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Correlation between multi-type human papillomavirus infections and viral loads and the cervical pathological grade

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Abstract

Objective: To investigate the relationship between single/multiple HPV infections and cervical lesions, and the correlation between viral load and the degree of cervical lesions.

Methods: A total of 27 284 patients who underwent testing for HPV were retrospectively screened and 3728 women were enrolled who tested positive for HPV when examined by liquid-based ThinPrep cervical smear cytology test and diagnosed by histopathology at the Shanxi Provincial People's Hospital between May 2017 and March 2019. The genotype and viral load of HPV were determined by fluorescence quantitative polymerase chain reaction. Based on the pathological grade, the cervical lesions were stratified into three groups: chronic cervicitis/cervical intraepithelial neoplasia (CIN) I; CIN II/CIN III; and cervical cancer.

Results: There were significant intergroup differences in the distribution of single and multiple HPV infections. There was a positive correlation between the viral load and cervical pathological grade when the infections were caused by HPV 16, 18, 31, 33, 51, 52, 53, and 58.

Conclusion: Multi-type HPV infections are more likely to aggravate the degree of cervical lesions than single-type infections. The HPV type-dependent viral load is associated with the cervical pathological grade.

KEYWORDS

Cervical pathological grade; Human papillomavirus; Multiple infections; Single infection; Viral load

1 | INTRODUCTION

Cervical cancer is one of the most common gynecological malignant tumors and has the highest incidence and mortality rate among the malignancies of the female reproductive tract, with serious detrimental impact on the physical and mental health of women.[1] Worldwide, there are 528 000 new cases and 266 000 deaths due to cervical cancer every year, and approximately 85% of these occur in low-income countries.[2] Infection with HPV is the main risk factor for cervical cancer and precancerous lesions.[3–5] HPV is a double-stranded, closed, circular DNA virus that specifically infects the human skin and mucosa, especially the vulva, reproductive system, anus, and oropharynx. More than 200 different HPV genotypes have been identified thus far and are divided into low-risk and high-risk types based on the clinical features of infection. The high-risk types include HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58,

59, 66, 68, 73, and 82, whereas the low-risk types include HPV 6, 11, and 81.[6]

Persistent HPV infection plays a causal role in the development of cervical cancer and precancerous lesions, and 99% of cervical cancer cases are associated with high-risk-HPV (hr-HPV) infection.[7] Moreover, the viral load of hr-HPV is surmised to be closely related to the progression of cervical lesions. In addition, an increased HPV viral load was associated with the severity and progression of cervical lesions in some studies,[8] although other studies have not found such a correlation.[9] Therefore, understanding the correlation between HPV viral load and the severity of cervical lesions will facilitate the prediction of risk and provide guidance for the prevention and treatment of cervical cancer. Due to the large number of HPV subtypes, co-infection by multiple HPV genotypes is common and accounts for 20%–50% of all patients with HPV.[10–12] Cancers caused by multiple infections have been shown to contain more carcinogenic E6/E7 HPV mRNA than a single-type HPV infection, suggesting that multi-type HPV infections may be more likely to cause cancer in precancerous lesions than single-type infections.[13] Prospective studies have shown that multi-type HPV infection plays a synergistic role in the development of cervical cancer and increases the severity of cervical diseases. However, other reports have found no significant differences in the risk of cervical cancer in women with single and multiple HPV infections.[14] Therefore, there is a need for clinical studies to analyze the impact of single and multiple HPV infections on cervical diseases.

