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Natural Antibodies to Human Papillomavirus 16 and Recurrence of Vulvar High-Grade Intraepithelial Neoplasia (VIN3)

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Objectives: Approximately 30% of women treated for squamous high-grade intraepithelial neoplasia (VIN3), often associated with human papillomavirus (HPV), have recurrent disease. In this study, we assess predictors of recurrence that may provide targets for early prevention or treatment.

Materials and Methods: Women with VIN3 who participated in a previous population-based case-control study with blood and tumor samples completed a follow-up telephone interview an average of 5 years after initial diagnosis. The risk of recurrence was determined by proportional hazards modeling.

Results: Women with VIN3 in the follow-up study ($n = 65$) were similar to women with VIN3 in the parent study ($n = 215$) with regard to age at primary diagnosis, level of current cigarette smoking (>60%), and lifetime number of partners. We found that 22 (33.8%) of 65 participants had a vulvar recurrence and that 73.4% recurred within 3 years of treatment. Recurrences occurred more often among women with common warts in the decade before diagnosis (hazard ratio [HR] = 2.5, 95% CI = 1.1–5.8) and among those with a previous anogenital cancer (HR = 2.7, 95% CI = 1.2–6.3). Interestingly, recurrence was less frequent among women who mounted a natural antibody response to HPV16 (HR = 0.4, 95% CI = 0.2–0.9).

Conclusions: These data provide strong preliminary evidence that VIN3 recurrence was less frequent among those with HPV16 antibodies. Vaccination with the currently licensed HPV vaccine as part of adjunctive therapy for VIN3 would increase antibody response and may decrease risk of recurrence. Randomized controlled trials are needed to determine whether HPV vaccination is effective against VIN3 recurrence.

Key Words: high-grade vulvar intraepithelial neoplasia (VIN3), HPV antibodies, vulvar recurrence

(*J Low Genit Tract Dis* 2016;20: 257–260)

High-grade squamous intraepithelial lesions of the vulva, specifically vulva intraepithelial grade 3 or in situ vulvar carcinoma (high-grade vulvar intraepithelial neoplasia [VIN3]) have the potential to progress to invasive vulvar cancer and therefore require histologic confirmation and treatment.[1] Incidence of VIN3 peaks at the ages of 40 to 49 years,[2] has a primarily warty/basaloid histology,[3] and is associated with human papillomavirus (HPV) infection. More than 90% of 67 VIN3 in a recent US study were associated with HPV,[4] consistent with HPV prevalence rate of 86.7% in a larger, worldwide study of 587 VIN3.[3] Another important risk factor for VIN3 is current cigarette smoking. Our previous Seattle area population-based case-control study reported that 63% of women with VIN3 were current smokers at the time of diagnosis compared with 25.4% current smokers in the general population (odds ratio [OR] = 6.4, 95% CI = 4.4–9.3).[5]

Surveillance, Epidemiology, and End Results registry data show that VIN3 is a rare disease. It has been increasing gradually in incidence in recent years, from 5.3 cases per 100,000 women in 1992–1998 to 5.7 in 2006–2012, with an annual percent change of 0.5% (95% CI = 0.2–0.9).[6] The HPV prophylactic vaccines, first approved for use in the United States in 2006,[7] are anticipated to become routinely administered to adolescents and young adults and will eventually impact the rate of VIN3.[8] Until then, given poor uptake of the vaccine, incidence of HPV-related lesions will likely continue to increase for several generations of women.[9]

High-grade vulvar intraepithelial neoplasia can be difficult to treat and recurs in approximately 30% of patients after surgery.[10–12] In contrast, studies of recurrence after treatment for HPV-related cervical lesions report a lower proportion of cervical intraepithelial neoplasia 3 recurrence, at 5% to 10%.[13,14] Progression is estimated to be 6.5% in a meta-analysis, which included 3.5% occult invasive disease.[12] Because of the high prevalence rate of recurrence and the potential for progression, surveillance after treatment for VIN3 is recommended every 6 months posttreatment for the first 5 years and then annually. Surgeries for VIN3, including repeat surgeries for recurrences, can be disfiguring and related to functional deficits and psychosexual trauma.[15,16]

We conducted a follow-up study to assess predictors of VIN3 recurrence including type of initial surgery, markers of HPV infection, medical history, and history of cigarette smoking. Our goal in this study was to identify factors associated with risk of VIN3 recurrence that may lead to improvement in surveillance or treatment.

