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Human Papillomavirus Status in Primary Lesions and Pelvic Lymph Nodes and Its Prognostic Value in Cervical Cancer Patients with Lymph Node Metastases

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Background: The aim of this study was to assess the presence of HPV DNA in cervical tissues and lymph nodes in patients who have uterine cervical neoplasms with lymphatic metastases and who underwent surgery for invasive cervical cancer and pelvic lymphadenectomy, to establish the utility of HPV type and viral load in predicting disease progression.

Material/Methods: We retrospectively assessed 88 patients with uterine cervical neoplasms with lymph node metastases. All 88 patients were in FIGO stage IA-IIB. A total of 316 paraffin-embedded archival tissues (88 cervical samples and 228 pelvic lymph node specimens) were acquired. All the samples were analyzed using real-time PCR to determine HPV DNA presence/type and to quantify viral load.

Results: In total, 17 HPV genotypes were detected in the cervical lesions and pelvic lymph nodes of the patients. The most common HPV type in all samples was HPV16, followed by HPV18. The existence of HPV16 DNA and low HPV16 viral load in cervical lesions were also significantly associated with disease recurrence. Furthermore, lymphovascular space involvement was also correlated with worse disease outcome.

Conclusions: HPV16 DNA presence and low viral load in primary lesions can be used to predict disease recurrence. HPV DNA is a favorable prognostic indicator in patients with uterine cervical neoplasms who have lymphatic metastases.

MeSH Keywords: Human Papillomavirus DNA Tests • Lymphatic Metastasis • Uterine Cervical Neoplasms • Viral Load

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Background

Carcinoma of the uterine cervix is the second leading cancer affecting women all over the world, and it is the principal cancer affecting women in developing countries [1]. Surgery is the standard therapy for cervical cancer, and lymphadenectomy is commonly used to treat invasive cervical and pelvic cancer. In carcinoma of uterine cervix patients with early clinical stage, the rate of relapse following hysterectomy is still high (15%), even with histologically negative lymph nodes [2]. Thus, continued investigation to develop methods and drugs to prevent, diagnose, and treat cervical cancer is essential.

Regardless of subtype, the first step towards the development of cervical cancer is exposure to the human papillomavirus (HPV). Indeed, the effects of high-risk HPV strains in the progression of carcinoma of uterine cervix are well known [3]. Uterine cervix carcinoma has a lengthy course involving HPV integration to the host DNA [4–6]. It is, therefore, not surprising that the existence of metastatic HPV deposits in pelvic lymph nodes is an important prognostic factor in cervical cancer [7]. The methods typically used to detect HPV DNA are sensitive but have low specificity, and the positive predictive value is very low [8]. Alternatively, quantitative HPV DNA (viral load) testing was found to reduce false-positive results and increase specificity and positive predictive value during detection of cervical lesions. Researchers have attempted to determine the relationship between viral load and cervical lesions, and it is suspected that the quantitative estimate may be an indirect indicator of active cellular replication [9]. However, although the clinical significance of HPV in cervical cancer development and diagnosis is widely accepted, the relationship between cancer progression and HPV status in cervical tissues and lymph nodes remains uncertain.

Therefore, in this study, we examined the relationship between HPV strain and gene expression levels in cervical samples and in lymph nodes in association with cancer recurrence in patients who have uterine cervical neoplasms with pelvic lymphatic metastases. To the best of our knowledge, this is the first time this relationship has been investigated in detail using HPV typing and viral load quantification.

Material and Methods

Only stage IA-IIB carcinoma of uterine cervix patients (diagnosed and treated at the International Federation of Gynecology and Obstetrics [FIGO]) undergoing surgery for invasive cervical cancer and pelvic lymphadenectomy were enrolled in our research. Subjects who were confirmed to have pelvic lymph nodes metastases were also recruited. Eligible women were not pregnant and had no history of chemotherapy, neoadjuvant treatments, or pelvic radiation.