Research has shown that the carcinogenesis of cervical cancer occurs through obvious gradual changes and most HPV infections resolve within 2 years. Only a few women develop persistent HPV infection and subsequent cervical intraepithelial neoplasia (CIN), which ultimately leads to cervical cancer over 8–10 years.[15] This duration provides a favorable opportunity for the screening of cervical cancer to reduce its incidence and mortality rate. The key to effective prevention of cervical cancer is to discover, diagnose, and treat CIN because of the lengthy duration of the persistence of the precancerous lesion before it evolves into cervical cancer.[16]

The aim of the present study was to analyze retrospectively the relationship between HPV viral loads, infection status, and the severity of cervical lesions. Moreover, the receiver operating characteristic (ROC) curve was applied to determine a cut-off value to predict CIN II+ stage, which has important clinical value for the prediction of risk, early diagnosis, prevention, and prognosis monitoring in cervical cancer.

2 | MATERIALS AND METHODS

A total of 27 284 women who underwent detection of HPV DNA in cervical exfoliated cells at the Shanxi Provincial People's Hospital between May 2017 and March 2019 were retrospectively screened. Among them, 4783 samples tested positive for HPV. The present study included women who were simultaneously examined by liquid-based ThinPrep cervical smear cytology test (TCT) and diagnosed by histopathology. The exclusion criteria were as follows: pregnant or menstruating patients; patients with a history of pelvic chemotherapy or radiotherapy; patients with a history of total hysterectomy or cervical resection; and patients who have received cervical physical therapy. Based on the study eligibility criteria, a total of 3728 patients were included in the study. The patients provided written informed consent to participate in the study.

Sampling was carried out with the patient in the lithotomy position. A professional gynecologist wiped away the cervical secretions with a cotton swab, extended the cervical sampling brush into the cervical squamocolumnar junction and rotated it 3–5 turns in a clockwise or counterclockwise direction, withdrew the brush, and pushed the brush head of the specimen collector into the specimen-fixing solution. Real-time fluorescence quantitative polymerase chain reaction (PCR) was used to design specific primers and probes for the 21 most common HPV subtypes, including 18 of the hr-HPV subtypes (i.e. HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and three of the low-risk HPV (lr-HPV) subtypes (i.e. HPV 6, 11, and 81), which were mainly targeted at the L1 region of the HPV genome. The number of copies of single-copy genes encoding DNA topoisomerase III in the exfoliated cells was calculated, and the HPV viral load (copy number/10^4 cells) in the same number of cells was reported.

The scraped specimens were evenly coated onto clean glass slides, fixed with 95% ethanol, and stained with Papanicolaou stain. The diagnostic criteria for TCT were defined in accordance with the Bethesda System of Classification: no intraepithelial lesions or malignant lesions (NILM), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, except for highly squamous intraepithelial lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). In the present study, patients with gradings other than NILM on the TCT test were defined as positive for HPV.

Cervical biopsy under colposcopy and histopathological diagnosis of cervical canal were undertaken for women positive for TCT with hr-HPV infection. According to the pathological results of the cervical lesions, the patients were stratified into three grades: chronic cervicitis/CIN I, CIN II/CIN III, and cervical cancer.

The viral load of HPV DNA was transformed into the log10 value before statistical analysis. Statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and $P<0.05$ was considered statistically significant. A comparative analysis of the positive rates was undertaken with the χ^2 test. The differences of viral loads between different cervical lesions were evaluated by Least Significant Difference (LSD) or the Dunnett T_3 post hoc test after one-way analysis of variance in SPSS. The correlation between the viral load and cervical pathological grade was evaluated by Spearman correlation analysis. The ROC curve method was used to evaluate the predictive efficiency of the HPV viral load, the sensitivity and specificity of diagnosis, and to explore the threshold for shunting of common HPV genotype viral loads in patients positive for HPV.

The research protocol was approved by the ethics committee of Shanxi Provincial People's Hospital (Taiyuan, Shanxi, China).

3 | RESULTS

In the present study, 27 284 women were screened for cervical cancer using the HPV test and 4783 (17.53%) were positive for HPV. However, 3728 patients, including 3364 patients with single HPV infection and 364 with multiple HPV infections, were selected for the present study. The age range of study participants was 18–83 years (mean age 45.5 ± 13.6 years).

