Australian and New Zealand Journal of Obstetrics and Gynaecology 2007; **47:** 141–144

Original Article

High-risk human papillomavirus DNA testing and high-grade cervical intraepithelial lesions

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Abstract

Objective: To explore the role of high-risk human papillomavirus (HPV) DNA testing in the improvement of the recognition of cervical cancer and precancerous lesions in women with abnormal cervical cytology.

Methods: A total of 2152 women with abnormal cervical cytology were submitted to both HPV DNA testing and biopsy guided by colposcopy and the results were correlated.

Results: Positive rate of high-risk HPV DNA in groups of atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, cannot exclude high-grade (ASC-H), low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions was 53.7, 53.2, 84.6 and 93.0%, respectively. In each group, the detection rate of grade 2,3 cervical intraepithelial neoplasia (CIN 2,3) or cervical cancer in patients with positive HPV DNA was significantly higher than that with negative HPV DNA ($P < 0.05$). In ASC-US group, the negative predictive value of high-risk HPV DNA testing for detection of CIN 2,3 and cervical cancer was 99.8% and the sensitivity 98%.

Conclusion: HPV DNA testing is a useful indicator in the management of patients with ASC-US and plays an important role in the evaluation of risk for CIN 2,3 and cervical cancer.

Key words: CIN, HPV, liquid-based cytology.

Introduction

Cervical cancer is one of the most common malignancies seriously threatening women population. There were about 466 000 new cases every year in the world, of which one third were in China.[1] Therefore, the prevention and treatment of cervical cancer in China are enormous. As early diagnosis of cervical cancer or precancerous lesions attributes to a better prognosis and better quality of living for patients, researchers and clinicians have been exploring an accurate and easy screening test to detect cervical cancer at early stage.

Since the application of cervical cytology test for screening of cervical cancer, incidence and morbidity of cervical cancer were decreased dramatically.[2,3] The liquid-based cytology, an improved cervical cytological technique, has recently become available in China. However, it was only a primary screening test, so in conditions of abnormal cervical cytology, clinical management is needed to confirm cervical cancer or precancerous lesions.[4] Biopsy guided by colposcopy is a common method that gynaecologists rely on, which, however, is invasive, more expensive and inconvenient.

Epidemiological and virological studies have clearly demonstrated that specific human papillomavirus (HPV) infection is a principal pathogenesis factor of cervical cancer.[5] Persistent exposure to high-risk HPV DNA is necessary for cervical precancerous lesions developing into cancer.[6] The detection of high-risk HPV may be useful for women with abnormal cytology. In China, studies on the role of HPV DNA testing are few.

We did a retrospective study on 2152 specimens of Chinese women who were confirmed as abnormal with liquid-based cytology testing (LCT). HPV DNA testing and biopsy guided by colposcopy were conducted on all the specimens and the results were correlated to evaluate the combination method for its maximum effectiveness and convenience of application.

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DOI: 10.1111/j.1479-828X.2007.00701.x

Received 09 September 2006; accepted 31 December 2006.

Methods

Samples

This retrospective study included patients from the Department of Gynecology, the Third Hospital of Peking University and the Maternal and Child Health Hospital of Haidian District of Beijing from October 2004 to February 2006. Patients first received LCT, and if abnormal, they were suggested to undergo HPV DNA testing and colposcope-directed biopsy to confirm possibility of grade 2,3 cervical intraepithelial neoplasia (CIN 2,3) or cervical cancer. Altogether, 2152 patients (out of about 20 000 patients) (aged 18–81 years, mean age 38.1 years) with abnormal cervical cytological results and further supplemented with biopsy and HPV DNA testing were included in this study.

LCT was adopted using the PrepStain™ system (Tripath Imaging Inc., Burlington, NC, USA) and was reported according to the 2001 terminology of Bethesda System (TBS). Each Pap smear was reviewed by an expert pathologist with more than ten years experience. If any obscurity arises, two pathologists would discuss to make a decision. Of the 2152 patients, 1171 (54.4%) had atypical squamous cells of undetermined significance (ASC-US), 109 (5.1%) atypical squamous cells, cannot exclude high-grade (ASC-H), 656 (30.5%) low-grade squamous intraepithelial lesions (LSIL), and 216 (10.0%) high-grade squamous intraepithelial lesions (HSIL).

This study was approved by the local ethics committee of Peking University Health Center.

