

Original Article

Viral DNA load of high-risk human papilloma virus is closely associated with the grade of cervical lesions

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Abstract: This study is to explore the correlation between the viral load of high-risk human papilloma virus (HPV) and the degree of cervical lesions, as well as the follow-up monitoring role of high-risk HPV measurements in the treatment of patients with cervical lesions. Hybrid capture-2 method was used to measure the amount of high-risk HPV load of 361 patients who were enrolled from January 2009 to December 2010 at the Affiliated Tumor Hospital of Xinjiang Medical University, including 76 cases of cervical squamous carcinoma, 119 cases of cervical intraepithelial neoplasia and 166 cases of cervicitis. The correlation between the viral load of high-risk HPV and the degree of cervical lesions was analyzed using correlation analysis. Patients with cervical intraepithelial neoplasia (CIN) and cervical squamous carcinoma were followed up until December 2013, with the follow-up time being 37-60 months. Statistically significant differences in the high-risk HPV load existed between cervicitis group, CIN group and cervical squamous carcinoma group ($P = 0.000$). In addition, the viral load was increased with the increase of the severity of cervical lesions, showing a positive correlation ($r = 0.436$, $P = 0.000$). During the follow-up, 6 cases of vaginal intraepithelial neoplasia, 3 cases of recurrence CIN and 1 case of vaginal squamous cell carcinoma of the vulva were found, which were shown to relate with the continuing high-risk HPV infection in vagina. Viral load of high-risk HPV were positively correlated with the severity of cervical lesions, playing an important role in the monitoring of patients with cervical lesions after treatment.

Keywords: Cervical squamous carcinoma, cervical intraepithelial neoplasia, human papilloma virus, viral load

Introduction

The incidence of cervical cancer is second only to that of breast cancer in women [1, 2]. A large number of epidemiological and basic researches showed that human papilloma virus infection is the major cause of cervical intraepithelial neoplasia (CIN) and cervical cancer. The incidence of cervical cancer is related to the prevalence of human papillomavirus (HPV). Generally, 50% women have HPV infection within two years after the first sexual life. In China, the positive rate of HPV in cervical cancer is about 87.7% [3], with this rate being 90.4% in sub-Saharan Africa [4]. After high-risk HPV infection, only a few cases can develop into cervical malignant tumor, while some cases have long latency period. Sustained infection by HPV is required for the occurrence of cervical cancer.

The abnormality of genomes in host cells plays important roles in the pathogenic process of high-risk HPV, by sustaining high-risk HPV infection and facilitating the occurrence and development of tumors. Cervical squamous cell carcinoma accounts for 80% of cervical cancer, with a reversible progressive precancerous lesion period. Therefore, the early diagnosis of precancerous cervical lesions is important for the prevention of cervical cancer. High-risk HPV infection is the direct cause for cervical squamous cell carcinoma and CIN. However, the relationship between HPV viral load and the degree of cervical lesions is still controversial. By analyzing the clinical data of cervicitis, CIN and cervical cancer patients, this study aims to explore the correlation of viral load of high-risk HPV with the severity of cervical squamous intraepithelial lesions, as well as the role of

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Table 1. Positive distribution rates of high-risk HPV in cervicitis, CIN and squamous cervical cancer groups

Diagnosis	No. of cases	No. of positive HR-HPV cases	No. of negative HR-HPV cases	χ^2	P values
Cervicitis	166	73	43.98		
CIN	119	102	85.71	83.958	0.000
Squamous cervical cancer	76	71	93.42		

Note: HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia.

Table 2. Comparison of viral loads of high-risk HPV among cervicitis, CIN and squamous cervical cancer groups

Diagnosis	HPV semi-quantitative values	Z values	P values
Cervicitis	114.51 ± 338.90	14.659	0.000
CIN	393.31 ± 585.41		
Squamous cervical cancer	648.43 ± 736.96		

Note: HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia. Data are means ± SD.

high-risk HPV measurement in the follow-up monitoring of patients with cervical lesions after treatment.

Materials and methods

Subjects

A total of 361 patients (23-83 years old) with cervical cytology-prompted atypical squamous cells of undetermined significance (ASCUS) were studied from January 2009 to December 2010 in the Department of Gynecological Surgery at the Affiliated Tumor Hospital of Xinjiang Medical University, including 170 cases of Han, 151 cases of Uygur, 12 cases of Hui, 12 cases of Kazak, 7 cases of Mongolian, 2 cases of ethnic Russian and 2 cases of Man. All of the 361 patients underwent high-risk HPV-DNA semi-quantitative measurement, colposcopy and biopsy. Among them, 166 cases were diagnosed as cervicitis, 119 cases were diagnosed as CIN, and 76 cases were diagnosed as cervical squamous carcinoma, according to pathological investigations. Written informed consent was obtained from all subjects. All experiments were approved by the Ethics Committee of Xinjiang Medical University.

Pathological examination

Cervical cytology was carried out by ThinPrep cytology test (TCT). Cytological diagnosis was made according to the Bethesda system clas-

sification. Histological diagnosis of cervix was made according to the criteria proposed by the 7th edition of "Obstetrics and Gynecology". The biopsy of pathological sections was reviewed by senior physicians.