This retrospective research was approved by the Medical Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University and adheres to the standards of the Declaration of Helsinki. The clinical, surgical, and follow-up information were collected for further analysis.

To test HPV viral load, 1 cervical tissue sample and 2–4 samples of the pelvic lymph nodes were acquired from each patient. We obtained 316 paraffin-embedded tissues (88 archival cervical samples and 228 pelvic lymph node samples) from 88 patients affected by cervical carcinoma. These paraffin-embedded samples were sectioned, and 2–8 tissue slices were used for analysis. These slices were rinsed twice in xylene and washed in ethanol 3 times, followed by digestion using cell lysis buffer and proteinase K. Further, a DNA Lysis kit (Hybribio, China) was used to extract DNA from the digested tissues.

HPV genotyping was performed with real-time fluorescence PCR (HPV Genotyping Real-time PCR Kit, Hybribio, China) following the manufacturer's instructions. Quantitative viral load was determined using real-time quantitative PCR. The amplified reaction was carried out with a 20-μL container including PCR primers, DNA polymerase, template DNA, and the TaqMan probe. There were 6 reaction tubes in the kit and 4 fluorescence probe detection channels (FAM, HEX/JOE, ROX/Red 610, and Cy5) in the sequence detector. The primers used for the detection of high- and low-risk HPV DNA in this study were synthesized according to registered patents at the Hybribio Company. The reaction cycle was programmed as follows: 95°C for 10 min; 45 cycles of 95°C for 15 s, and 60°C for 1 min; and finally, 38°C for 5 s. A 178-bp fragment of the β-globin gene was introduced to assess the quality of the extracted sample DNA. The β-globin gene was then quantitated for each sample and used for normalization. The number of copies per mL of inoculum was used to express the HPV load.

Continuous variables are presented as the mean ± standard deviation (SD) when they followed a normal distribution pattern. If they were not normally distributed, they are represented by the median and 4 quantiles. Comparisons between groups were made by *t* test, analysis of variance (ANOVA), or non-parametric Wilcoxon and Kruskal-Wallis rank sum test. Categorical variables are expressed numerically and by percentage. Comparisons between groups were performed by chi-square test or Fisher's test. Multivariate analyses were performed with Cox regression method. Hazard ratios (HR) with 95% confidence intervals (95%CI) are shown. Kaplan-Meier curves were also determined. The difference of survival probabilities was calculated by the log-rank test. Statistical analysis was carried out with the SAS program (SAS, Release 9.4). $P<0.05$ was considered to show significance.

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Table 1. Characteristics of the 88 cervical cancer patients enrolled in this study.

Median age, years (range)	48	(25–76)
Histologic type, n (%)		
Squamous cell carcinoma	82	(93.2%)
Adenocarcinoma	5	(5.7%)
Adenosquamous carcinoma	1	(1.1%)
FIGO stage, n (%)		
IA	2	(2.3%)
IB	36	(40.9%)
IIA	46	(52.3%)
IIB	4	(4.5%)
Histological grade		
Well differentiated	7	(7.9%)
Moderately differentiated	68	(77.3%)
Poorly differentiated	13	(14.8%)
Deep stromal invasion	83	(94.3%)
Parametrial invasion	7	(7.9%)
Vaginal margin involvement	3	(3.4%)
Lymphovascular space involvement	69	(78.4%)
Total lymph node number, n (%)	228	
Histopathological positive lymph nodes	136	(59.6%)
Histopathological negative lymph nodes	92	(40.4%)
Follow-up data(months), median(range)	26	(7–78)
Recurrent patients	34	(38.6%)
Non-recurrent patients	48	(54.6%)
Lost to follow-up	6	(6.8%)

FIGO – The International Federation of Gynecology and Obstetrics.

Results

Patient characteristics

The age distribution of patients was from 25–76 years (mean, 48±10.8). FIGO staging identified 2 patients with IA, 36 patients with IB, 46 patients with IIA, and 4 patients with IIB. After surgery, we histologically confirmed 82 cases of squamous carcinoma, 5 patients had adenocarcinoma, and the remaining 1 patient had adenosquamous carcinoma. The subjects with existing lymphatic metastases underwent further histopathological examination for confirmation. Over the follow-up period (mean, 26 months), 34 subjects had disease recurrence and 2 subjects died following distant metastasis.