HPV DNA test

High-risk HPV DNA testing was conducted using the commercial HC II system (Digene Diagnostics Inc., Galthersburg, MD, USA) according to the manufacturer's instructions. Results were expressed as value of relative light units (RLU) versus cut-off (CO). RLU/CO ≥ 1.0 pg/mL was considered as positive.

Statistical analysis

SPSS software package (version 12.0; SPSS Inc., Chicago, IL, USA) was used. Correlation of the HPV-positive rate and the result of biopsy was evaluated by trend χ^2. Kruskal–Wallis test was used to reveal the relation of high-risk HPV load and pathological diagnosis. Chi-squared test was performed to correlate the result of cytology with that of HPV test. $P < 0.05$ was considered significant.

Results

Relationship of high-risk HPV DNA-positive and virus load with CIN or cervical cancer

The positive rate of HPV DNA and the median of virus load of RLU/CO in CIN group were significantly higher than those in chronic inflammation group, and increased gradually with the increase of grades of CIN (Table 1, Fig. 1). There was a significant difference among groups ($P < 0.05$).

Correlation of the results of high-risk HPV DNA test and detection of CIN 2,3 or cervical cancer in patients with abnormal cytology

The histology from colposcopy revealed 21 women having invasive squamous cell carcinoma, 104 having CIN 3, 151 CIN 2, and 253 CIN 1. For the 21 cases, cytology revealed that three were ASC-US and the rest 18 were HSIL.

The positive rate of high-risk HPV DNA was 53.7% in ASC-US, 53.2% in ASC-H, 84.6% in LSIL and 93.0% in HSIL, respectively.

Correlating the results of cytology with those of biopsy and HPV DNA testing, we found that the detection rate of CIN 2,3 or cervical cancer characterised by positive HPV DNA was significantly higher than that characterised by negative HPV DNA in ASC-US, ASC-H, LSIL or HSIL groups ($P < 0.05$) (Table 2).

Biopsy-confirmed CIN 2,3 or cervical cancer was detected in 4.4% of ASC-US, 18.3% of ASC-H, 12.3% of LSIL and 57.4% of HSIL.

Table 1 Relation of high-risk human papillomavirus load and pathological diagnosis

		Virus load (pg/mL)		
Pathological diagnosis	*n*	Median	Minimum	Maximum
Chronic inflammation	1134	1.07	0	6019.29
Condyloma	489	82.04	0.07	6804.43
CIN 1	253	267.89	0.07	4589.14
CIN 2	151	392.90	0.12	4070.23
≥ CIN 3†	125	364.46	0.17	6825.73

Kruskal–Wallis $\chi^2 = 428.999$, $P < 0.01$.

n, number of patients.

†, refers to cervical intraepithelial neoplasia (CIN) 3 and invasive cervical cancer.

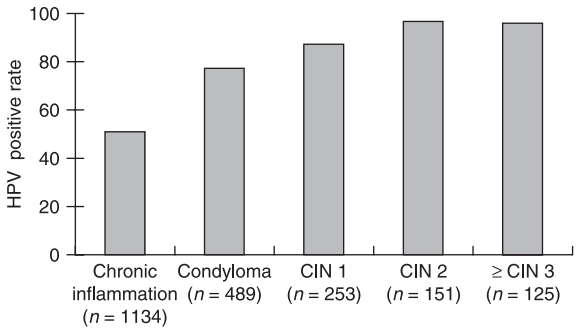

Figure 1 Positive rates of high-risk human papillomavirus (HPV) DNA in chronic inflammation (51.0%), condyloma (77.3%), cervical intraepithelial neoplasia (CIN; 87.3% in CIN 1, 96.7% in CIN 2 and 96.0% in CIN 3) and cervical cancer (96.0%). Trend $\chi^2 = 272.098$, $P < 0.01$.

Table 2 High-risk HPV DNA test and detection of CIN 2,3 or cervical cancer confirmed by biopsy in patients with abnormal cytology

		Biopsy *n* (%)			
Cytology	*n*	< CIN 2	≥ CIN 2	χ^2	*P*
ASC-US					
HPV DNA(+)	629	579 (92.1)	50 (7.9)		
HPV DNA(–)	542	541 (99.8)	1 (0.2)	42.137	< 0.01
ASC-H					
HPV DNA(+)	58	39 (67.2)	19 (32.8)		
HPV DNA(–)	51	50 (98.0)	1 (2.0)	17.181	< 0.01
LSIL					
HPV DNA(+)	555	478 (86.1)	77 (13.9)		
HPV DNA(–)	101	97 (96.0)	4 (4.0)	7.759	< 0.01
HSIL					
HPV DNA(+)	200	80 (40.0)	120 (60)		
HPV DNA(–)	16	12 (75.0)	4 (25.0)	7.422	< 0.01
Total	2152	1876	276		

ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells of undetermined significance – cannot exclude high-grade intraepithelial lesion; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

The negative predictive value of high-risk HPV DNA testing for detection of CIN 2,3 or cervical cancer in patients with ASC-US was as high as 99.8%, the sensitivity being 98%.

