HPV prevalence and Detection Sensitivity in Vaginal Intraepithelial Neoplasia: a hospital-based study

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Abstract

Vaginal intraepithelial neoplasia (VaIN) always diagnosed through coloscopy accidentally when the cervical cancer screening was abnormal. A precise estimate of the detection rate of cervical cancer screening for VaIN is limited. This study to investigated the characteristics and screening history of VaIN, and compared the sensitivity of cytology and human papillomavirus tests on the cervix against vaginal and cervical high-grade squamous intraepithelial lesion (HSIL) or cancer. A total of 1200 patients with definitive histopathologic diagnoses of VaIN were included in this study. Among them, 22.5% were diagnosed with VaIN2+, and 50.4% were concomitant with cervical lesions. The median age of VaIN2+ patients was 41.5 years old , while VaIN1 reported a median age of 53 years old , p¡0.001. This study reported that VaIN was significantly and positively correlated with cervical lesions (r=0.387). The high-risk human papillomavirus (hr-HPV) detection rate was 88.5% (883/998) in VaIN and 95.4% in VaIN2+. HPV 16 was the most prevalent HPV type in VaIN2+, which accounted for 54.5%, followed by HPV58 (17.0%), HPV52 (14.8%), HPV51 (11.4%), and HPV18 (10.2%). The sensitivity of hr-HPV and cytology tests on the cervix for detecting VaIN2+ was 95.0% and 84.9%, respectively. Both tests were not significantly different from detecting CIN2+. When the cervical cancer screening is abnormal and referring to colposcopy, acetic acid and Lugol's iodine need to cover the whole vaginal mucosa as well as the fornix, attention need to be paid for the abnormal images of vagina in order to find VaIN.

1. Introduction

Vaginal intraepithelial neoplasia (VaIN) is an uncommon gynecologic disease that accounts for only 1% of all lower genital tract intraepithelial neoplasia.[1]Human papillomavirus (HPV) infection is implicated as a causative agent of VaIN.[2] As with cervical lesions, persistent high-risk HPV (hr-HPV) infection can induce the progression of VaIN to vaginal cancer. Other risk factors include first sexual intercourse at age younger than 17, having more than 5 sexual partners, immunosuppression, smoking, pelvic radiation therapy, exposure to diethylstilbestrol in utero, and history of cervical precancer or cancer.[3,4]

Epidemiologic information about VaIN is limited, with only one population-based study in the United States in 1977 estimating that VaIN may occur in 0.2- 0.3 per 100,000 women.[5] VaIN is often discovered during colposcopy when cervical cancer screening is abnormal. A missed diagnosis of VaIN may occur if colposcope practitioners solely concentrate on examining the cervix and neglect to observe the vagina for potential vaginal lesions. There are currently no screening programs for VaIN except for women who have had a hysterectomy for cervical precancerous lesions or cervical cancer. Thus, the true incidence rates of VaIN may be higher than reported. According to the 2014 WHO classification of tumors of the female reproductive organs (4^{th} Edition)[6],VaIN was classified into the low-grade squamous intraepithelial lesion (LSIL), which refers to VaIN1, and high-grade squamous intraepithelial lesion (HSIL), which includes VaIN2 and VaIN3. Compared with cervical intraepithelial neoplasia (CIN), the possibility of VaIN progression to invasive vaginal cancer is much lower, and most VaIN will regress. However, the risk of progression to invasion vaginal cancer

remains, especially for HSIL.[7-9] Hence, it is crucial to discover vaginal HSIL and treat it promptly prevent vaginal cancer.

Currently, there is no FDA-approved hr-HPV test available for use in vaginal cancer screening. In general population screening, excluding women post-hysterectomy with vaginal stump, both cytological tests and HPV test sample above the surface of cervix. Assessing the sensitivity of cervical cancer screening against VaIN can be helpful in developing an effective screening protocol and managing screen-detected abnormalities. Additionally, in the post-HPV-vaccine era, knowing the prevalence and distribution of HPV genotypes can aid in selecting the most suitable vaccine among the available options to protect against VaIN and vaginal cancer. Due to its rarity, the estimate of accuracy of cervical cancer screening to detect VaIN2 is limited by the availability of few studies.