There were 3364 cases of HPV single-type infection, wherein 78.3%, 18.7%, and 3.0% were graded as chronic cervicitis/CIN I, CIN II/CIN III, and cervical cancer, respectively. Of the 364 patients with multiple HPV genotype infections, 51.6%, 42.6%, and 5.8% had chronic cervicitis/CIN I, CIN II/CIN III, and cervical cancer, respectively. There were significant differences in the quantities of single infections and multiple infections for the different cervical pathological grades (χ^2 127.21, P<0.001; Table 1).

The most common genotype identified in the single HPV type infections was HPV 16, followed by HPV 52, 58, and 53. The HPV genotypes in chronic cervicitis, CIN I and CIN II, and CIN III were HPV 16, 52, 58, and 53. The percentages of HPV 16, 52, 58, and 53 infections in chronic cervicitis/CIN I were 17.05%, 12.91%, 9.08%, and 8.89%, respectively. Moreover, the percentages of HPV 16, 52, 58, and 53 infections in CIN II/CIN III were 32.22%, 10.32%, 8.41%, and 5.87%, respectively. The common HPV genotypes in cervical cancer were HPV 16 and HPV 18 (81.19% and 6.93%, respectively; Table 2).

TABLE 1 Analysis of single and multiple HPV infection and cervical pathological grade.[a]

HPV infection status	No. of cases	Distribution of cervical pathological grading			χ^2	P
		Chronic cervicitis/CIN I	CIN II/CIN III	Cervical carcinoma		
Single infection	3364	2633 (78.3)	630 (18.7)	101 (3)	127.21	<0.001
Multiple infection	364	188 (51.6)	155 (42.6)	21 (5.8)		

Abbreviation: CIN, cervical intraepithelial neoplasia.
[a]Values are given as number (percentage).

TABLE 2 Proportion of single HPV subtypes in different pathological grades of cervical lesions.[a]

Types of HPV	Total number of single infections	Chronic cervicitis/CIN I	CIN II/CIN III	Cervical cancer
16	734	449 (17.05)	203 (32.22)	82 (81.19)
52	406	340 (12.91)	65 (10.32)	1 (0.99)
58	294	239 (9.08)	53 (8.41)	2 (1.98)
53	271	234 (8.89)	37 (5.87)	0 (0)
39	157	153 (5.81)	4 (0.63)	0 (0)
81	175	146 (5.55)	28 (4.44)	1 (0.99)
56	172	140 (5.32)	32 (5.08)	0 (0)
66	157	129 (4.9)	28 (4.44)	0 (0)
51	138	114 (4.33)	24 (3.81)	0 (0)
59	121	106 (4.03)	14 (2.22)	1 (0.99)
68	125	99 (3.76)	26 (4.13)	0 (0)
18	134	96 (3.65)	31 (4.92)	7 (6.93)
33	120	93 (3.53)	25 (3.97)	2 (1.98)
31	94	80 (3.04)	11 (1.75)	3 (2.97)
35	86	59 (2.24)	27 (4.29)	0 (0)
6	56	49 (1.86)	7 (1.11)	0 (0)
45	33	31 (1.18)	2 (0.32)	0 (0)
11	33	31 (1.18)	2 (0.32)	0 (0)
82	33	28 (1.06)	4 (0.63)	1 (0.99)
73	18	15 (0.57)	3 (0.48)	0 (0)
26	7	2 (0.08)	4 (0.63)	1 (0.99)
Total	3364	2633	630	101

Abbreviation: CIN, cervical intraepithelial neoplasia.
[a]Values are given as number (percentage).

