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ORIGINAL RESEARCH

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Significance of HR-HPV E6/E7 mRNA detection to screen the severity of cervical cancer in relation to age

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Abstract

In order to determine the significance of HR-HPV (high risk HPV, HR-HPV) E6/E7 mRNA detection for screening the severity of cervical cancer in relation to age, a total of 308 patients were admitted to the Department of Gynecology at The Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University between July 2017 and July 2018 and underwent LBC (Liquid Based Cytology) detection and HR-HPV E6/E7 mRNA detection. Data were then compared with the criteria laid down by the Pathology Department. The positive rate of HR-HPV E6/E7 mRNA detection was significantly higher in a CIN (cervical intraepithelial neoplasia) group than in a chronic cervicitis group. The sensitivity of LBC was higher than that for HR-HPV E6/E7 mRNA detection, while the specificity of LBC detection was higher than that of HR-HPV E6/E7 mRNA detection ($p < 0.05$). For women under 30 years old, the specificity of HR-HPV E6/E7 mRNA detection was lower than that of LBC detection, while the sensitivity of HR-HPV E6/E7 mRNA detection was higher than that of LBC detection. For women 30 years-of-age and above, the specificity of HR-HPV E6/E7 mRNA detection was significantly lower than that for LBC. HR-HPV E6/E7 mRNA detection has significant advantages for the screening of cervical cancer, especially in women under 30 years old.

Keywords

Cervical cancer; HR-HPV; E6/E7 mRNA; LBC; Age

1. Introduction

Cervical cancer is the fourth most common and fatal malignant cancer in female in the world, which is the second leading cause of cancer-related death in women from 20 to 39 [1]. Each year, approximately 750,000 new cases of cervical cancer occur in China, thus ranking third in terms of the number of new cervical cancer cases globally [2]. Therefore, early diagnosis and treatment are important to prevent the spread of cervical cancer and to control the mortality rate of cervical cancer. In cervical cancer screening, Liquid Based Cytology (LBC) is currently a common way. However, the LBC is associated with false negative or false positive results due to its reliance on several subjective factors. Existing studies have shown that most cervical cancer patients are caused by high-risk human papilloma virus infection (high risk HPV, HR-HPV), such as type 16, 18, 31, 33 or 35. For the development of cervical and pre-cervical lesions, persistent infection of HR-HPV is a necessary factor [3–6]. Currently, the methods used for HPV DNA detection are highly sensitive but lack specificity. Previous studies have shown that HPV DNA detection is only able to identify a response to the cause of the disease. This method of detection cannot reflect the degree of lesions generated by cervical cells when infected by HPV virus; furthermore, the specificity of this detection method is low [7]. This means that HPV DNA detection cannot detect the transient or non-clinical significance of HPV infection, thus leading to over-diagnosis and over-treatment; this practice can also increase the economic and psychological burden on patients and medical resources. Recently, the HR-HPV E6/E7 method has been widely deployed as a novel test. Related research suggest that persistent HR-HPV E6/E7 infection can initiate carcinogenesis by up-regulating the expression levels of the HPV E6 and E7 oncogenes which are closely related to the severity of cervical lesions [8–10]. In 2014, the NCCN (National Comprehensive Cancer Network) guidelines highlighted the fact that HPV DNA typing is not a routine method for screening cervical precancerous lesions in women under the age of 30. In this study, we divided our patients in two groups at the age of 30 and compared HR-HPV E6/E7 mRNA detection with LBC detection in order to define the significance of these methods for cervical cancer screening.

2. Methods

We acquired 23,785 LBC detection specimens, 4082 E6/E7 detection specimens and 1696 cervical histological specimens from The Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University from July 2017 to July 2018. These patients had been treated for contact bleeding, abnormal

leucorrhea, external itching and other symptoms. A total of 308 patients were selected; then, we performed LBC detection, HR-HPV E6/E7 mRNA detection and pathological diagnosis. We excluded patients with a history of acute inflammation of the genital tract, cervical disease, hysterectomy, cervical surgery, pelvic radiotherapy, pregnancy, menstruation, sexual intercourse in the three days prior to specimen collection, and those with a history of vaginal washing. Patient's age ranged from 19 to 80, with a median age of 39.

3. Results

3.1 Comparison of positive rates for the two methods with respect to cervical lesions with different severities

Of the 308 cases with cervical lesions, there were 40 cases of cervical intraepithelial neoplasia (CIN) I, 216 cases of chronic cervicitis, and 52 cases of high-grade CIN lesions, thus accounting for 70.13%, 12.99% and 16.88% of the study group, respectively. The detection rate for HR-HPV E6/E7 mRNA was 63.9%, 72.5% and 61.5%, respectively, and there were 75, 10 and 18 cases that were positive for LBC detection (34.7%, 25.0% and 34.6%, respectively). Statistical analysis showed that the positive rate for HR-HPV E6/E7 mRNA detection in the CIN group was significantly higher than that in the chronic cervicitis group. There was no significant difference in terms of LBC detection, although there was a significant difference for HR-HPV E6/E7 mRNA detection ($p < 0.001$), as shown in Table 1. Statistically, the positive rates of the two methods were different when compared between in the chronic cervicitis, CIN I and CIN II+ groups, as shown in Table 2.