The present study aims to analyze the clinical data of 1200 VaIN patients at the largest cervical disease center in Fujian province, China. The characteristics and screening history of VaIN were revealed, the result may help better understand and detect VaIN.

2. Materials and Methods

2.1 Study Population

This study included patients with a definitive histopathologic diagnosis of VaIN who were referred for colposcopy from February 2013 to November 2022 at the Cervical Disease Diagnosis and Treatment Health Center at Fujian Maternity and Child Health Hospital dueto abnormal cytology results and/or a positive hr-HPV test, and underwent biopsy from suspicious regions. The pathological diagnosis of tissue and cytology specimens were independently performed by two senior pathologists. The interval between cytological tests/ HPV test and colposcopy were all less than 3 months. Patients with a history of VaIN or cervical cancer metastasis were excluded from the study. Clinical information, including age, gravidity, parity, HPV genotypes, cytology results, cervical lesion history, hysterectomy history cervical pathology and vaginal pathology, was obtained from the medical records of the department.

This study was conducted in compliance with the Declaration of Helsinki (as revised in 2013) and was endorsed by the Ethics Committee at Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University (2023KY003). An exemption of informed consent was obtained considering the retrospective nature of the study.

2.2 Specimen Collection

For patients with a cervix, a special TCT brush and/or HPV special brush was used to scrape the cervical exfoliated cells at the junction of the squamous column epithelium and the cervical canal. For patients who had undergone total hysterectomy, brush-cell samples were from vaginal stump. The cell samples were stored in the corresponding cell preserve solution for cytology and HPV testing.

2.3 Cytology Screening Testing

Liquid-based method was used as cytology screening test. The results were reported based on the 2001 Bethesda system.[10]Atypical squamous cells of unknown significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells, cannot rule out HSIL (ASC-H), atypical glandular cells (AGC), endocervical adenocarcinoma in situ (AIS), squamous cell carcinoma (SCC) and adenocarcinoma were defined as abnormal cytology results.

2.4 HPV Testing

Three types of HPV testing were performed: Hybrid capture-2 testing (Qiagen, Hilden, Germany) which detecting HPV DNA of 13 high-risk oncogenic types (i.e.,HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68), PCR-RDB HPV genotyping (Yaneng Biotech) which detecting 18 types of hr-HPV (i.e.,HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) and five types of low-risk HPV (lr- HPV) (6, 11, 42, 43, and 81), and Aptima (Gen-Probe Inc., San Diego, CA) which detecting HPV

E6/E7 mRNA from 14 high-risk oncogenic types (i.e., HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68).

2.5 Statistical Analysis

Statistical analyses were performed using SPSS 23.0 (IBM Corp). Two-tailed p-values less than 0.05 were considered statistically significant. Categorical variables were presented as frequencies (percentages) and analyzed using chi-squared tests. Numerical variables were tested for normality using the Shapiro–Wilk normality test. Date with normal distribution were expressed as mean ± standard deviation and analyzed with t-test. Non-normal data distribution was expressed as median (interquartile range) and analyzed with the Mann-Whitney test. The correlation of VaIN and CIN was evaluated using Kendall's correlation test.

3. Results

3.1 Characteristics of study objects

A total of 1200 patients who met the inclusion and exclusion criteria and were included in the study. The characteristics of patients are presented in Table 1. The overall median age was 44.17 years old from a range between 17 and 90 years old. Of the total, 930 (77.5%) patients had VaIN 1, 194 (16.2%) patients had VaIN 2, 67 (5.5%) patients had VaIN3 and 9 (0.8%) patients had vaginal cancer.