Vaginoscopy

Cervical biopsy was carried out in suspicious lesion areas using Olympus electronic colposcopy. If no abnormality was found or the image was dissatisfied, multiple biopsies or endocervical curettage were performed.

Semi-quantitative measurement of high-risk HPV-DNA

HPV-DNA testing was carried out using the second generation of hybrid capture signal amplification gene detection system (hybrid capture-2) developed by Digene (USA). The test results were determined using the following criteria: positive, ≥ 1.0 mg/L; negative, < 1.0 mg/L. All specific values were recorded.

Follow-up

Follow-up was carried out once every 3 months during the postoperative two years, and once every six months from the third year to the fifth year.

There were 69 cases of cervical squamous carcinoma patients, including 4 cases at stage I a1, 5 cases at stage I a2, 11 cases at stage I b1, 8 cases at stage I b2, 22 cases at stage II a1 and 19 cases at stage II a2. According to another classification method, the 69 cases were classified into 14 cases of well differentiated squamous carcinoma, 30 cases of moderately differentiated squamous carcinoma and 25 cases of poorly differentiated squamous

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Table 3. The clinical data of CIN patients

Primary disease	CIN III	CIN III [#]	CIN II ^{##}
Surgery time	August 2009	January 2010	September 2012
Surgery method	Cold knife conization	Loop electrosurgical excision procedure	Loop electrosurgical excision procedure
HR-HPV values before surgery	587.35	1149.02	706.14
Time of CIN diagnosis	CIN II in March 2012	CIN II in April 2013	CIN I in June 2013
High-risk HPV values of recurrence	79.69	1530.83	570.0
Time of the continuing high-risk HPV infection	14 months	19 months	8 months
Treatment	Cervicectomy	Extrafascial hysterectomy	Extrafascial hysterectomy
Results of patients until the end of follow-up	TCT (-) HPV 8.41	TCT (-) HPV 0.33	Three months after surgery

Note: HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia. [#], the patient (43 years old) also had adenomyosis of uterus and heavier symptom, with extrafascial hysterectomy being performed during recurrence. ^{##}, the patient (50 years old) had menopause for 6 years, with the uterus being removed as insisted.

carcinoma. All patients received different surgical methods for treatment, including 2 cases of cervical cold knife conization, 2 cases of extrafascial hysterectomy, 12 cases of radical resection of cervix and pelvic lymph node dissection, and 53 cases of radical hysterectomy and pelvic lymph node dissection with or without abdominal aortic side lymph node dissection.

There were 112 cases of CIN patients, including 12 cases at stage I, 43 cases at stage II and 57 cases at stage III. Among patients at stage III, 11 patients with other benign uterine disorders received extrafascial hysterectomy, 8 patients underwent loop electrosurgical excisional procedure, and 38 patients received cervical cold knife conization. All patients at stages I and II received loop electrosurgical excisional procedure.

Statistical analysis

All statistical analyses were performed using SPSS version 13.0 (USA). Differences between each group were compared using the χ^2 test. The measurement data were presented as means \pm SD. Completely randomized comparison of multiple groups, if meeting the normality, was performed using homogeneity of variance, and then analyzed by variance. If it did not meet these conditions, the analysis was performed using rank sum test. The relationship between variables was studied by correlation analysis. $P < 0.05$ was considered statistically significant.

Results

The viral load of high-risk HPV is positively correlated with cervical lesions

To determine the correlation between viral load of high-risk HPV and cervical lesions, we first

investigated the positive distribution rates of high-risk HPV in cervicitis, CIN and squamous cervical cancer groups, and then compared the viral loads of high-risk HPV between these groups. The high-risk HPV-positive rate was increased as the severity of cervical lesions was increased ($P = 0.000$) (**Table 1**). In addition, the viral load was also increased with the increase of the severity of cervical lesions ($r = 0.436$, $P = 0.000$) (**Table 2**). These data suggested that a positive correlation existed between viral load of high-risk HPV and cervical lesions.

High-risk HPV infection is a risk factor for recurrence

To investigate the role of high-risk HPV measurement in the follow-up monitoring of patients with cervical lesions after treatment, 112 cases of CIN and 69 cases of cervical squamous carcinoma were followed up before December 2013, with the follow-up duration being from 37 months to 60 months. Among these patients, 6 cases of vaginal intraepithelial neoplasia (VAIN), 3 cases of recurrence CIN and 1 case of vaginal squamous cell carcinoma of the vulva were found (**Tables 3** and **4**). These clinical data analysis demonstrated that the continuing high-risk HPV infection was a risk factor for cervical lesions.