TABLE 3 Analysis of HPV viral load and cervical pathological grade.[a]

Types of HPV	No. of cases	Viral load in chronic cervicitis/CIN I	Viral load in CIN II/CIN III	Viral load in cervical cancer	P_1[b]	P_2[c]
6	56	3.49 ± 1.22	3.76 ± 1.84	—	0.612	—
11	33	3.76 ± 1.76	4.01 ± 1.22	—	0.846	—
16	734	3.51 ± 1.21	4.35 ± 1.03	5.04 ± 0.63	<0.001[d]	<0.001[d]
18	134	3.52 ± 1.53	4.22 ± 1.16	5.68 ± 0.30	0.028[d]	<0.001[d]
26	7	2.44 ± 0.63	4.22 ± 1.16	3.58	0.110	—
31	94	3.81 ± 1.48	6.00 ± 0.83	5.13 ± 1.05	<0.001[d]	0.349
33	120	3.57 ± 1.39	4.26 ± 1.28	5.34 ± 0.09	0.028[d]	0.286
35	86	3.71 ± 1.49	3.81 ± 1.40	—	0.767	—
39	157	3.87 ± 1.60	4.57 ± 2.87	—	0.053	—
45	33	3.21 ± 0.96	3.41 ± 0.35	—	0.780	—
51	138	3.92 ± 1.42	4.83 ± 1.38	—	0.005[d]	—
52	406	3.54 ± 1.28	4.08 ± 1.33	3.25	0.008[d]	—
53	271	3.78 ± 1.53	4.45 ± 1.47	—	0.015[d]	—
56	172	3.83 ± 1.66	4.19 ± 1.76	—	0.267	—
58	294	3.68 ± 1.39	4.55 ± 1.10	4.68 ± 1.03	<0.001[d]	0.897
59	121	3.87 ± 1.81	3.99 ± 1.69	2.18	0.622	—
66	157	3.66 ± 1.60	4.19 ± 1.79	—	0.124	—
68	125	3.75 ± 1.65	4.45 ± 1.50	—	0.052	—
73	18	3.56 ± 1.79	4.34 ± 2.28	—	0.517	—
81	175	3.41 ± 1.60	3.55 ± 1.24	6.50	0.132	—
82	33	3.25 ± 1.44	3.07 ± 1.37	3.07	0.969	—

Abbreviation: CIN, cervical intraepithelial neoplasia.
[a]Values are given as mean ± standard deviation.
[b]Differences in viral load between chronic cervicitis/CIN I and CIN II/CIN III.
[c]Differences in viral load between CIN II/CIN III and cervical cancer.
[d]Statistically significant differences in viral loads among different cervical pathological grades ($P<0.05$).

In infections with the genotypes HPV 16 and 18, there were significant differences in viral loads among the different cervical pathological grades ($P<0.05$). There was a positive correlation between the viral load and the cervical pathological grade, with correlation coefficients of 0.441 and 0.343, respectively. In infections with HPV 31, 33, 51, 52, 53, and 58, there were significant differences in viral load between chronic cervicitis/CIN I and CIN II/CIN III ($P<0.05$), and the viral loads positively correlated with the cervical pathological grade (correlation coefficients of 0.442, 0.256, 0.234, 0.142, 0.156, and 0.265, respectively). There were no significant differences in viral load among different cervical pathological grades for infections with other HPV genotypes (HPV 6, 11, 26, 35, 39, 45, 56, 59, 66, 68, 73, 81, and 82; $P>0.05$; Tables 3 and 4).

When the viral loads of HPV 31, 18, 33, and 16 were higher than the critical value, the sensitivity of predicting CIN II+ was 98%, 92.1%, 77.8%, and 70.5%, respectively (Table 5 and Fig. 1).

4 | DISCUSSION

Cervical cancer is the second most common malignant tumor and the leading cause of cancer-related deaths in women worldwide.[17] The hr-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 59 are closely related to cervical cancer and precancerous lesions.[18–20] In the present study, fluorescence quantitative PCR was used to detect the HPV genotype and viral load in cervical exfoliated cells.