Patient characteristics are displayed in Table 1.

HPV subtype existence and HPV gene quantitation in cervical lesions and pelvic lymph nodes

In 82 (93.2%) cases, both the cervical tissues and pelvic lymph nodules contained HPV DNA. In 1 out of 88 (1.1%) cases, none of the known HPV types were detected in either the primary lesions or the pelvic lymph nodes. Alternatively, in 4 patients, HPV was detected in the primary tumors, but not the lymph nodes, while the opposite (HPV positive lymph nodes, HPV negative primary lesions) was observed in only 1 patient (Table 2).

A total of 17 HPV genotypes, including HPV16, 18, 31, 33, 39, 45, 51, 52, 58, 59, 68, 73, 82, 6, 43, 44, and 81, were detected in the cervical lesions and pelvic lymph nodes. Among the tested specimens, 70.9% were positive for HPV16, 14.6% for HPV18, 4.7% for HPV58, 3.5% for HPV33, 2.2% for HPV68, 1.3% for HPV52, and 1.3% for HPV59. Furthermore, 0.6% were positive for HPV31, HPV45, HPV73, and HPV82, respectively, while only 0.3% of patients were positive for HPV39, 51, 43, 44, 81, and 6, respectively. There were 49 samples that were infected by multiple HPV types.

Focusing on the primary lesions, we found that the most prominent HPV genotypes were HPV16 (81.8%), followed by HPV18 (17.0%) and HPV58 (6.8%). Infection with more than 1 HPV type was observed in 17.0% of the cervical lesions. Of the 136

Table 2. Distribution of patient numbers according to HPV genotyping in primary lesions and lymph nodes.

Primary lesions	Lymph nodes	n	%
–	–	1	1.1%
–	+	1	1.1%
+	–	4	4.6%
+	+	82	93.2%

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Table 3. Proportion of different HPV types in cervical lesions and pelvic lymph nodes.

	Cervical lesions (n, %)	Metastatic lymph nodes (n, %)	Non-metastatic lymph nodes (n, %)
Total	88, 100%	136, 100%	92, 100%
HPV16	72, 81.8%	93, 68.4%	59, 64.1%
HPV18	16, 18.2%	19, 13.9%	11, 11.9%
HPV58	6, 6.8%	6, 4.4%	3, 3.2%
HPV33	2, 2.3%	5, 3.7%	4, 4.3%
HPV68	3, 3.4%	4, 2.9%	0
HPV52	1, 1.1%	0	3, 3.2%
HPV59	2, 2.2%	2, 1.5%	0
Other HPV types*	6, 6.8%	5, 3.7%	3, 3.2%
Double co-infection	22, 25%	19, 13.9%	8, 8.7%

* Other HPV types included HPV39, 31, 45, 73, 82, 51, 43, 44, 81, and 6.

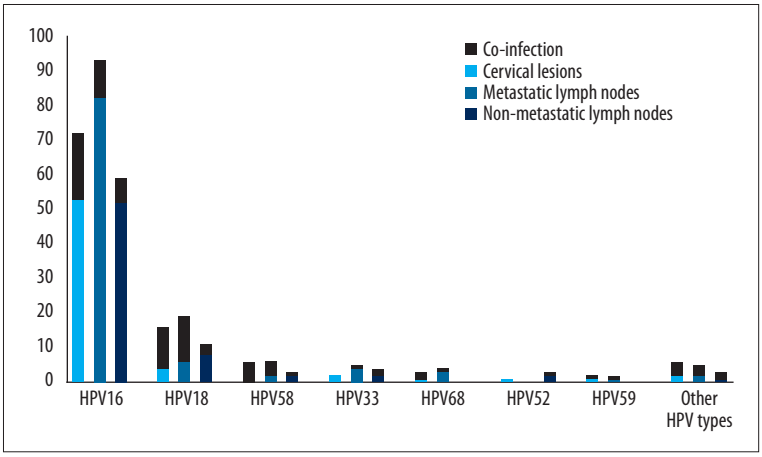

Figure 1. Distribution of different HPV types in the cervical lesions and pelvic lymph nodes isolated from the 88 cervical cancer patients enrolled in this study.