Discussion

High-risk HPV infection is the direct cause of cervical squamous carcinoma and CIN. Multicenter studies in Southeast Asia showed that [5] the detection rate of high-risk HPV in cervical cancer patients was 94%-98.7%, and that in CIN II/III patients was 93.7%-100%. Multicenter studies in Portugal also found that

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Table 4. The clinical data of vaginal intraepithelial neoplasia (VAIN) patients

	CIN III	CSCC II a2	CSCC I a2 [#]	CSCC II a1	CSCC II a1	CSCC I b1
Operation time	Aug. 2009	Jan. 2009	Jan. 2009	Feb. 2009	Mar. 2010	May. 2010
Preoperative high-risk HPV	374.96	807.36	1084.62	380.25	1430.04	951.07
VAIN diagnosis time	VAIN II in Mar. 2012	VAIN I in Nov. 2010	VAIN III in Oct. 2011 VAIN III recurrence in Jun. 2012	VAIN III in Jun. 2011	VAIN III in Feb. 2011	VAIN III in Apr. 2013
High-risk HPV value in VAIN	0.87	1530.83	1520.92 1154.61 during 2nd surgery	53.46	5.38	947.24
Continuous infection time by high-risk HPV	-	5 months	42 months	18 months	- ^{##}	35 months
Therapies	Laser therapy	Interferon therapy	Resection by 2 surgeries	Resection by surgery	Resection by surgery	Resection by surgery
Patient outcomes after follow-up	TCT (-) HPV 0.26	HPV 0.33 and TCT (-) in Apr. 2011; Died of intestinal obstruction in Jun. 2012	TCT (-) HPV 5.33	TCT (-) HPV 1.35	TCT (-) HPV 0.51	TCT (-) HPV 54.3

Note: CIN, cervical intraepithelial neoplasia; CSCC, cervical squamous cell carcinoma. [#], this patient had sustained infection by high-risk HPV before and after surgery. In month 22 after the surgery, 2 × 2.5 erosion surface was found on the left side of vaginal cuff. TCT result indicated squamous cell carcinoma and colposcopy confirmed VAIN III. After surgical resection, TCT result was negative, but the patient still had infection by high-risk HPV. Seven months later, VAIN III was diagnosed again before surgical treatment was carried out. ^{##}, This patient had a semi-quantitative HPV-DNA value of 0.25 and negative TCT result 3 months after the surgery. Eleven months after the surgery, high-risk HPV-DNA was 5.38, TCT result was HSIL, and VAIN III was diagnosed by colposcopy. Time of sustained infection by high-risk HPV was unclear.

[6] the total detection rate of high-risk HPV in patients with CIN stage II and above was 97.9%, that in invasive cervical cancer patients was 96.9%, that in CIN III patients was 99.4%, and that in CIN II patients was 95.5%. In this study, the positive rate of high-risk HPV was 43.98% in the cervicitis group, 85.71% in the CIN group and 93.42% in the cervical carcinoma group, with statistically significant differences between these three groups ($P = 0.000$), indicating that the positive rate of high-risk HPV was increased with the increase of the severity of cervical lesions.

The removal of high-risk HPV was related to the viral load level of high-risk HPV [7, 8]. In general, over 90% of HPV infections may subside on their own within two years, and low copy number of high-risk HPV can be easily cleared by human immune system. Dalstein *et al* [9] performed hybrid capture-2 assay and found that in 5% to 10% of cases, cervical lesions could occur in women patients with continuing high-risk HPV infection for over 12 months. In addition, at a low viral load of HPV-DNA below 10 pg/mL, the high-risk HPV was be easily cleared by the immune system. By contrast, the continuing high-risk HPV infection persisted, when the viral load was ≥ 10 pg/mL. Studies also showed that [10, 11] high levels of HPV-DNA loads might promote mild cervical lesions to severe disease, and the cumulative incidence rate of CINII/III patients was gradually increased with the increase of viral load, suggesting a significantly positive correlation between viral load and the severity of cervical lesions. Cervical cancer studies in Xinjiang Uygur showed that [12] higher viral load led to higher stage of cervical cancer. These results indicated that high-risk HPV-DNA load was an effective risk predictor in the development of cervical cancer.

Vaginal intraepithelial neoplasia (VAIN), a rare precancerous lesion of the lower genital tract, accounts for 1% of female lower genital tract epithelial neoplasia. The occurrence of VAIN is related to HPV infection, abnormal Pap smear, a history of genital warts, a history of radiation therapy and low immune function [13, 14]. HPV16 is detected in about 70% VAIN cases, with high-risk HPV infection probably being the main cause of VAIN [11, 12]. High-risk HPV infects the vagina at the same time when it infects the cervix. Squamous metaplasia

occurs during vaginal epithelium healing when HPV infection occurs. Because no clinically significant symptom appears, missed diagnosis may occur. In the follow-up of this study, we found that the high-risk HPV DNA load in most patients before the surgery was more than 100, and the reduced postoperative HPV viral load indicated good prognosis. If positive results were observed for postoperative high-risk HPV, recurrence of the lesions was likely to happen.

In conclusion, viral load and continuing high-risk HPV infection are not only important causes of cervical cancer, but also effective indicators for the follow-up after treatment of cervical lesions. Cases from the high incidence area of cervical cancer in Xinjiang were mainly patients with advanced cancers. High-risk HPV-DNA testing may help identify the high-risk group, and strengthen the monitoring and management of this group, which may reduce cancer mortality in this region.

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Disclosure of conflict of interest

None.

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