METHODS

Study Participants

We randomly selected 90 women with VIN3 of 270 who participated in our previous population-based case-control study of vulvar cancer and VIN3,[5] which included an extensive in-person interview, tissue retrieval, and blood draw. A total of 65 women (72.2% of 90 women we recontacted) agreed to a follow-up telephone interview for this study, called the Violet Study. Participants had originally been diagnosed with VIN3 between 1991 and 1996 in the Seattle area (King, Pierce, and Snohomish Counties). Women diagnosed with VIN2 or high-grade squamous intraepithelial lesion without mention of VIN3 or carcinoma in situ on the pathology report were not included in this study. Informed consent was obtained from all study participants before the study began, and the study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Data Collection

Questions on a follow-up telephone survey focused on history of anogenital treatments and biopsies, cigarette smoking

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The authors have declared they have no conflicts of interest.

This study was supported by Public Health Sciences Division, Fred Hutchinson Cancer Research Center and NIH/NCI Grant Number P01 CA42792.

This study was approved by the Fred Hutch IRB (#4846).

history, and general health since the first interview. Medical records were retrieved and abstracted, including pathology and surgery reports for women who indicated that they had an anogenital biopsy after their primary VIN3 diagnosis. Information on initial treatment that led to the primary diagnosis and subsequent biopsies and surgeries was also collected.

Recurrence

Recurrence was defined as a histologically confirmed vulvar lesion of VIN2 or worse occurring 3 months or more after diagnosis of the primary VIN3, among women who self-reported a subsequent surgery. Medical records of biopsies and related treatment were available for 22 of 23 women (1 woman refused medical records release). Recurrence of VIN2/3 or worse occurring 3 months or more after the primary resection was confirmed for 18 (81.8%) of 22 women; 1 woman had a vulvar LSIL recurrence, and 3 women had anal or cervical surgeries after their initial VIN3 diagnosis. We included all 23 women who self-reported a recurrence more than 3 months after primary resection for VIN3 as having a recurrence for this study.

Assays

Serum was drawn at the time of the parent interview, after the initial diagnosis and treatment for VIN3. Enzyme-linked immunosorbent assay results for HPV16L1 virus-like particles were available for 62 (95.4%) of the 65 participants. The original VIN3 tumor tissue was available for 54 (83.1%) of 65 participants, and HPV DNA type was assessed by polymerase chain reaction using MY09/11 primers, followed by restriction-fragment length polymorphism on those with tumor tissue available. All HPV DNA genotyping was performed on the initial VIN3 tumor tissue. The methods for DNA typing and serology are described in more detail in a previous study.[17]

Human leukocyte antigen genotyping was available for a subset (49/65, 75.4%) of the Violet participants. We selected 3 human leukocyte antigen types associated with cervical cancer, a priori, as potentially associated with recurrence.[18] Specifically, we assessed risk of recurrence associated with carriage of DQB1*0301, DRB1*13-DQB1*06, or DRB1*1501-DQB1*0602 alleles.

Statistical Analysis

For each woman, we computed the risk of recurrence during the interval between the month and year of initial VIN3 diagnosis as well as month and year of last follow-up or self-reported recurrence, whichever occurred first. Our analysis focused on the relationship between potential risk factors for recurrence and was adjusted for other characteristics to calculate adjusted hazard ratios (HRs) estimates and associated 95% CI using Cox proportional hazards. We performed a subanalysis restricted to women with HPV16 DNA in their tumors, to assess the specificity of the enzyme-linked immunosorbent assay for presence or absence of HPV16 antibodies in women with HPV16-containing tumors. Smoking after initial VIN3 diagnosis was evaluated as a time-dependent variable.

RESULTS

In Table 1, the 65 women in the Violet follow-up study were compared with other women with VIN3 in the parent case-control study not chosen for the Violet study ($n = 215$). Mean age at reference was 46.8 in the parent study and 46.0 in the Violet study. The Violet study was also similar to the parent study with respect to current smoking status and total number of partners.

Recurrences of the initial vulvar lesion were reported by 23 (35.4%) of 65 women during the telephone interview. A medical record review including pathology reports and description of treatment was performed and confirmed recurrence among 18 of 22 women with records available for review, for a total of 29.2% recurrence with histologic confirmation of a VIN3 recurrence. Given the high proportion of confirmed reports (82.6%), the analyses in Tables 2 and 3 included all 23 self-reported recurrences.

Mean follow-up time for the 65 participants in the Violet study was 61.2 months (see Table 2). Recurrence occurred within 3 years of initial resection for 17 (26.2%) of 65 women and within 5 years for 21 (32.3%) of 65 women. Recurrence frequency was not observed to be different by type of initial treatment. Among those tested with tissue available for testing, HPV16 was detected in the initial lesion in approximately 72% of those without recurrence and only 54.5% of those with recurrence, but this difference could be attributed to chance ($p = .296$). One woman had a recurrence at 4 years and progressed to invasive cancer 1.5 years after her recurrence (5.5 years from initial treatment).