[insert Table 1]

3.2 Clinical characteristics comparison between VaIN1 and VaIN2+

The median age of VaIN2+ patients was 53 years (range: 42-59.25 years)which was older than that of VaIN1 patients with a median age of 41.5 years (range: 32-52 years) (p¡0.001). Moreover, VaIN2+ patients were more likely to have concomitant cervical lesions (67.7% vs. 46.2%, p¡0.001), higher gravidity (p=0.021) and parity (p¡0.001) compared to VaIN1 patients. There were also more post-menopausal women in VaIN2+ group the VaIN1 group (52.3% vs. 24.8%, p¡0.001). The hr-HPV positive rate among VaIN2+ patients was higher than VaIN1 patients (95.4% vs.86.8%, p¡0.001), with HPV16/18 detected more frequently in VaIN2+ patients(62.2%) than in VaIN1 patients (27.9%) (p¡0.001).There was no statistically significant difference in single and multiple hr-HPV infections between VaIN1 and VaIN2+ patients. VaIN1 group was more likely to have a history of low-grade cervical intraepithelial neoplasia than VaIN2 group (6.9% vs. 3.0%, p¡0.001), while VaIN2 group was more likely to have a history of high-grade cervical intraepithelial neoplasia than VaIN1 group (29.3% vs. 14.4%, p¡0.001). Furthermore, ASCUS and LSIL were more frequantly detected in VaIN1 patients, while ASC-H\HSIL\SCC\AGC were more likely to be detected in VaIN2 patients, the difference is statistically significant (p¡0.001) (Table 2). Additionally, the analysis showed a weak correlation between vaginal and cervical lesions (Kendall's Tau correlation=0.387, p¡0.001).

[insert Table 2]

3.3 Distribution of hr-HPV among different grades of VaIN

A total of 832 cases underwent HPV genotyping, with the most common HPV genotypes detected in VaIN being HPV16、HPV52、HPV58、HPV51、HPV56、HPV18,accounting for 26.9%, 21.0%, 17.7%, 12.7%, 11.7%, 10.6%, 10.1%, respectively(Figure 1A).The detail numbers was displayed in Supplementary Table S1. In VaIN 1, the most frequently detected HPV types were HPV52、HPV16、HPV58、HPV53、HPV56、HPV51、HPV59、HPV18, accounting for 22.7%, 19.5%, 17.8%, 13.6%, 12.0%, 11.7%, 10.2%, 10.1%, respectively (Figure 1B).While in VaIN2+, the most common HPV types were HPV16、HPV58、HPV52、HPV51、HPV18、HPV53, accounting for 54.5%, 17%, 14.8%, 11.4%, 10.2%, respectively(Figure 1C).

[insert Figure 1]

3.4 Comparison of the sensitivity of cervical cancer screening against VaIN and CIN

The sensitivities of cytology, hr-HPV and cotesting is displayed in Table 3. Among patients without total hysterectomy, cytology had a sensitivity of 84.9% for VaIN2+, which was slightly lower than the sensitivity of 87.4% for CIN2+; however, this difference was not statistically significant (P=0.495). The sensitivities of hr-HPV testing for detecting VaIN2+ and CIN2+ were 95.0% and 96.7%, respectively (P=0.480). The cotesting sensitivities for detecting VaIN2+, CIN2+, and VaIN2+ after hysterectomy were all 100%. The sensitivity of hr-HPV testing for detecting VaIN2+ was significantly higher than that of cytology (95.4% vs. 88.1%, p=0.007). However, there was no statistical difference between the two tests for detecting VaIN2+ after hysterectomy (97.1% vs. 96.4%, p=0.829).

