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Joint Detection of High-Risk HPV E6/E7 mRNA and HC2 HPV-DNA in Cervical Lesions Screening

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Abstract: *Objective* To explore the value of joint detection of high-risk HPV E6/E7 mRNA and HC2 HPV-DNA to the screening of cervical lesions. *Methods* Totally 869 patients were divided into low-grade squamous intraepithelial lesions negative group including normal, inflammation and low-grade squamous intraepithelial lesions, and high-grade squamous intraepithelial lesions (HSIL) positive group including HSIL, squamous cell carcinoma and adenocarcinoma confirmed by ThinPrep cytologic test, HC2 HPV-DNA, high-risk HPV E6/E7 mRNA detection and colposcopy biopsy. The pathological result on HSIL positive was as the gold standard to evaluate the value of joint detection of HC2 HPV-DNA and high-risk HPV E6/E7 mRNA to the screening of cervical cancer. *Results* The positive rates of HPV E6/E7 mRNA and HPV-DNA detection in 869 patients were 46.1% and 61.3%, and increased with the severity of cytological grade and cervical level (P<0.05). The sensitivity and specificity of HPV E6/E7 mRNA were 97.4% and 64.9%, higher than those of HPV-DNA (89.0%, 44.6%) (P<0.05). The sensitivity and specificity of joint detection of HPV E6/E7 mRNA and HPV-DNA were 99.3% and 56.7%. The overall consistency rate and positive consistency rate were higher for HSIL positive (87.6%, 87.0%) than those for HSIL negative (70.5%, 30.5%) (P<0.05). *Conclusion* The positive expressions of HPV E6/E7 mRNA and HPV-DNA are closely correlated with the severity of cervical lesions, and the joint detection of them two has high sensitivity and specificity.

Keywords: Cervical Lesions, Human Papillomavirus, HPV E6/E7 mRNA, HPV-DNA

1. Introduction

High-Risk Human Papillomavirus (HR-HPV) continuous infection was closely related to cervical cancer [1], and HPV-DNA was integrated to the host chromosomes, causing excessive expression of two cancer genes of E6/E7, thereby initiating carcinogenic cancer process [2-4]. HPV16 E6/E7's excessive expression could promote the migration of cervical cancer cells by increasing the activity of MMP-2/9 [5] This study analysed the HR-HPV E6/E7mRNA and HC2 HPV-DNA results of 869 patients with cervical falling cell specimens and combined with the results of the TCT, the pathological results of the tissues. The aim was to explore the role of joint detection of HR-HPV E6/E7mRNA and HC2 HPV-DNA in cervical lesion screening.

2. Materials and Methods

2.1. Study Population

From July 2017 to December 2018, 869 patients aged 21-77 (47.4±10.4) years, underwent cervical opportunistic screening in Taicang Hospital Affiliated to Soochow University were enrolled in the clinical study. Inclusion criteria: (1) sexual life; (2) non-pregnant women; (3) No history of cervical coning, hysterectomy or chemoradiotherapy; (4) No acute genital tract inflammation; (5) HR-HPV E6/E7 mRNA, HC2 HPV-DNA and TCT were detected; (6) Colposcopic biopsy was performed for pathological examination; (7) Good compliance could be followed up. Exclusion criteria: (1) incomplete data; (2) lactating or menstruating women; (3) previous immune system disease or immunosuppressive therapy history; (4) Patients with

severe cardiac, liver, kidney and lung dysfunction; (5) Patients with acute infectious diseases or serious mental diseases; (6) Patients with CIN, cervical cancer or VIN, VaIN; (7) Patients with other malignant tumors. The study was approved by the institutional review board of Taicang Hospital Affiliated to Soochow University (Ethics No. KJ-2016-13), All the participants provided written informed consent.

2.2. TCT Test

ThinPrep automatic ultra-thin liquid-based cytology test system (Hologic, USA) was used for cytological diagnosis by professional gynecologists, and the results were determined by reference [6]. The diagnosis was cytological positive except for the absence of negative for intraepithelial lesion or malignancy (NILM).

2.3. HR- HC2 HPV-DNA Detection

Using HC2 system (DIGENE Company, USA), hybridization capture method qualitative detection of 13 kinds of HR-HPV in cervical samples (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) [7]. HPV≥1.0 ng/L is positive.