Discussion

This study demonstrated that in patients with ASC-US indicated by cytology, high-risk HPV DNA testing should be performed, and the positive result predicted an obvious risk of CIN 2,3 or cervical cancer. In our study, two-thirds of patients with ASC-US were confirmed as having inflammation by biopsy. Therefore, there was the potential of overdiagnosis in these patients if colposcopy was performed immediately; and there might be missed diagnosis of CIN 2,3 or cervical cancer if colposcopy was not performed. The negative predictive value of HPV DNA testing for detection of CIN 2,3 and cervical cancer was 99.8% in ASC-US group, suggesting that patients with negative HPV DNA have low risk of CIN 2,3 or cervical cancer. However, a positive HPV DNA result indicates a comparatively high risk of CIN 2,3 or cervical cancer (53.7%), and could be regarded as a signal for further pathological test. So we consider that HPV DNA testing could be used to screen out high-risk population of CIN 2,3 or cervical cancer in patients with ASC-US.

Moreover, in most reports, the sensitivity of HPV DNA testing for the detection of biopsy-confirmed CIN 2,3 in women with ASC is 0.83–1.0.[7] The American Society of Colposcopy and Cervical Pathology introduced three protocols for the management of ASC-US, namely, repeated cytology test, immediate colposcopy, and high-risk HPV DNA detection. If liquid-based cytology test is performed, it is suggested that remnant liquid-based samples be prepared for detection of high-risk HPV DNA, which will help differentiate patients of varied status so as to facilitate further management. Accurate HPV DNA testing could be helpful for the management of ASC-US in two ways. First, the type of HPV (low-risk or cancer-associated) is associated with the severity of squamous intraepithelial lesions and their natural history. Second, the presence or absence of cancer-associated types of HPV can help predict the accuracy of the original cytological diagnosis of equivocal and low-grade lesions, in that HPV-negative patients are more likely to have false-positive cytological diagnoses.[8] The present study also indicated the necessity of high-risk HPV DNA detection in patients with ASC-US. For ASC-US patients with negative HPV DNA (46.3%), about half could avoid colposcopy, saving them from the pain and cost of colposcopy. Besides, the rate of missed diagnosis of CIN 2,3 or cervical cancer can be lowered. Moreover, considering the fact that approximately 50% of the 2152 Pap tests were diagnosed as ASC-US, the combined use in clinics of cytology testing and HPV testing could save medical resources and avoid unnecessary treatment for a considerable group of patients. As LCT and HPV DNA testing are widely available in Beijing and other cities in China, their combined use is recommended.

Although the incidence of CIN 2,3 or cervical cancer confirmed by biopsy in HPV DNA-positive patients was significantly higher than that in HPV DNA-negative patients in groups of ASC-H, LSIL and HSIL, much more CIN 2,3 or cervical cancer cases were detected in these groups than in ASC-US. Therefore, being negative for HPV DNA should not be interpreted as avoidance of colposcopy in ASC-H, LSIL and HSIL groups, but all should immediately receive colposcopy. Meanwhile the rate of HPV DNA infection in LSIL was high, which coincided with the ALTS study, in which 83% of women referred for the evaluation of an LSIL cytology result tested positive for high-risk HPV types.[9] Hence high-risk HPV DNA detection was slightly helpful in the management of LSIL, and consequently patients with LSIL should receive colposcopy immediately, and HPV DNA testing could be waived.

The epidemiological and molecular biological data all confirmed that high-risk HPV DNA infection was a principal pathogenesis factor of CIN and cervical invasive cancer.[5] The rate of HPV DNA infection in patients with CIN 2 and even worse amounts to proximately 100%.[10,11] In this study, the positive rate of high-risk HPV infection and virus load was higher in patients with CIN than that in inflammation patients, and elevated with the increase of the degree of CIN.

In summary, HPV DNA test result is a useful indicator in the management of patients with ASC-US. High-risk HPV DNA test plays a very important role in the evaluation of risk of CIN 2,3 and cervical cancer and can be done on residual sample aliquots.

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