[insert Table 3]

4.Discussion

In the present study, the HR-HPV detection rate was 88.5% (883/998) in all cases of VaIN. Specifically, the detection rate was 95.4% in cases of VaIN2+.The most common hr-HPV genotypes found in VaIN were HPV16, HPV52, HPV58, HPV53. The median age of patients with VaIN2+ was 11.5 years older than VaIN1. The proportion of postmenopausal women in the population with VaIN2+ disease was higher than that in the population with VaIN1 disease. Patients with VaIN2+ tended to have more pregnancies and deliveries than those with VaIN1. Additionally, they were more likely to have a history of CIN. VaIN grade was significantly positive correlated with cervical lesions, but correlation was weak (r=0.387). HPV 16 was present in 54.5% cases of VaIN2+ making it the most prevalent HPV type in VaIN2+, followed by HPV58 (17.0%), HPV52 (14.8%), HPV51 (11.4%), and HPV18 (10.2%).. The sensitivity of hr-HPV testing for VaIN2+ and CIN2+ were significantly higher than that of cytology testing. However, for VaIN of vaginal stumps, they had no difference. Furthermore, the sensitivity of cervical cytology and hr-HPV testing for detecting VaIN2+ was not significantly different from their sensitivity for detecting CIN2+.

In previous studies, the HPV detection rate in patients with VaIN2/3 ranged from 50% to 100%, and from 25% to 89% in vaginal cancer.[11-13] The different results may be attributed to variations in study sample sizes, HPV detection methods, and sample site selections (cervix or vaginal wall). HPV 16 was the dominated HPV type in VaIN especially in VaIN2+ in all former studies.[13-17] In a global multicenter study[17] that performed HPV DNA detection and typing in 597 vaginal precancerous and cancerous lesions, HPV 16 accounted for 59% of VaIN2+, which is very close to the result of this study.. Aside from HPV 16, the following HPV genotypes were not consistent in different studies, which may due to the regional differences in HPV type prevalence. HPV18, 33, 45, 31 were the following common HPV types in VaIN2+ in Europe and North America[13], while HPV 58, 52, 39, 33 were the following common in Taiwan, China[14]. The result of this study show the HPV prevalent in VaIN2+ is similar to HPV prevalent in CIN2+ in our previous study[18], but HPV 58 exceeded HPV 52 to become the second most common HPV type in VaIN 2+ in present study, however, the difference was not great. HPV vaccination provides an excellent opportunity to prevent VaIN2+. Denmark, a country with high HPV vaccination coverage, found that the incidence rate of high-grade VaIN decreased by nearly 16% per year among women younger than 30 years old after the introduction of HPV vaccine.[19] As China is a country with poor HPV vaccine coverage[20], the results of HPV prevalent presents near baseline distribution and inspire the use of HPV vaccines that cover HPV 16, 58, 52, 51, and 18 to provide excellent protection against VaIN2+.

Research has shown that a history of high-grade cervical lesions and cervical cancer increases the risk of developing noncervical HPV-related anogenital premalignancies and carcinomas for at least 20 years[21, 22], even after total hysterectomy[23-25]. Consistent with these findings, the present study revealed that patients with VaIN2+ were more likely to have a history of high-grade cervical lesions or cervical cancer than those with VaIN1. Moreover, postmenopausal women, those with gravidity and parity more than three times, and those with hr-HPV infection are all at increased risk of developing VaIN2+. These results underscore the importance of screening high-risk populations for VaIN2+. Currently, both cytology and HPV testing are performed on the cervix except for vaginal stumps. It is important to evaluate the sensitivity of cervical cancer screening for detecting VaIN2+ and how it compares to CIN2+. Previous studies have shown that the sensitivity of cytology for detecting CIN2+ ranged from 47% to 72.9%, while the sensitivity of HPV testing