Table 4. Viral load of HPV16, 18, 58, and 33 in cervical lesions and pelvic lymph nodes.

	Cervical lesions	Pelvic lymph nodes
HPV16	8,909,106,901.73±26,185,097,229.31	688,934,926.37±2,103,613,710.70
HPV18	8,054,772,073.01±29,366,849,231.21	4,130,408.89±7,278,874.45
HPV58	11,363,100,644.69±25,407,687,655.31	1,017,860.64±125,222.61
HPV33	41,516,466,917.05±35,767,008,259.22	3,192,152,602.27±4,506,471,956.35

Table 5. Univariate analysis evaluating the relationship between disease recurrence and HPV viral load in cervical lesions and pelvic lymph nodes.

	Recurrence (median)	Non-recurrence (median)	P value
HPV16 viral load in cervical lesions	207,102,496.00	2,491,783,170.00	0.0040
HPV18 viral load in cervical lesions	12,841.16	645,914.75	0.1013
HPV16 viral load in pelvic lymph nodes	1,708,033.50	14,939,166.57	0.4285
HPV18 viral load in pelvic lymph nodes	181,268.63	401,969.28	0.1653

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Table 6. Univariate analysis evaluating the relationship between disease recurrence and clinicopathological factors as well as HPV status in cervical lesions and pelvic lymph nodes.

	Recurrence (n, %)	Non-recurrence (n, %)	P value
Age			0.7838
<40	6 (17.6%)	10 (20.8%)	
≥40	28 (82.4%)	38 (79.2%)	
Histologic type			0.3020
Squamous carcinoma	31 (91.2%)	47 (97.9%)	
Adenocarcinoma	3 (8.8%)	1 (2.1%)	
Adenosquamous carcinoma	0	0	
FIGO stage			0.1152
Ia	0	2 (4.2%)	
Ib	10 (29.4%)	23 (47.9%)	
IIa	21 (61.8%)	22 (45.8%)	
IIb	3 (8.8%)	1 (2.1%)	
Differentiated degree			0.7420
Well differentiated	2 (5.9%)	2 (4.2%)	
Moderately differentiated	26 (76.5%)	40 (83.3%)	
Poorly differentiated	6 (17.6%)	6 (12.5%)	
Deep stromal invasion	33 (97.1%)	45 (93.8%)	0.6382
Parametrial invasion	4 (11.8%)	3 (6.3%)	0.4411
Vaginal margin involvement	1 (2.9%)	1 (2.1%)	1.0000
Lymphovascular space involvement	30 (88.2%)	33 (68.8%)	0.0394
High-risk HPV positivity in cervical lesions	33 (97.1%)	47 (97.9%)	1.0000
HPV16 positivity in cervical lesions	31 (91.2%)	35 (72.9%)	0.0498
HPV18 positivity in cervical lesions	3 (8.8%)	10 (20.8%)	0.2203
HPV58 positivity in cervical lesions	1 (2.9%)	5 (10.4%)	0.3927
HPV33 positivity in cervical lesions	0	2 (4.2%)	0.5086
HPV16 and HPV18 co-infection in cervical lesions	3 (8.8%)	5 (10.4%)	1.0000
High risk HPV positivity in pelvic lymph nodes	31 (91.2%)	46 (95.8%)	0.6444
HPV16 positivity in pelvic lymph nodes	30 (88.2%)	38 (79.2%)	0.3768
HPV18 positivity in pelvic lymph nodes	6 (17.6%)	16 (33.3%)	0.1354
HPV58 positivity in pelvic lymph nodes	0	7 (14.6%)	0.0378
HPV33 positivity in pelvic lymph nodes	1 (2.9%)	5 (10.4%)	0.3927
HPV16 and HPV18 co-infection in pelvic lymph nodes	5 (14.7%)	11 (22.9%)	0.4089