3.2 Comparison of positive rates for the two detection methods in cervical lesions in patients of different ages

The 308 patients were divided into two groups: women under 30 years old (56 cases) and women aged 30 years and above (252 cases). For the women under 30 years old, there was only a significant difference between the two detection methods in the chronic cervicitis group ($p < 0.05$). There was a significant difference between the two detection methods across the three groups of women aged 30 years and above ($p < 0.05$), as shown in Table 3.

3.3 Diagnostic value of the two detections for different pathological grades of cervical cancer

Next, we used pathological analysis as a gold standard and compared this with the two detection methods with regards to sensitivity, specificity, positive predictive value, and negative predictive value. We found that the sensitivity of the LBC method was significantly lower than that for HR-HPV E6/E7 mRNA detection, while the specificity of the LBC detection method was significantly higher than that of HR-HPV E6/E7 mRNA detection ($p < 0.05$), as shown in Table 4.

3.4 Diagnostic value of the two detection methods for cervical lesions in patients of different ages

The 308 patients were divided into two groups: women under 30 years old (56 cases) and women aged 30 years and above (252 cases). Using pathological analysis as a gold standard, we compared the sensitivity, specificity, positive predictive value, and negative predictive value between groups. We found that in women under 30 years old, the specificity of the HR-HPV E6/E7 mRNA detection method was lower than that of LBC detection, while the sensitivity of HR-HPV E6/E7 mRNA detection was higher than that of LBC detection. For women aged 30 years and above,the specificity of the HR-HPV E6/E7 mRNA detection method was significantly less than that of the LBC method, as shown in Table 5.

4. Discussion

In the female reproductive system, HPV is one of the common sexually transmitted viruses [11]. However, although HPV infection is common, most infections caused by HPV are transient. Approximately 90% of those infected with HPV can clear the virus, and it takes several years from HPV infection to progress to cervical cancer. With the development of science and medical technology, significant progress has been made in the treatment of cervical cancer; however, there is still a lack of biomarkers to identify cervical cancer in its early stages. Therefore, in the prevention and treatment of cervical cancer, high sensitivity and specific detection methods are very important. HR-HPV E6/E7 mRNA detection has become a significant research hotspot [12] and now represents an important method for the early detection, diagnosis and treatment of cervical cancer in clinical scenarios.

Research has shown that the E6/E7 gene is an oncogene that is located in the early coding region of HPV, which is very important in the development of cervical cancer. E6 protein can bind to and inactivate P53 protein, thus inducing the malignant proliferation of cells. E7 protein can regulate many proteins, such as the Rb family and cell cycle regulatory proteins, such that cells can proliferate indefinitely. Once HPV is integrated into the host genome, the expression of the E6/E7 gene will be unrestricted and increase to a level at which cervical cells become to deteriorate. Without repeated HPV testing, we can only identify subjects who are at high risk of repeated infection by testing for the transcription of cancer genes [13, 14].

LBC detection has become the most common method used to detect cervical cancer in the clinic. However, LBC detection is largely subjective and often leads to over-referral or over-treatment, especially in young women whose results suggest low-grade cellular abnormalities. Therefore, this remains a challenge from a clinical point of view. In contrast to LBC detection, HR-HPV E6/E7 mRNA testing is objective, directly applicable to the mRNA generated in most HPV-based screening workflows, and highly reproducible. Moreover, in actual clinical work, loop electrosurgical excision procedure (LEEP) will be selected for the vast majority of cervical lesions in young women. Although this can reduce the incidence

TABLE 1. Comparison of positive rates of two detections in cervical lesions between chronic cervicitis an CIN.

Pathology	Cases	LBC detection abnormal	E6/E7 positive	χ^2	*p*
CIN	92	28 (30.4)	61 (66.3)	23.699	<0.001
Chronic cervicitis	216	75 (34.7)	138 (63.9)	36.757	<0.001

LBC: Liquid Based Cytology; CIN: cervical intraepithelial neoplasia.

TABLE 2. Comparison of positive rates of two detections in cervical lesions at different.

Pathology	Cases	LBC abnormal	E6/E7 positive	χ^2	*p*
CIN II+	52	18 (34.6)	32 (61.5)	7.550	0.006
CIN I	40	10 (25.0)	29 (72.5)	18.061	<0.001
Chronic cervicitis	216	75 (34.7)	138 (63.9)	36.757	<0.001
Sum	308	103	199	59.876	<0.001

LBC: Liquid Based Cytology; CIN: cervical intraepithelial neoplasia.

TABLE 3. Comparison of the positive rates of two detections in cervical lesions of patients at different ages (%, 95% CI).