2.4. HR-HPV E6/E7 mRNA Detection

Application of Aptima HPV E6/E7 mRNA Diagnostic kit for HR-HPV E6/E7 mRNA detection (Hologic, USA), 14 types of HR-HPV were detected (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

2.5. Histopathological Examination

If colposcopy is abnormal, cervical biopsy will be performed, and the histopathological results will be diagnosed by two professional pathologists. Diagnostic criteria refer to WHO classification criteria [6]. Pathological results were normal, inflammatory, low-grade squamous intraepithelial lesions (LSIL) were group LSIL (-), high-grade squamous intraepithelial lesions, squamous carcinoma and adenocarcinoma were group HSIL (+).

2.6. Statistical Analysis

SPSS 22.0 software was used for statistical analysis, measurement data were expressed as mean ± standard deviation (x±s), and t test was used for comparison between the two groups. Counting data were compared by x2 test. Test level α=0.05.

3. Results

3.1. The Positive Rate of HPV E6/E7 mRNA and HPV-DNA

In 869 patients, the positive rate of HPV E6/E7 mRNA was 46.1% (401/869) and HPV-DNA was 61.3% (533/869) respectively. The positive rates of HPV E6/E7 mRNA and HPV-DNA increased with the upgrade of abnormal cytological diagnosis (χ2=93.360, P<0.001; χ2=55.710, P<0.001). The histopathological results showed that 715 cases (82.3%) was LSIL (-) and 154 cases (+) was HSIL (17.7%), and the positive rates of HPV E6/E7 mRNA and HPV-DNA increased with the severity of cervical lesions (χ2=259.284, P<0.001; χ2=137.135, P<0.001). The detection positive rate of HPV-DNA in LSIL (-) patients (55.4%, 396/715) was higher than that of HPV E6E7 mRNA detection (35.1%, 251/715), and the detection positive rate of HPV E6E7 mRNA in HSIL (+) patients (97.4%, 150/154) was higher than HPV-DNA test (89.0%, 137/154), as shown in Tables 1-2.

Table 1. *Comparison of positive rates of HR-HPV E6/E7 mRNA and HC2 HPV-DNA in different cytology.*

Cytological results	E6/E7 mRNA	HPV-DNA	x2	*P*
	cases positive rate (%)	cases positive rate (%)		
NILM	248 47.4	349 66.7	39.806	<0.001
ASCUS	36 20.6	70 40.0	15.643	<0.001
AGC	1 50.0	0 0	—	—
LSIL	68 60.2	69 61.1	0.019	0.892
ASC-H	7 63.6	5 45.5	0.733	0.669
HSIL	40 90.9	39 88.6	0.124	0.725
SCC	1 100.0	1 100.0	—	—

ASC-US (atypical squamous cell of undetermined significance, ASC-US); AGC (atypical glandular cell); ASC-H (atypical squamous cell, cannot exclude high-grade squamous intraepithelial lesion); LSIL (low-grade squamous intraepithelial lesions); HSIL (high-grade squamous intraepithelial lesions); SCC (Squamous cell carcinoma)

Table 2. *Comparison of positive rates of HR-HPV E6/E7 mRNA and HC2 HPV-DNA in different histopathology.*

Histopathology	E6/E7 mRNA	HPV-DNA	x2	P
results	Cases positive rate (%)	Cases positive rate (%)		
Inflammation	141 26.6	244 46.0	43.273	<0.001
LSIL	110 59.5	152 82.2	23.066	<0.001
HSIL	146 98.0	133 89.3	9.500	0.002
SCC	3 100.0	3 100.0	—	—
AC	1 50.0	1 50.0	—	—

LSIL (low-grade squamous intraepithelial lesions); HSIL (high-grade squamous intraepithelial lesions); SCC (Squamous cell carcinoma); AC (Adenocarcinoma)

3.2. Comparison of Diagnostic Efficiency of Two Detection Methods

Using histopathologic results as the gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of HSIL (+) for HPV E6/E7 mRNA detection were higher than those for HPV-DNA detection ($\chi2$=8.636, P=0.003; $\chi2$=59.348, P<0.001; $\chi2$=14.724, P<0.001; $\chi2$=13.594, P<0.001). The sensitivity, specificity, positive predictive value and negative predictive value of HPV E6/E7 mRNA combined with HPV-DNA were higher than those of HPV-DNA alone ($\chi2$=13.063, P<0.001; $\chi2$=17.401, P<0.001; $\chi2$=15.256, P<0.001; $\chi2$=12.244, P <0.001), specificity was lower than E6/E7 mRNA detection alone ($\chi2$=8.293, P=0.004), sensitivity, positive predictive value and negative predictive value were not statistically significant compared with E6/E7 mRNA detection alone ($\chi2$=0.571, P= 0.450; $\chi2$=0.035, P=0.852; $\chi2$=0.137, P=0.110). See Table 3.

