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Original Article

Performance of Aptima HPV E6/E7 mRNA Test for Detection of Cervical Lesions in a Large Chinese Population

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Abstract

Background: We aimed to examine the effectiveness of Aptima HPV E6/E7 mRNA test for detection of cervical lesions in a large Chinese population.

Methods: Overall, 4,350 women, who received simultaneously Aptima HPV E6/E7 mRNA test and HPV DNA test, followed by cervical biopsy in the Department of Gynecology of the Second Affiliated Hospital of Soochow University, Jiangsu Province, China from 2016-2020, were recruited. The detection of cervical lesions was compared between Aptima HPV E6/E7 mRNA test and HPV DNA test.

Results: Overall, HPV DNA test exhibited a higher detection of all cervical lesions than Aptima HPV E6/E7 mRNA test (P<0.05), and showed a higher efficacy for detection of normal tissues and chronic cervicitis (P<0.05) and low-grade squamous intraepithelial lesions (LSILs) (P<0.05) than Aptima HPV E6/E7 mRNA test; while Aptima HPV E6/E7 mRNA test showed a greater detection of high-grade squamous intraepithelial lesions (HSILs) (P<0.05) and invasive cervical carcinoma than HPV DNA test. Aptima HPV E6/E7 mRNA test exhibited a higher specificity P<0.05), positive and negative prediction rates than HPV DNA test for detection of cervical lesions, and the sensitivity was comparable between the two tests (P>0.05).

Conclusion: Aptima HPV E6/E7 mRNA test gradually improves the detection of cervical lesions with disease severity, and shows a higher specificity, positive and negative prediction rates and comparable sensitivity for detection of clinical cervical lesions as compared with HPV DNA test.

Keywords: Cervical lesion; Screening; Human papillomavirus DNA; Diagnostic performance

Introduction

Cervical cancer is one of the most frequent malignant tumors and is among the most common causes of cancer-related mortality in women worldwide (1). Each year, there are more than half a million new cases diagnosed with cervical cancer and a woman dies from this malignancy every 2 minutes across the world (2). Although the morbidity and mortality of cervical cancer shows a steady decline among women living in developed countries, this gynecologic cancer remains a leading cause of cancer-related death in a large number of countries in the world, notably in Africa, India and China (3, 4). Great strides have been made to achieve the ambitious goal of global elimination of cervical cancer as a public

health problem; however, significant challenges remain to be overcome (5-7).

Although the exact pathogenesis of cervical cancer remains unclear (8), infection with human papillomavirus (HPV) has been proved to be the most important risk factor for cervical cancer (9,10). HPV is responsible for more than 90% cervical cancer (11). To date, more than 200 types of HPV have been identified, including approximately 40 causing reproductive tract infections and 14 causing cancers, and these cancer-causing HPV types are also known as high-risk HPV (12). Persistent high-risk HPV infections will develop histological low-grade squamous intraepithelial lesions (LSILs), followed by high-grade squamous intraepithelial lesions (HSILs), and may finally progress into invasive cervical cancer (10,11). Early and precision identification of high-risk HPV is therefore of great significance to reduce the incidence of cervical cancer.

Currently, cytology and HPV DNA test are the two most common approaches used for screening high-risk groups of cervical cancer (13). Cytological screening is sensitive to screen precancerous cervical lesions, which shows great values in reducing the morbidity and mortality of cervical cancer; however, the test may cause harms due to invasive procedures and misdiagnosis (14). Although HPV DNA test is highly sensitive to identify high-risk groups of cervical cancer, this test alone fails to detect cervical cancer, since HPV infection is extremely common and mostly transient (15, 16). Aptima HPV E6/E7 mRNA test, a novel qualitative nucleic acid amplification assay that detects E6/E7 mRNA collectively from 14 high-risk HPV types, including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68, has shown potential for identification of high-risk HPV (17). However, there is little knowledge on the effectiveness of HPV E6/E7 mRNA test for large-scale screening of cervical lesions among Chinese populations.

The present study was designed with aims to examine the performance of Aptima HPV E6/E7 mRNA test for the evaluation of cervical lesions in a large Chinese population, so as to provide more clinical evidence for the use of this new nucleic acid amplification assay for cervical cancer in clinical practices.

Methods

Study subjects

During the period from Jul 2016 to Feb 2020, a total of 10,000 women at ages of 21 to 68 yr underwent cervical cancer screening with liquid-based cytology, HPV DNA test and Aptima HPV E6/E7 mRNA test in the Department of Gynecology of the Second Affiliated Hospital of Soochow University (Suzhou, China), and among them, 4,350 women simultaneously underwent colposcopy and biopsy, which were included in the analysis. HPV DNA test and Aptima HPV E6/E7 mRNA test were performed prior to cervical biopsy, which served as a gold standard in this study.