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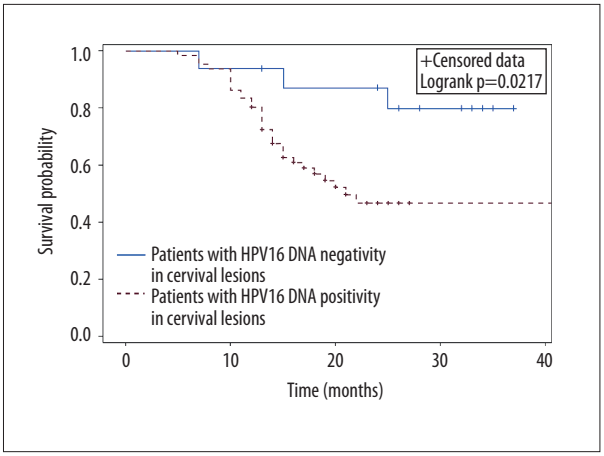

Figure 2. Kaplan-Meier analysis highlighting the relationship between disease-free survival and HPV16 positivity in cervical lesions.

metastatic lymph nodes removed, 117 (86.0%) were positive for high-risk HPV, while 71 (77.1%) of the 92 non-metastatic lymph nodes dissected were positive for high-risk HPV. The most prominent HPV genotypes in the pelvic lymph nodes were HPV16 (66.7%), HPV18 (13.2%), HPV58 (3.9%), and HPV33 (3.9%). The percentage of HPV positive lymph nodules in all lymph node samples was 83.3% (190/228). The existence of these different HPV subtypes is displayed in Table 3 and Figure 1.

Quantification of viral load was also performed. In this analysis, we focused on HPV16, 18, 58, and 33 gene expression in cervical lesions and pelvic lymph nodes because these were the most common types in both tissues. These data (expressed as the mean ±SD) are shown in Table 4.

The relationship between prognosis and viral load

Low HPV16 viral load in cervical lesions was significantly associated with disease recurrence in univariate analysis (P=0.004) (Table 5). Other parameters related to prognosis included changes in lymphovascular space (P=0.0394) and the detection of HPV16 during cervical lesion genotyping (P=0.0498). HPV58 existing in the lymph nodules of non-recurrence patients appears to be a protective factor (P=0.0378). However, this relationship was not observed in our multivariate analysis, highlighted below. Notably, age, histology type, FIGO stage, degree of differentiation, deep stromal invasion, parametrial invasion, and vaginal margin involvement were not significantly correlated with disease recurrence in our univariate analysis (Table 6).

The relationship between HPV58 and lymph nodes prognosis was not statistically significant in our multivariate analysis (P=0.9884). However, this analysis did identify HPV16 positivity in cervical lesions as an independent risk indicator of cancer recurrence (P=0.0233; HR, 4.025; 95%CI, 1.208, 13.406). Kaplan-Meier analysis also revealed that disease-free survival rate was distinctly higher for patients negative for HPV16 in their cervical lesions than those that were positive for HPV16 in their cervical lesions (P=0.0217) (Figure 2). Alternatively, lymphovascular space involvement indicated a significant negative relationship with disease outcome (P=0.0398; HR, 3.057; 95%CI, 1.053, 8.872). The results of our multivariate analysis are shown in Table 7.

Discussion

Genital HPV strains have been subdivided into high-risk types, which are typically associated with cervical cancer, and low-risk types, which primarily cause genital warts. There are 15 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), and 11 low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81) [10]. While the relationship between HPV exposure and genome expression and cervical cancer pathogenesis is widely accepted, that between HPV type and expression levels and disease recurrence is unclear. In this study, we analyzed the HPV type and viral load in cervical lesion and pelvic lymph node samples from patients with cervical cancer with lymphatic metastases. Thus, we sought to establish the significance of prognosis involving HPV subtypes, HPV viral load, and various known histological indicators. We assessed the effects of HPV subtypes in cervical lesions, HPV viral load in cervical lesions, HPV subtypes in pelvic lymph nodes, and HPV viral load in pelvic lymph nodes. To the best of our knowledge, this is the first time this relationship has been assessed using these particular methods.