In Table 3, we present risk of recurrence associated with smoking and markers of HPV status. We noted that more women (41.5%) had a recurrence among those who were current smokers at the time of initial diagnosis compared with those who were former (20%) or never (28.6%) smokers, although this difference was not significantly associated with risk for recurrence (HR = 1.2, 95% CI = 0.4–3.6). There was also a higher risk of recurrence among women who continued to smoke after their initial diagnosis, (HR = 2.1, 95% CI = 0.8–5.4), although this estimate was not statistically significant.

Recurrence occurred more frequently among those with a history of HPV-related lesions, including common (nongenital) warts in the decade before initial diagnosis, (HR = 2.5, 95% CI = 1.1–5.8), and a history of anogenital cancer at sites other than the vulva (HR = 2.7, 95% CI = 1.2–6.3). Interestingly, we found that recurrence was less frequent among women who had a detectable HPV16 antibody response (22.9%) compared with women who were HPV16 antibody negative (52.0%), suggesting a reduced risk among those with immune response to HPV16 (HR = 0.4, 95% = CI 0.2–0.9, adjusted for age).

Further adjustment by smoking as a time-dependent covariate did not change the estimate of reduced risk among those with HPV16 antibody positivity. A similar result (HR = 0.4, 95% CI = 0.1–1.0) was observed when the outcome was limited to the 18 women with histologic confirmation of recurrence. In addition, the reduced hazard of recurrence among those with HPV16

TABLE 1. Characteristics of Violet Study Participants With VIN3 Compared With VIN3 Cases in the Parent Case-Control Study

	Parent study ($n = 215$)	Violet ($n = 65$)	
	n (%)	*n* (%)	*p*
Age at diagnosis			.955
18–39	73 (34.0)	21 (32.3)	
40–59	94 (43.7)	33 (50.8)	
60–79	48 (22.3)	11 (16.9)	
Mean age	46.8	46.0	
Cigarette smoking			.396
Never	40 (18.6)	14 (21.5)	
Former	54 (25.1)	10 (15.4)	
Current	121 (56.3)	41 (63.1)	
Total sexual partners			.155
1	32 (14.9)	3 (4.6)	
2–4	54 (25.1)	30 (46.2)	
5+	129 (60.0)	32 (49.2)	

TABLE 2. Violet Study Follow-Up Time and Type of Surgery for Primary Lesion

	No recurrence (n = 42)	Recurrence (n = 23)
	n (%)	n (%)
Follow-up time		
Mean, mo	61.2	24.0
<3 y	4 (9.5)	17 (73.9)
3–<5 y	16 (38.1)	4 (17.4)
5+ y	22 (52.4)	2 (8.7)
Surgery[a]		
No surgery	1 (2.4)	0 (0.0)
Cryosurgery	4 (9.5)	3 (13.0)
Biopsy/excision	25 (59.5)	13 (56.5)
Vulvectomy	12 (28.6)	7 (30.4)
HPV DNA		
Negative	2 (6.3)	4 (18.2)
16 positive	23 (71.9)	12 (54.5)
18/33/45 positive	7 (21.9)	6 (27.3)
Not tested	10	1

HPV, human papillomavirus.

[a]Refers to primary treatment for VIN3; individuals are counted only once as having the most extensive procedure.

antibodies was more pronounced when the analysis was restricted to those who had HPV16 DNA positive tumors (HR = 0.2, 95% CI = 0.1–0.9).

Carriage of human leukocyte antigen haplotypes DRB1*13-DQB1*06 or DRB1*1501-DQB1*0602 was not associated with recurrence. However, we did detect an increased risk of recurrence among women with DQB1*0301 compared with recurrence among women without DQB1*0301 (HR = 3.2, 95% CI = 1.3–8.1).

DISCUSSION

In this follow-up study of 65 women with VIN3, 23 women self-reported a recurrence and 18 were histologically confirmed as a repeat VIN3 through medical chart review. Importantly, recurrence was less frequent among those seropositive for HPV16 (22.9%) compared with seronegative for HPV16 (52%), leading to a reduced risk of recurrence (HR = 0.4, 95% CI = 0.2–0.9). When restricted to women with HPV16 DNA–positive tumors, the impact of HPV16 antibodies was more pronounced (HR = 0.2, 95% CI = 0.1–0.9), suggesting specificity of the association. Furthermore, recurrence was more common among women with previous evidence of susceptibility to various types of persistent HPV infection, from common cutaneous warts in the decade before VIN3 diagnosis through history of other types of anogenital cancers. Recurrence was also more frequent among those with DQB1*0301, which was previously associated with increased risk of cervical cancer,[18] and may be a surrogate for lack of recognition of HPV antigens by the acquired immune response. Taken together, these observations suggest that a poor host immune response to HPV is associated with increased risk of VIN3 recurrence.