HPV DNA test

HPV isolates were genotyped using the PCR-reverse hybridization genotyping kit (Qiagen; Hiden, Germany). Such a kit is active to identify 23 HPV genotypes, including 17 high-risk HPV genotypes (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73 and -82) and 6 low-risk genotypes (HPV-11, -42, -43, -54, -81 and -83). Briefly, genomic DNA was extracted from cervical epithelial cells and PCR assay was performed. The flow-through hybridization was performed on the gene chip, and finally the color was developed.

Aptima HPV E6/E7 mRNA test

High-risk HPV genotyping was performed using the Aptima HPV Assay (Gen Probe, Inc.; San Diego, CA, USA), which is used to quantitatively detect 14 high-risk HPV, including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68. The detection probe was specific for the E6/E7 mRNA of 14 high-risk HPV genotypes. If any type was detected, the result would be positive. All procedures were done in strict accordance with the manufacturer's instructions, and the test results were automatically interpreted according to the copy number of E6/E7 mRNA

and the clinical threshold. A test value of 0.5 and greater was defined positive, and the test value of < 0.5 defined negative.

Colposcopy and histopathological examinations

If there were atypical squamous cells of undetermined significance, higher cytological results, positive HPV DNA test or HPV E6/E7 mRNA test results, patients should undergo colposcopy plus cervical biopsy and pathological examinations. The histopathological diagnosis results were divided into normal tissues and chronic cervicitis, LSIL, HSIL, invasive cervical carcinoma.

Ethical statement

This study was approved by the Ethical Review Committee of the Second Affiliated Hospital of Soochow University (approval number: JD-LK-2018-015-02). All procedures were done in accordance with the Declaration of Helsinki and international and national guideline.

Data management and analysis

All data were managed using the software Microsoft Excel 2010 (Microsoft; Redmond, WA, USA, and all statistical analyses were done with the statistical software SPSS 16.0 (SPSS, Inc.; Chicago, IL, USA). Difference of proportions was tested for statistical significance with chi-square test, and a *P*-value of <0.05 was considered statistically significant.

Results

Among 4,350 subjects receiving colposcopy and biopsy, pathological examinations showed normal tissues and chronic cervicitis in 3,140 women, LSIL in 640 women, HSIL in 360 women, and invasive cervical carcinoma in 210 women. The overall detection of cervical lesions by HPV DNA test (46.67%) was higher than that (35.17%) by Aptima HPV E6/E7 mRNA test (χ^2 = 11.89, *P*<0.05). In addition, HPV DNA test showed a higher efficacy than Aptima HPV E6/E7 mRNA test for the detection of normal tissues and chronic cervicitis (38.21% vs. 22.93%; χ^2 =17.28, *P*<0.05) and LSIL (71.88% vs. 51.56%; χ^2 = 5.59, *P*<0.05); however, HPV DNA test exhibited a significantly lower detection rate of HSIL (58.33% vs. 80.56%; χ^2 = 4.19, *P*<0.05) and invasive cervical carcinoma (76.19% vs. 90.47%; χ^2 = 0.73, *P*>0.05) than Aptima HPV E6/E7 mRNA test (Table 1).

Table 1: Comparison of HPV DNA test and Aptima HPV E6/E7 mRNA test for detection of cervical lesions with different degrees of severity

Cervical lesion	*No. of subjects*	*Positive HPV DNA test (%)*	*Positive HPV E6/E7 mRNA test (%)*	*χ^2 value*	*P-value*
Normal tissues and chronic cervicitis	3,140	1,200 (38.21)	720 (22.93)	17.28	< 0.05
Low-grade squamous intraepithelial lesions	640	460 (71.88)	330 (51.56)	5.59	< 0.05
High-grade squamous intraepithelial lesions	360	210 (58.33)	290 (80.56)	4.19	< 0.05
Invasive cervical carcinoma	210	160 (76.19)	190 (90.47)	0.73	> 0.05

To compare the clinical performance of Aptima HPV E6/E7 mRNA test and HPV DNA test, we estimated the sensitivity, specificity, negative prediction rate and positive prediction rate of these two tests for detection of cervical lesions. The overall specificity of Aptima HPV E6/E7 mRNA test was significantly higher than HPV DNA test for detection of cervical lesions (*P*<0.05); however, the overall sensitivity, negative prediction rate and positive prediction rate were comparable between Aptima HPV E6/E7 mRNA test and

HPV DNA test for detection of cervical lesions (P>0.05) (Table 2).