Table 3. *Diagnostic effectiveness Comparison of HPV E6/E7 mRNA、HPV-DNA and E6/E7 mRNA+HPV-DNA.*

Detection method	sensitivity	specificity	PPV	NPV	Consistency rate	Kappa value
E6/E7 mRNA	97.4	64.9	37.4	99.1	70.7	0.346
HPV-DNA	89.0	44.6	25.7	95.0	52.5	0.229
E6/E7 mRNA+HPV-DNA	99.3	56.7	38.1	99.7	48.3	0.397

3.3. Consistency Analysis of HPV E6/E7 mRNA and HPV-DNA Tests

Of the 869 patients, 715 cases were LSIL (-), 154 cases were HSIL (+); In the 715 LSIL (-) cases, both tests showing positive were 218 cases and both showing negative were 286. The overall coincidence rate was 70.5% (504/715), the positive coincidence rate was 30.5% (218/715), and the negative coincidence rate was 40.0% (286/715). The results were inconsistent in 211 cases (29.5%, 211/715), of which 178 were positive for HPV-DNA but negative for HPV E6/E7 mRNA, and 33 were positive for HPV E6/E7 mRNA but negative for HPV-DNA. In the 154 HSIL (+) cases, 134 cases with both positive and 1 case with both negative. The overall coincidence rate was 87.7% (135/154), the positive coincidence rate was 87.0% (134/154), and the negative coincidence rate was 0.6% (1/154). Results were inconsistent in 19 cases (12.3%, 19/154), of which 3 were positive for HPV-DNA but negative for HPV E6/E7 mRNA, and 16 were positive for HPV E6/E7 mRNA but negative for HPV-DNA.

4. Discussion and Conclusion

Continuous infection of high-risk HPV is an important cause of cervical cancer, which is caused by E6/E7 genes. At the early stage of HPV infection, the HPV gene is free in the cell, and at this time, the E6/E7 gene is in the silent period, with no expression or low expression of E6/E7 mRNA. Transient infection is mostly in this stage. When high-risk HPV is continuously and repeatedly infected, the oncogene E6/E7 is in the active state of replication, the E6/E7 mRNA is expressed in large quantities and increased the risk of cancer [7].

Clad et al. [8] showed that for both Aptima HPV mRNA detection and HC2 HPV-DNA detection, the positive rate increased with the degree of cytological and histological lesions (from normal to highly squamous intraepithelial lesions or cervical cancer). The positive rates of HPV mRNA were 25%, 57.9%, 94.4%, 100% in Cytological result normal, LSIL, HSIL, cervical cancer, and positive rates of HC2 HPV-DNA were 32.9%, 75.4%, 94.8% and 100%, respectively. The positive rates of HPV mRNA and HPV-DNA were 7.4% vs 22.2%, 54.7% vs 68.8%, respectively, in normal and LSIL histology specimens. The positive rates of HPV mRNA were lower than those of HPV-DNA.

The positive rates of HPV mRNA Compared to HPV-DNA were similar or higher in HSIL+ lesions (CIN2: 79.8% and 83.1%, CIN3: 98.7% and 96.7%, cervical cancer: 92.3% and 84.6%). The results of this study showed that the positive rates of HPV E6/E7 mRNA and HPV-DNA increased with the increase of cytology and cervical lesion grade. In patients with cytological ASCUS, the positive rate of HPV-DNA detection (40%) was higher than that of HPV E6/E7 mRNA detection (20.6%), suggesting that HPV-DNA was prone to positive results in cytological ASUCS. In patients with LSIL (-), HPV-DNA was also more likely to have positive test results, and the positive detection rate (55.4%) was higher than that of HPV E6/E7 mRNA detection (35.1%). The possible reason is that HPV virus may exist in a free state, infection may be transient, and oncogene transcription did not start. It indicates that the infection may clear spontaneously, but it also suggests that such patients should be focused on monitoring. Different from the general population, the occurrence of cervical cancer should be vigilant during close follow-up.