Table 7. Multivariate analysis evaluating the relationship between disease recurrence and clinicopathological factors as well as HPV status in cervical lesions and pelvic lymph nodes.

Factor	P value	Hazard ratio	HR (95%CI)
Lymphovascular space involvement	0.0398	3.057	(1.053, 8.872)
HPV16 positivity in cervical lesions	0.0233	4.025	(1.208, 13.406)
HPV18 positivity in pelvic lymph nodes	0.1652	0.530	(0.216, 1.299)
HPV58 positivity in pelvic lymph nodes	0.9884	0.000	–

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Our data indicate that the most common HPV type in all samples was HPV16 (81.8% in primary lesions and 66.7% in dissected pelvic lymph nodes). The prevalence of HPV16 is supported by previous work [10,11]. This was followed by HPV 18 (18.2% and 13.1% in primary lesions and pelvic lymph nodes, respectively). Tortora et al. and De Vuyst et al. also suggested that the most prevalent genotypes in cervical cancer patients were HPV16 and HPV18, supporting our findings [12,13]. Furthermore, the use of a PCR assay system in the present study also lead to the identification of co-infections. Notably, the most common HPV type involved in co-infection of both types of samples was also HPV16.

While HPV16 was the most prevalent strain affecting cervical cancer patients, other high-risk types were also observed. Historically, high-risk types have been associated with cervical cancer metastasis. In this study, the percentage of high-risk HPV types was 86.0% in all the metastatic lymph nodes analyzed, while the percentage was 77.1% in non-metastatic lymph nodes. This is supported by previous work indicating the presence of HPV DNA in more than 50% of lymph nodes in patients with metastatic involvement, while patients with non-metastatic nodes were observed to have a greater range of HPV DNA positivity, from 35.7% to 90.1% [14–19]. Taken together, these data indicate that high-risk HPV DNA is not solely an indicator of lymph node metastasis, as it is also found in non-metastatic pelvic lymph nodes. Presumably, these findings highlight the important role of other events necessary for metastasis. For example, the immune system likely plays a significant role in transporting viral DNA from the cervical lesion to the lymph node, but requires certain invasive cell types for complete metastasis. Indeed, both CD4+/CD8+ T cells (able to recognize HPV antigens) and phagocytes have been shown to transport HPV DNA from cervical cells to lymphaden cells [20]. However, transport alone does not appear to indicate an early or significant risk of lymphatic extension of cervical cancer. Notably, Landro et al. found that HPV DNA is primarily present in the cell nucleus and/or cytosol of the leukomonocyte, endotheliocyte, macrophagocyte, and matrix cells in non-metastatic lymphaden, while in lymphaden with metastasis, HPV is found in all of these same cell types as well as in squamous invasive cells [17].

Notably, we observed that HPV39, 43, 45, 73, 81, and 6 were present only in the pelvic lymph nodes and were not detected in the primary lesions. All these HPV types are considered to be low risk. Paccagnella et al. reported similar cases in which HPV DNA was not detectable in primary lesions but was found in pelvic lymph nodes [21]. They suggest that the HPV DNA was possibly carried by "scavenger" cells to the lymph nodes where it continued to replicate, while viral reproduction was unable to be perpetuated in the cervical lesions after the neoplastic cells reached high dedifferentiation. However, this phenomenon is rare, only occurring in 1 patient in the present study.