Table 2: Comparison of the performance of HPV DNA test and Aptima HPV E6/E7 mRNA test for detection of cervical lesions

Diagnostic index	*HPV DNA test (%)*	*Aptima HPV E6/E7 mRNA test (%)*	*χ^2 value*	*P-value*
Sensitivity	68.60	66.94.	0.092	> 0.05
Specificity	61.78	77.07	5.307	< 0.05
Positive prediction rate	40.89	52.94	2.89	> 0.05
Negative prediction rate	83.62	85.51	0.157	> 0.05

Discussion

Cervical cancer is the most common malignant tumor in the female reproductive system (1). Although the Global Cervical Cancer Elimination Initiative launched by WHO in 2018 has achieved great strides in elimination of cervical cancer as a global public health problem (18), this malignancy remains a high burden in developing countries, where more than 85% of cervical cancer patients would die (4). In recent years, the incidence of this cancer seems to appear a rise in younger women (19). Effective cervical screening facilitates early detection of squamous intraepithelial lesions and therefore prevents the development of cervical cancer (16).

Currently, multiple approaches have been employed for screening cervical cancer (16). Pap cytology screening is mainly based on smears; however, its sensitivity is relatively poor because the screening results are likely to be affected by multiple factors (15). Liquid-based cytology screening (Thinprep cytologic test, TCT), a more complete approach based on smear detections, stores the collected specimens in a container with cell preservation solutions, which reduces the false negative rate caused by smears; however, the inability to distinguish the structure of tissues limits its applications in clinical practices (15). Additionally, cytologists have subjective judgments on abnormal cell states, leading to subjective diagnosis (15). HPV DNA test is becoming more and more popular; however, the test fails to determine whether cervical lesions have occurred, which may increase the probability of missed diagnosis if it is used as a tool for screening cervical cancer alone (15). Colposcopy is a clinical pathological diagnosis. Clinicians observe the cervical transformation zone under direct vision of the colposcopy in abnormal areas, and cervical biopsy is performed if necessary. It is characterized by a high true positive rate; however, it is more suitable for observing the disease development in patients with cervical lesions rather than routine screening because of its invasiveness (15).

Cervical cancer has been lined with persistent infections with high-risk HPV, which integrates viral oncogenes with the cervical basal cell genome (11). This causes the deletion of the E2 fragment in the E region, leading to aberrant expression of E6 and E7 virus oncogenes (20). High E6/E7 mRNA expression interferes with the cell cycle, and the cell genome is unstable to produce E6 and E7 oncoproteins (21). HPV E6 and E7 have been identified as the main oncogene proteins that induce cell proliferation and immortalization (22). Aptima HPV E6/E7 mRNA test is an indicator of HPV virus carcinogenic activity and a predictor of cervical epithelial cell carcinogenesis (15). HPV E6/E7 mRNA expression suggests that HPV is integrated into host cell DNA, and the uncontrolled transcription of oncogenes leads to overexpression of E6 and E7 oncoproteins. The initiation of malignant transformation of cells indicates that HPV oncogene E6/E7 is in the active stage of expression and promotes the cancerous progression of cervical epithelial cells. Previous studies reported that HPV E6/E7 mRNA test might serve as a reliable test for both primary cervical cancer

screening and the triage of borderline cytological abnormalities (17); however, there is little knowledge on the performance of HPV E6/E7 mRNA test in large-scale screening of cervical lesions among Chinese populations until now.

In this study, we examined the performance of Aptima HPV E6/E7 mRNA test for the evaluation of cervical lesions, and compared with HPV DNA test. We detected a gradual increase in the detection of cervical lesions with the disease severity by using Aptima HPV E6/E7 mRNA test. HPV DNA test exhibited a higher detection rate of normal tissues and chronic cervicitis than Aptima HPV E6/E7 mRNA test, which indicates that HPV DNA test detects a transient infection that has not been integrated into cells, leading to negative Aptima HPV E6/E7 mRNA test. This also suggests that the false positive rate of Aptima HPV E6/E7 mRNA test is lower than that of HPV DNA test. In addition, Aptima HPV E6/E7 mRNA test showed a significantly higher detection rate of HSIL and a numberically greater detection rate of invasive cervical carcinoma than HPV DNA test. Overall, Aptima HPV E6/E7 mRNA test showed significantly higher specificity, numberically greater positive and negative prediction rates and comparable sensitivity in detection of clinical cervical lesions than HPV DNA test, indicating that Aptima HPV E6/E7 mRNA test may be used for large-scale identification of cervical lesions. Nevertheless, Aptima HPV E6/E7 mRNA test, which reduces the panic of patients suffering from transient HPV infections and the cost of further colposcopy as compared with HPV DNA test, may serve as an additional test to currently commonly used HPV DNA test for screening cervical cancer.

Conclusion

Aptima HPV E6/E7 mRNA test gradually improves the detection of cervical lesions with disease severity, and shows a higher specificity, positive and negative prediction rates and comparable sensitivity for detection of clinical cervical lesions as compared with HPV DNA test. Our data indicate that Aptima HPV E6/E7 mRNA test may serve as an addition test to HPV DNA test for cervical screening in Chinese populations with a low missing diagnosis. Further multi-center, prospective, diagnostic clinical trials to investigate the performance of Aptima HPV E6/E7 mRNA test for detection of cervical lesions seem justified.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare no conflict of interest.

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