ranged from 89.9% to 95%.[26, 27] Meanwhile, the sensitivity of cytology for detecting VaIN2+ ranged from 61.5% to 83.8%, the sensitivity of HPV testing ranged from 82.5% to 97.3%, and cotesting had a sensitivity ranging from 95.2% to 100%.[28, 29] Although these findings suggest that cervical cancer screening has a higher sensitivity for detecting VaIN2+ compared to CIN2+, direct comparison between studies is not possible due to differences in HPV testing methods and cytologic quality, as well as the object of study. This study has a unique advantage in that it directly compares the sensitivity of cervical cancer screening (cytology or HPV test) for VaIN2+ and CIN2+ within the same study. The results indicate that the sensitivity of cervical cytology and hr-HPV testing are equivalent for detecting both VaIN2+ and CIN2+, with cotesting having the highest detection rate. However, for patients who have undergone hysterectomy, the sensitivity of cytology for VaIN2+ was comparable to that of HPV testing, but the small sample size may have biased the results. Vaginal cytology testing has a reported sensitivity of 83% for predicting vaginal HSIL[30], while vaginal hr-HPV testing has a sensitivity of 100%[31], but a direct comparison between these tests is not appropriate. Thus, future well-designed controlled studies are necessary to further investigate these findings.

Most of the vaginal lesions occur in the upper one-third of the vagina. A colposcopy is a necessary procedure for diagnosing VaIN. Acetic acid should be applied to the entire vaginal mucosa, including the fornix, for 1-2 minutes, followed by the application of Lugol's iodine to confirm the presence or absence of abnormal epithelium throughout the vaginal mucosa and fornix. In this study, 32.3% of VaIN2+ cases were not accompanied by CIN. Therefore, when cervical cancer screening is abnormal and there are no abnormal images on the cervix, attention should be paid to the vaginal wall. According to the 2019 American Society of Colposcopy and Cervical Pathology (ASCCP) guidelines[32], for non-pregnant patients [?]25 years old with HSIL cytology, expedited treatment (treatment without colposcopic biopsy) for cervix is preferred. However, this type of management of abnormal cervical cancer screening may lead to a missed diagnosis of VaIN. Therefore, when choosing expedited treatment, colposcopic examination should also be performed not only on the cervix but also on the vaginal wall, and it is important to take a biopsy where a lesion is identified on the vagina.

The limitations of this study are as follows: firstly, this is a single hospital-based retrospective analysis, and selection bias may exist. Secondly, three types of HPV testing were used in this study, which may have resulted in heterogeneity of the results. Thirdly, since all the patients included in this study were diagnosed because of abnormal cervical cancer screening, VaIN without abnormal TCT or positive hr-HPV results were not taken into account, so the sensitivity of cervical cancer screening may be over-estimated.

Conclusion

Vaccines that cover HPV 16, 58, 52, 51, and 18 are likely to provide excellent protection against VaIN2+. The rate of multiple HPV infection in VaIN1 is not statistically different from that in VaIN2+. Cervical cancer screening has similar sensitivity for VaIN2+ as for CIN2+, with hr-HPV testing showing higher sensitivity than cytology, and cotesting showing the highest detection rate. However, in populations after hysterectomy, cytology and hr-HPV testing have similar sensitivity for detecting VaIN2+. During colposcopy, acetic acid and Lugol's iodine should be applied to cover the entire vaginal mucosa and fornix, and attention should be paid to abnormal images in the vagina.

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Author Contributions

Yusha Chen and Qiaoyun Chen were involved in the design of the study. Huifeng Xue and Jinwen Zheng carried out data collection and analysis. Yusha Chen assisted in drafting the manuscript. Xiangqin Zheng and Jiancui Chen revised the manuscript. All the authors reviewed and approved the final manuscript.

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Figure 1 The prevalence of hr-HPV genotype in total VaIN(A) , VaIN1(B) and VaIN2+(C). hr-HPV, high-risk human papillomavirus

Table 1 Clinical characteristics of patients with vaginal intraepithelial neoplasia