Because there are many prevalent HPV types, patients may have single-type or multi-type HPV infections. There are currently different theories on the development of cervical lesions by multi-type HPV infections. Mazarico et al.[21] reported that multi-type HPV infections promote the development of cervical lesions and the occurrence of cervical cancer, and there are HPV-related factors that affect the prognosis of cervical cancer, such as the type of cancer cells and the depth of cervical interstitial infiltration among others. However, there are counter-perspectives that multi-type HPV infections will not increase the incidence of cervical cancer and that the severity of cervical lesions is unrelated to the number of HPV infection types, but rather, is related to the virulence of the HPV genotype.[22] The present study compared the relationship between single- and multi-type HPV infections and different cervical pathological grades and obtained findings that were consistent with the results reported by Mazarico et al.[21] There were significant differences in the distribution of single- and multi-type HPV infections among the different cervical pathological

TABLE 4 Correlation between viral load and cervical pathological grade.

Types of HPV	No. of cases	Correlation coefficient	*P*
6	56	−0.002	0.990
11	33	0.040	0.825
16	734	0.441	<0.001
18	134	0.343	<0.001
26	7	0.219	0.637
31	94	0.442	<0.001
33	120	0.256	0.005
35	86	0.045	0.681
39	157	0.116	0.149
45	33	0.067	0.712
51	138	0.234	0.006
52	406	0.142	0.004
53	271	0.156	0.010
56	172	0.084	0.272
58	294	0.265	<0.001
59	121	−0.014	0.883
66	157	0.116	0.152
68	125	0.170	0.058
73	18	0.101	0.691
81	175	0.093	0.222
82	33	−0.039	0.828

grades. Thus, it can be concluded that single-type infections occur more frequently than multi-type HPV infections in chronic cervicitis/CIN I, whereas multi-type HPV infections are more likely to occur than single-type infections in CIN II/CIN III and cervical cancer. Therefore, with the increased degree of cervical pathological changes, the number of types of HPV infection also increases. Thus, multi-type HPV genotype infections are more likely to aggravate the degree of cervical pathological grade than a single-type HPV infection. This suggests that the number of HPV types is related to the development of cervical lesions. The phenomenon of multiple HPV infections indicates the involvement of a recombination mechanism in the evolution of HPV, and gene recombination is an important mechanism to accelerate the aggressive and carcinogenic evolution of HPV and, thus, to form a new HPV genotype with a higher pathogenicity.

Differences in rates of HPV infection are observed in various regions, and the distribution of HPV subtypes in different regions and different populations is quite different. The present study evaluated the rate of infection and distribution of different HPV subtypes and the results showed that the most common HPV type was HPV 16, followed by HPV 52, 58, and 53. The common genotypes of HPV infection in chronic cervicitis, CIN I and CIN II, and CIN III were HPV 16, 52, 58, and 53. The percentages of HPV 16, 52, 58, and 53 infections in chronic cervicitis and CIN I were 17.05%, 12.91%, 9.08%, and 8.89%, respectively, whereas the percentages of HPV 16, 52, 58, and 53 infections in CIN II and CIN III were 32.22%, 10.32%, 8.41%, and 5.87%, respectively. The most common HPV infection types in cervical cancer were HPV 16 and HPV 18 (81.19% and 6.93%, respectively). Studies have shown that HPV 16 and 18 were the most common HPV carcinogenic subtypes. Cervical cancer caused by these two subtypes accounted for 85% of cervical cancers in Chinese women,[23] which is consistent with the results of the present study.

Studies have shown that the viral load of hr-HPV is related to the severity of cervical lesions[24]; therefore, the HPV viral load is recommended to be a potential biomarker for candidates. The present study analyzed the relationship between the viral load of 18 hours-HPV subtypes and three lr-HPV subtypes and the degree of cervical pathological grade. The results showed that there were significant differences in viral load among different cervical pathological grades in HPV 16 and 18 infections ($P<0.001$ and $P=0.028$, respectively). There was a positive correlation between viral load and cervical pathological grade. Therefore, it can be inferred that the viral load in CIN II/CIN III was higher than that in chronic cervicitis/CIN I, and the viral load of cervical cancer was higher than that of CIN II/CIN III. The viral loads of HPV 31, 33, 51, 52, 53, and 58 showed significant differences between chronic cervicitis, CIN I and CIN II, and CIN III ($P<0.001$, $P=0.028$, $P=0.005$, $P=0.008$, $P=0.015$, and $P<0.001$, respectively). The viral load is positively correlated with the cervical pathological grade; therefore, it could be

TABLE 5 Predictive effect of HPV viral load on cervical lesions.