In patients with HSIL (+), the positive detection rate of HPV E6/E7 mRNA (97.4%) was higher than that of HPV-DNA detection (89.0%), suggesting that the activation state of HPV oncogenes could be detected by HPV E6/E7 mRNA transcripts. PV E6/E7 mRNA testing can reduce interference from transient HPV infection, more accurately predict the risk of disease progression, and accurately shunt patients with HPV-DNA positive.

Studies [9-13] showed that the sensitivity and specificity of HPV E6/E7 mRNA detection were 83%~96% and 51%~80%, and the sensitivity and specificity of HPV-DNA detection were 92%~94% and 20%~63%. The results of this study showed that the sensitivity (97.4%) and specificity (64.9%) of HPV E6/E7 mRNA for HSIL (+) detection were higher than those of HC2 HPV-DNA (89.0% and 44.6%) with histopathological examination as the gold standard, which was

different from related studies. The reasons might be the differences in the level of cervical exhumation cell collection, HPV test quality control, difference of colposcopy and biopsy. However, it was consistent that HPV E6/E7 mRNA detection had a high diagnostic specificity for patients with high-grade cervical lesions, which could significantly reduce false positive results and improve negative predictive value, while HPV-DNA detection had a high sensitivity, and the combined use of the two methods had complementary advantages, which could improve the accuracy of screening.

Castle et al. [14] found that the detection consistency of HPV E6/E7 mRNA and HPV-DNA in high-grade lesions and cervical cancer was higher than that in low-grade lesions, and E6/E7 mRNA was a specific marker for high-grade lesions. The results of this study showed that the overall coincidence rate (87.7%) and positive coincidence rate (87.0%) of HSIL (+) detected by the two methods were higher than those of LSIL (-) patients (70.5% and 30.5%), and there was little difference between HPV-DNA and HPV E6/E7mRNA when the disease was severe. Both showed high positive rate. The results of this study showed that 3 cases with positive HPV-DNA test and negative HPV E6/E7 mRNA test were diagnosed as HSIL by histropathological examination, suggesting that while emphasizing the superiority of HPV E6/E7 mRNA detection, the possibility of missed diagnosis should not be ignored, and the application of combined HPV-DNA detection can reduce the risk of missed diagnosis. The results of this study showed that 16 patients with HSIL had positive HPV E6/E7 mRNA detection but negative HPV-DNA detection, which might be caused by E6/E7 gene mutation, or the loss of some gene segments during the integration of HPV virus with human DNA, resulting in the failure of DNA probe. Benevolent O et al. [15] had also found that such phenomenon exists, but the specific mechanism remains unclear. More research is being done to develop better monitoring methods [16].

In terms of detection of cervical adenocarcinoma, HPV E6/E7 mRNA and HPV-DNA detection are insensitive due to the presence of subtypes of adenocarcinoma unrelated to HPV infection (such as cervical mucinous adenocarcinoma gastric type and mesrenal duct carcinoma, etc.) [17]. The results of this study showed that 1 adenocarcinoma patient was negative for two HPV detection methods, but cytology indicated abnormalities, and histopathological examination diagnosed the patient as gastric adenocarcinoma, suggesting that even if HPV test was negative, close follow-up is still needed clinically to guard against missing special types of cervical cancer.

The results of this study suggested that detection of HPV E6/E7 mRNA transcripts can detect the activation state of HPV oncogenes, which had high sensitivity and specificity when combined with HPV-DNA detection, and its expression was closely related to the degree of cervical lesions, and could accurately predict the risk of disease progression caused by high-risk HPV persistent infection in early stage. It is expected to be an effective method for screening cervical lesions.

The detection of E6/E7 mRNA could identify the potential of high-risk recurrent infections without repeated detection, which was a potentially valuable tool in monitoring the expression potential of oncogenic proteins after HPV infection. Moreover, a high sensitivity and high specificity detection method was needed clinically to diagnose cervical cancer in an early, efficient and accurate manner and avoid missed diagnosis and over-diagnosis.

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