Most recurrences, 73.9%, were observed within 3 years of primary resection. We found no evidence that recurrence differed by type of treatment or HPV type in the initial tumor, although treatment information in our study was limited to self-report. A larger study of 303 women with VIN2/3 and 28.7% recurrence found decreased recurrence-free survival with excision plus laser compared with excision alone.[19]

Current smoking was strongly associated with risk of VIN3 in the parent study and another study of vulvar precancer and cancer.[5,20] We investigated whether recurrence was more frequent among women who smoked after VIN3 diagnosis and found that those who continued to smoke had an elevated but not significant risk of recurrence compared with those who did not smoke (HR = 2.1, 95% CI = 0.8–5.4). Cigarette smoking may contribute to a poor immune response by lowered immune response markers[21] or limiting antibody response.[22]

Our previous study and others have observed an increased risk of VIN3 and vulvar cancer associated with antibodies to HPV16.[5,20] In contrast, this study suggests that recurrence is less frequent among women who have antibodies to HPV16. Similar to the current study, high levels of HPV16 antibodies at study start were associated with a reduced risk of subsequent HPV16 DNA detection among women in the control arm of an HPV vaccine trial.[23] It may be that higher level of antibodies is associated with decreased risk of disease in our study as well, but we were not able to estimate titers from our serologic assay.

The small sample size of our study may have limited our power to detect differences in risk of recurrence within some groups. However, the sample is a representative of the women in the larger population-based study of VIN3 from which participants in this study were randomly chosen (see Table 1). Another limitation is that this study lacked medical chart reviews for those individuals who did not report a biopsy or excision during follow-up. However, it is unlikely that women would not report a vulvar surgery. Surgery information was self-reported in Table 2, but there was a good agreement between self-reported surgery and medical record information on surgery among those with recurrences.

TABLE 3. Risk of Recurrence (HR) Associated With Smoking and HPV16 Antibodies

	No recurrence (n = 42)	Recurrence (n = 23)		
	n (%)	n (%)	HR	95% CI
Smoking at initial diagnosis				
Never	10 (71.4)	4 (28.6)	1.0	Ref
Former	8 (80.0)	2 (20.0)	0.6	0.1–3.2
Current	24 (58.5)	17 (41.5)	1.2	0.4–3.6
Smoking after initial diagnosis[a]				
No	20 (76.9)	6 (23.1)	1.0	Ref
Yes	22 (56.4)	17 (43.6)	2.1	0.8–5.4
Ever have genital warts				
No	25 (67.6)	12 (32.4)	1.0	Ref
Yes	17 (60.7)	11 (39.3)	1.1	0.5–2.7
Common warts in previous decade				
No	31 (75.6)	10 (24.4)	1.0	Ref
Yes	11 (45.8)	13 (54.2)	2.5	1.1–5.8
Previous anogenital cancer				
No	36 (72.0)	14 (28.0)	1.0	Ref
Yes	6 (40.0)	9 (60.0)	**2.7**	1.2–6.3
HPV16 antibody				
Negative	12 (48.0)	13 (52.0)	1.0	Ref
Positive	27 (77.1)	8 (22.9)	0.4	0.2–0.9

All HR are adjusted for age.

HPV, human papillomavirus 16; Ref, reference category.

[a]Smoking after initial diagnosis was assessed as a time-dependent variable.

In conclusion, this study is the first to report fewer VIN3 recurrences among those who mounted a detectable antibody response to HPV16. The licensed HPV vaccines recommended for prophylaxis work by provoking high levels of HPV antibodies in HPV naive individuals. Data from 2 observational studies found lower recurrence among vaccinated versus unvaccinated individuals treated for high-grade cervical and anal lesions.[24,25] These studies and the results from the current study suggest that a clinical trial to assess the impact of the licensed vaccine as an adjunct to treatment for VIN3 is warranted. A proof of principal trial is needed, and individuals with VIN3 would be good candidates for such a trial for several reasons, including the following: their high recurrence proportion, short time to recurrence, and reduced risk associated with HPV antibody as confirmed in this study.

ACKNOWLEDGMENTS

The authors thank the women with VIN3 who participated in this follow-up study.

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