Types of HPV	Best critical point (cut-off)	Sensitivity (%)	Specificity (%)	Area under the curve	95% CI
31	3.311E + 004 (4.52)	98.00	66.20	0.863	0.784 – 0.942
18	5.240E + 002 (2.72)	92.10	34.40	0.638	0.539 – 0.737
33	5.248E + 003 (3.72)	77.80	57.00	0.673	0.559 – 0.788
16	1.175E + 004 (4.07)	70.50	65.50	0.743	0.708 – 0.778
58	2.951E + 004 (4.47)	63.60	72.00	0.696	0.627 – 0.765
53	5.012E + 004 (4.70)	59.50	71.40	0.629	0.533 – 0.725
51	2.089E + 005 (5.32)	50.00	81.60	0.678	0.560 – 0.797
52	7.413E + 004 (4.87)	37.90	85.30	0.612	0.536 – 0.688

Abbreviation: CI, confidence interval.

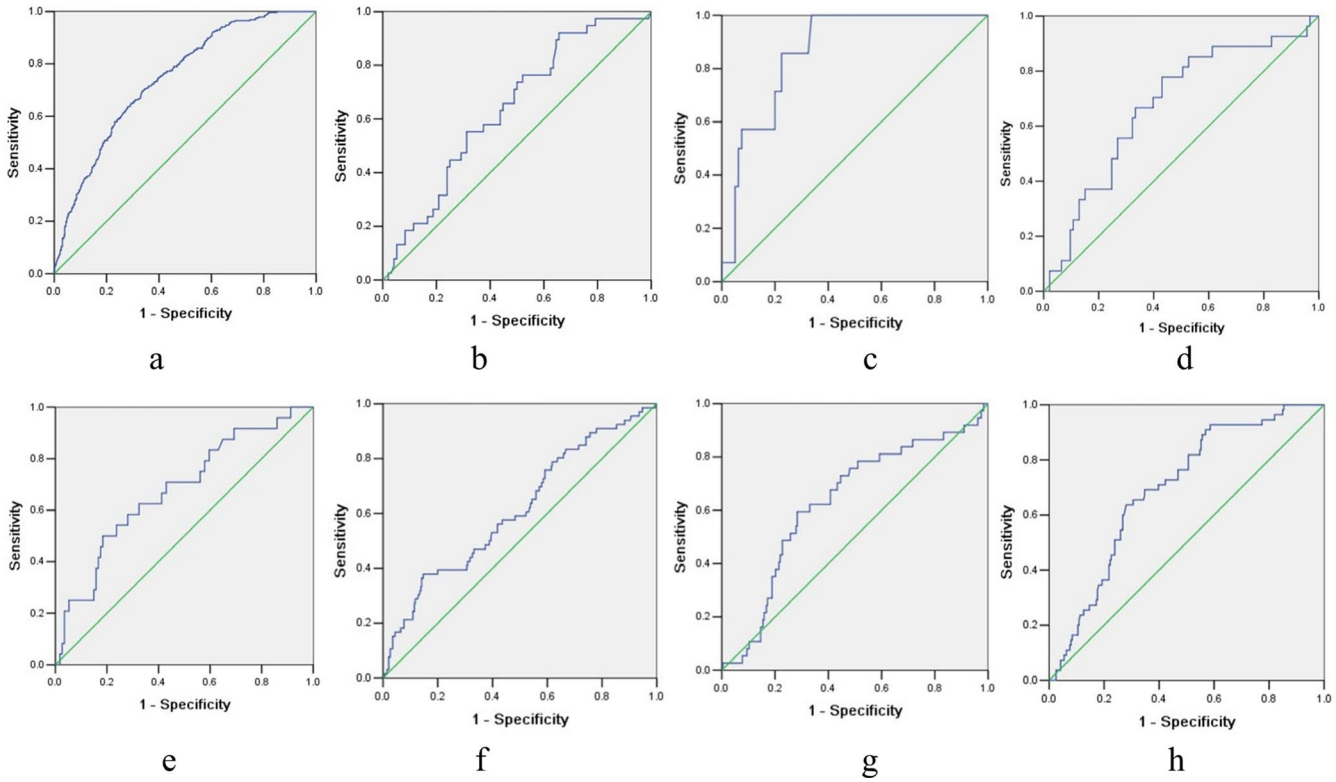

FIGURE 1 Receiver operating characteristics curves of HPV genotype viral loads: (a) HPV 16; (b) HPV 18; (c) HPV 31; (d) HPV 33; (e) HPV 51; (f) HPV 52; (g) HPV 53; (h) HPV 58

considered that the viral load in CIN II/CIN III was higher than that in chronic cervicitis/CIN I. However, in infections with HPV 31, 33, 51, 52, 53, and 58, there were no significant differences in the viral loads between cervical cancer and CIN II/CIN III, and this may be related to the low incidence of HPV 31, 33, 51, 52, 53, and 58 in the pathogenesis of cervical cancer.

Hr-HPV infection promotes the progression of CIN into cervical cancer. The risk of carcinogenesis of cells in patients with hr-HPV is approximately 300 times higher than that in uninfected patients. Therefore, early detection, early diagnosis, and early treatment are of great significance in improving the rate of survival of patients with cervical cancer. With the development of methods of screening, detection of HPV DNA combined with TCT and cervical biopsy under colposcopy have been gradually applied in clinical practice. As an increasing number of patients with precancerous lesions are identified and receive treatment, the prognosis of patients with cervical cancer has significantly improved. In routine clinical practice, it is necessary to determine the critical value of the HPV viral load to enable accurate prediction of CIN II+ and selection of patients for treatment. In the present study, the