Major limitation is the use of embedded vulvar tissue requiring multiple steps to obtain sample available for HPV genotyping. This may lead to unexposed HPV and underestimation of results. Similar methodological approach using PapilloCheck microarray was previously presented and it has shown prevalence of HPV as high as 90% [15]. Withal, the main purpose of the study was to appraise the presence of HPV among women undergoing vulvar sampling.

CONCLUSIONS

Finally, it is important to gain more knowledge on vulvar intraepithelial neoplasia as the incidence of VIN has

increased in recent decades. uVIN almost doubled from 1.2/100 000 patients in 1992 to 2.1/100 000 in 2005 and dVIN increased nine-fold from 0.013/100 000 patients to 0.121/100 000 [16]. Life-time risk of HPV infection is around 80% [17]. In light of recent findings, in which 9-valent HPV vaccine comparing to historic placebo proved to diminish by 94% incidence of vulvar or vaginal cancers, it seems necessary to further study the presence of HPV in women with vulvar intraepithelial neoplasia [18].

Compliance with ethical standards

This study was funded by the University Grant n^0 KNW-1--168-N/4/0.

Conflict of interest

All authors state no conflict of interest.

REFERENCES

1. de Freitas AC, Coimbra EC, Leitão Md. Molecular targets of HPV oncoproteins: potential biomarkers for cervical carcinogenesis. Biochim Biophys Acta. 2014; 1845(2): 91–103, doi: 10.1016/j.bbcan.2013.12.004, indexed in Pubmed: 24388872.
2. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol. 2014; 26: 13–21, doi: 10.1016/j.semcancer.2013.11.002, indexed in Pubmed: 24316445.
3. Bryant D, Rai N, Rowlands G, et al. Human papillomavirus type distribution in vulval intraepithelial neoplasia determined using PapilloCheck DNA Microarray. J Med Virol. 2011; 83(8): 1358–1361, doi: 10.1002/jmv.22107, indexed in Pubmed: 21618551.
4. Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. Int J Gynecol Pathol. 2001; 20(1): 16–30, doi: 10.1097/00004347-200101000-00003, indexed in Pubmed: 11192069.
5. Kowalewska M, Szkoda MT, Radziszewski J, et al. The frequency of human papillomavirus infection in polish patients with vulvar squamous cell carcinoma. Int J Gynecol Cancer. 2010; 20(3): 434–437, doi: 10.1111/IGC.0b013e3181d320f1, indexed in Pubmed: 20375810.
6. Nowakowski A, Arbyn M, Turkot MH, et al. A roadmap for a comprehensive control of cervical cancer in Poland: integration of available solutions into current practice in primary and secondary prevention. Eur J Cancer Prev. 2020; 29(2): 157–164, doi: 10.1097/CEJ.0000000000000528, indexed in Pubmed: 31517672.
7. Võrno T, Lutsar K, Uusküla A, et al. Cost-effectiveness of HPV vaccination in the context of high cervical cancer incidence and low screening coverage. Vaccine. 2017; 35(46): 6329–6335, doi: 10.1016/j.vaccine.2017.08.083, indexed in Pubmed: 28899625.
8. Hamsikova E, Smahelova J, Ludvikova V, et al. The prevalence of HPV infections in HPV-vaccinated women from the general population. APMIS. 2017; 125(6): 585–595, doi: 10.1111/apm.12677, indexed in Pubmed: 28295672.
9. Pils S, Gensthaler L, Alemany L, et al. HPV prevalence in vulvar cancer in Austria. Wien Klin Wochenschr. 2017; 129(21-22): 805–809, doi: 10.1007/s00508-017-1255-2, indexed in Pubmed: 28884278.
10. Woo YL, Damay I, Stanley M, et al. The use of HPV Linear Array Assay for multiple HPV typing on archival frozen tissue and DNA specimens. J Virol Methods. 2007; 142: 226–230.
11. Serrano B, de Sa, Tous S, et al. Human papillomavirus genotype attribution for HPVs 6, 11, 16, 18, 31, 33, 45, 52, 58. Eur J Cancer. 2015; 51: 1732–1741.
12. Wakeham K, Kavanagh K, Cuschieri K, et al. HPV status and favourable outcome in vulvar squamous cancer. Int J Cancer. 2017; 140(5): 1134–1146, doi: 10.1002/ijc.30523, indexed in Pubmed: 27864932.
13. Riethdorf S, Neffen EF, Cviko A, et al. p16INK4A expression as biomarker for HPV 16-related vulvar neoplasias. Hum Pathol. 2004; 35(12): 1477–1483, doi: 10.1016/j.humpath.2004.09.004, indexed in Pubmed: 15619206.
14. Srodon M, Stoler MH, Baber GB, et al. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). Am J Surg Pathol. 2006; 30(12): 1513–1518, doi: 10.1097/01.pas.0000213291.96401.48, indexed in Pubmed: 17122506.
15. Mazellier S, Dadone-Montaudie B, Chevallier A, et al. Papillomavirus genotyping on formaldehyde fixed paraffin-embedded tissue in vulva intraepithelial neoplasia. Arch Gynecol Obstet. 2017; 296: 811–817.
16. van de Nieuwenhof HP, Massuger LF, van der Avoort IAM, et al. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur J Cancer. 2009; 45(5): 851–856, doi: 10.1016/j.ejca.2008.11.037, indexed in Pubmed: 19117749.
17. Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis. 2005; 191(2): 182–192, doi: 10.1086/426867, indexed in Pubmed: 15609227.
18. Giuliano AR, Joura EA, Garland SM, et al. Nine-valent HPV vaccine efficacy against related diseases and definitive therapy: comparison with historic placebo population. Gynecol Oncol. 2019; 154(1): 110–117, doi: 10.1016/j.ygyno.2019.03.253, indexed in Pubmed: 30982556.