Characteristics	Total Number (%)
Age, median (range)	44.17(17 to 90)
Menstrual status	
premenopausal	708(59.0%)
menopausal	305(25.4%)
hysterectomy(subtotal/total)	187(15.6%)
History of cervical lesions	
hysterectomy for benign disease	56(4.7%)
hysterectomy for CIN/SCC	111(9.3%)
hysterectomy cancers other than cervical cancer	13(1.0%%)
LEEP for HSIL	100(8.3%)
LSIL	72(6%)
hysterectomy for unknown reasons	8(0.7%)
none	840(70.0%)
Cytology results	
NILM	162(13.5%)
ASCUS	330(27.5%)
LSIL	492(41.0%)
HSIL	110(9.2%)
ASC-H/ AGC/AIS	51(4.2%)
SCC/ACC	3 (0.3%)
not performed	52(4.3%)
HR-HPV resutls	
positive	883(73.6%)
negative	115(9.6%)
Not tested	202(16.8%)
HR-HPV infection pattern	
single type	489 (40.8%)
multiple types	340 (28.3%)
untyped	371(30.9%)
Concomitant CIN	
no	504(49.6%)
CIN1	340(33.5%)

CIN2	101(9.9%)
CIN3	47(4.6%)
Cervical cancer	24(2.4%)
VaIN	
VaIN1	930(77.5%)
VaIN2	194 (16.2%)
VaIN3	67 (5.5%)
vaginal cancer	9 (0.8%)

Abbreviations: CIN, cervical intraepithelial neoplasia; SCC, cervical squamous cell carcinoma; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy (NILM); ASC-US, atypical squamous cells of unknown significance; ASC-H, atypical squamous cells, cannot rule out HSIL; AGC, atypical glandular cells; AIS, endocervical adenocarcinoma in situ; SCC, squamous cell carcinoma; hr-HPV, high-risk HPV; VaIN, vaginal intraepithelial neoplasia

Table 2 Comparison of clinical characteristics between VaIN1 and VaIN2 patients.

	VaIN1	VaIN2+	Z/x	P值
Age	41.5(32,52)	53(42,59.25)	-9.806	¡0.001
Concomitant CIN				
no	440(53.8%)	64(32.3%)	29.386	¡0.001
yes	378(46.2%)	134(67.7%)		
Cervical lesions histogy				
none	729(78.7%)[a]	180(67.7%)[a]	34.654	¡0.001
LSIL	64(6.9%)[a]	8(3.0%)[b]		
HSIL	133(14.4%)[a]	78(29.3%)[b]		
Menstrual status				
premenopausal	614(75.2%)	94(47.7%)	57.151	¡0.001
menopausal	202(24.8%)	103(52.3%)		
Cytology results				
NILM	132(14.7%)[a]	30(11.9%)[a]	191.936	¡0.001
ASCUS/LSIL	703(78.5%)[a]	119(47.0%)[b]		
ASC-H/AGC/HSIL/SCC	60(6.7%)[a]	104(41.1%)[b]		
HR-HPV results				
negative	106(13.2%)	9(4.6%)	11.343	0.001
positive	697(86.8%)	186(95.4%)		
HR-HPV infection pattern				
single	381(58.2%)	108(62.1%)	0.865	0.352
multiple	274(41.8%)	66(37.9%)		
Gravidity				
¡3	407(52.7%)	86(43.4%)	5.358	0.021?¿?
3	366(47.3%)	112(56.6%)		
Parity				
¡3	674(87.2%)	140(70.4%)	32.973	¡0.001?¿?
3	99(12.8%)	59(29.6%)		
HPV genotype				
HPV 16/18	183(27.9%)	108(61.4%)	68.342	¡0.001
non-type 16/18 hr-HPV	473(72.1%)	68(38.6%)		

Note: [a,b] different alphabet means statistically significant (pj0.05)

Table 3 Comparison of Sensitivity for Cervical Cancer Screening against VaIN2+ and CIN2+

	VaIN2+ (without total hysterectomy)	CIN2+	P-value (row)	VaIN2+ (after total hysterectomy)
Cytology	84.9%(157/285)	87.4%(139/159)	0.495	97.1%(66/68)
Hr-HPV	95.0%(133/140)	96.7%(119/123)	0.480	96.4%(53/55)
P-value (column)	0.004*	0.005*		0.829
Cotesting	100%(134/134)	100%(91/91)		100%(50/50)

Note: * denotes statistical significance ($p < 0.05$).