predictive value of viral loads of HPV 16, 18, 31, 33, 51, 52, 53, and 58 was evaluated. The results showed that when the viral loads of HPV 31, 18, 33, and 16 were higher than their critical values, the sensitivity of predicting CIN II+ was 98%, 92.1%, 77.8%, and 70.5%, respectively. By obtaining the appropriate thresholds of the viral loads of HPV 16, 18, 31, 33, 51, 52, 53, and 58, the appropriate risk and optimal management can be predicted for CIN II+.

The present study has some potential limitations. The detection of viral DNA by PCR has been the gold standard for the diagnosis of HPV, although the results may be misleading because the detection of DNA can only indicate the presence of the virus but not the active phase of the viral expression of oncoprotein. Therefore, understanding the gene expression pattern of HPV during the progression of the disease will help address the ineffectiveness of DNA testing. Therefore, methods of mRNA detection to analyze the expression of protein types and levels could provide insights for treating infections on a clinically meaningful basis.

In conclusion, multi-type HPV infections are more likely to aggravate the degree of cervical lesions than single-type infections, and the HPV type-dependent viral load is associated with cervical pathological grade. Thus, the treatment and monitoring of patients with hr-HPV infection, especially those with multi-type mixed hr-HPV infections and high viral loads, should be strengthened.

AUTHOR CONTRIBUTIONS

YFL contributed to the literature search, study design, study conduct, data analysis, and drafting of the manuscript. HRW and YBH performed the study experiments. NW and YJZ contributed the initial material and data collection. XJ contributed to the study design, data collection, and data analysis. CHH conducted the statistical analysis and edited the manuscript. All authors were involved in the manuscript revision and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors have no competing or financial interests.

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