The relationship between HPV type and cervical cancer patient prognosis is controversial. Kang et al. previously studied 204 cases, finding that HPV18 is a reliable prognostic factor of early-stage cervical cancer [22]. Alternatively, Plich et al. showed that HPV16 positivity, but not HPV18 positivity, was associated with poor prognosis [23]. The significance of HPV load in cervical cancer is also still controversial [24]. Some authors have suggested that the quantity of HPV in the cervical lesion demonstrates the tendency for invasion [25]. However, it was reported that low initial HPV viral load was a poor prognostic indicator in carcinoma of uterine cervix patients who underwent surgery for invasive cervical cancer [26]. In a study by De Boer et al., HPV DNA copy number did not appear to have any prognostic value in cervical cancer [27]. Furthermore, Kim et al. reported that disease-free survival also appears to be unaffected by the level of pretreatment HPV load [28], and concluded that when cancerous malignancy was established, the biological characteristics of the tumor may play a more important role in disease prognosis compared to HPV load [28]. However, in the present study, our follow-up data showed a significant association between the existence of HPV16 in primary lesions and low HPV16 viral load in cervical lesions with the recurrence of cervical cancer. Furthermore, HPV16 positivity in cervical lesions also appears to be an independent prognostic risk indicator of disease relapse. Thus, while age, tumor types, FIGO stage, differentiated degree, deep matrix infiltration, paracervical infiltration, and vaginal margin involvement were unrelated to disease recurrence, HPV16 in primary lesions significantly affected progression-free survival of carcinoma of uterine cervix patients with pelvic lymphatic metastases.

In recent years, various molecular biology studies have confirmed that high-risk HPV DNA exists in the genome of the host cells in an integrated pattern [12]. HPV DNA is then carried by the malignant cervical cells to the lymph nodes through the lymph-vascular drainage system [29]. However, the role of HPV DNA once it is delivered to the pelvic lymph nodes has been a point of debate for the last 2 decades. It has been suggested to be a sensitive marker that can be used for monitoring disease relapse in carcinoma of uterine cervix patients [12]. Furthermore, while Füle et al. demonstrated that lymph nodes with tumor infiltration more frequently contained high-risk HPV than lymph nodes without metastasis; HPV status in pelvic lymph nodes themselves was not a predictor of survival [30]. This is supported by a study investigating HPV in the lymph nodes and plasma of 28 patients of uterine cervical neoplasms, which also failed to indicate HPV in pelvic lymph nodes as a prognostic indicator [31]. In the present study, we also failed to observe a statistically significant association between HPV status in pelvic lymph nodes and disease recurrence.

With regards to histology, the histologic subtype, size of malignant neoplasm, invasive depth, parametrial invasion, space of

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lymphatic and vessel infiltration, and lymphatic metastasis are the key indicators used to evaluate disease outcome [32–34]. In this study, we noted that lymphovascular space invasion was remarkably associated with disease recurrence, and this feature was also an independent risk indicator of nodal involvement in carcinoma of uterine cervix patients. These data support the use of lymphovascular space analysis in selecting appropriate treatment after surgery in metastatic patients with cervical cancer.

Conclusions

We evaluated HPV type and viral load in cervical cancer lesions and lymph nodes isolated from patients of uterine cervical neoplasms to establish the prognostic value of these characteristics. Our data indicate that presence of HPV16 and low quantity of HPV16 DNA in primary lesions can be used to predict disease recurrence, particularly for patients with uterine cervical neoplasms who have lymphatic metastases. Although this study is retrospective in nature, it is the first to explore the significance of prognosis of HPV type and viral load in patients with uterine cervical neoplasms with pelvic lymphatic metastases undergoing surgery for invasive cervical cancer and pelvic lymphadenectomy. Thus, while larger-scale and prospective research is necessary, this study significantly adds to our current understanding of cervical cancer pathogenesis and highlights the diagnostic value of HPV type and viral load, which can ultimately be used to advise clinicians and enhance patient care.

Acknowledgement

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Ethics approval

This retrospective study was approved by the Medical Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University. For this type of study, formal consent is not required. The clinical, surgical, and follow-up data were collected from the Medical Records Department for each patient.

Conflict of interests

None.

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