Ages	Pathology	Cases	LBC detection abnormal	E6/E7 positive	χ^2	*p*
<30 yr						
	CIN II+	14	5 (35.71)	7 (50.00)	0.583	0.445
	CIN I	6	2 (33.33)	5 (83.33)	-	0.242
	Chronic cervicitis	36	12 (33.33)	30 (83.33)	21.799	<0.001
≥30 yr						
	CIN II+	38	13 (34.21)	25 (65.79)	7.579	0.006
	CIN I	34	8 (23.53)	24 (70.59)	15.111	<0.001
	Chronic cervicitis	180	63 (35.00)	108 (60.00)	22.556	<0.001
Sum		308	103 (33.44)	199 (64.61)	21.677	<0.001

LBC: Liquid Based Cytology; CIN: cervical intraepithelial neoplasia.

TABLE 4. Diagnostic value of two detections for different histopathological grades (%, 95% CI).

Subject	LBC detection	E6/E7 detection	*p*
Sensitivity	0.30 (0.25∼0.35)	0.66 (0.56∼0.76)	0.004
Specificity	0.65 (0.58∼0.72)	0.36 (0.30∼0.43)	<0.001
Positive predictive value	0.27 (0.19∼0.37)	0.31 (0.24∼0.38)	0.642
Negative predictive value	0.69 (0.62∼0.75)	0.72 (0.62∼0.80)	0.832

CI: Confidence interval; LBC: Liquid Based Cytology.

TABLE 5. Diagnostic value of two detections in cervical lesions of patients at different ages (%, 95% CI).

Ages	Subject	LBC detection	E6/E7 detection	*p*
<30 yr				
	Sensitivity	0.35 (0.16∼0.59)	0.60 (0.36∼0.80)	0.343
	Specificity	0.67 (0.49∼0.81)	0.17 (0.07∼0.33)	0.005
	Positive predictive value	0.37 (0.17∼0.61)	0.29 (0.16∼0.45)	0.644
	Negative predictive value	0.65 (0.47∼0.79)	0.43 (0.19∼0.70)	0.453
≥30 yr				
	Sensitivity	0.29 (0.19∼0.41)	0.68 (0.56∼0.78)	0.006
	Specificity	0.65 (0.58∼0.72)	0.40 (0.33∼0.48)	0.008
	Positive predictive value	0.25 (0.16∼0.36)	0.31 (0.24∼0.39)	0.450
	Negative predictive value	0.70 (0.62∼0.76)	0.76 (0.66∼0.84)	0.668

LBC: Liquid Based Cytology.

of cervical cancer, but there are many side effects. The most significant side effect is that the removal of the diseased cervix is associated with a significant increase in the risk of pregnancy-related morbidity caused by premature birth, for young women who have had fertility requirements [15]. It suggested that the positive rates of HR-HPV E6/E7 mRNA detection and LBC detection for chronic cervicitis, CIN I and CIN II+ were statistically significant ($p < 0.05$) in this article. Furthermore, the sensitivity of the LBC method was lower than that of HR-HPV E6/E7 mRNA detection, while the specificity of the LBC detection method was higher than those of the HR-HPV E6/E7 mRNA detection method, respectively ($p < 0.05$), thus indicating that these two detection methods have a certain clinical value for the screening of cervical lesions.

Our results also showed that the positive rate of HR-HPV E6/E7 mRNA detection was significantly higher in the CIN group than in the group of patients with inflammation. There was no statistically significant difference for LBC detection between groups, while there was a statistically significant difference for HR-HPV E6/E7 mRNA detection, thus indicating that HR-HPV E6/E7 mRNA detection was superior to LBC detection in terms of the aggravation of cervical lesions.

Over recent years, increasing research effort has focused on shunt management and the effect of age with regards to screening for cervical cancer. In the present study, we showed that when taking age into account, the specificity of HR-HPV E6/E7 mRNA detection was lower for women who were 30 years-of-age and younger than those of LBC detection, while the sensitivity of HR-HPV E6/E7 mRNA detection was significantly higher than that of LBC detection; for women aged 30 years and above, the specificity of HR-HPV E6/E7 mRNA detection was significantly lower than that of the LBC method. We believe that for patients with early cervical lesions, the low positive rate of HR-HPV E6/E7 mRNA detection may be due to the relative silence period of HPV virus. In other words, young women who have been infected with HPV for a short period of time (that is, patients with mild cervical lesions, such as CIN I patients) can mostly be cured, largely because cervical cells are still in the integration stage with HPV infection, and have not progressed to the point where E6/E7 oncogenes are highly transcribed and replicated. Therefore, the positive test rate is low. Therefore, when screening women who are younger than 30 years-of-age, we suggest that the HR-HPV E6/E7 mRNA detection method is used. This is because the sensitivity of HR-HPV E6/E7 mRNA detection is higher than that of LBC detection; however, this difference was not significant due to the limited sample size.

However, this study involved a limited sample size of 308 women with abnormal gynecology manifestations; this was not a routine screening population. Thus, the HR-HPV E6/E7 mRNA detection rate may be higher in our population than in a normal population, thus warranting further investigation.

5. Conclusions

In summary, the expression of HR-HPV E6/E7 mRNA is closely related to cervical lesions; our analysis showed that HR-HPV E6/E7 mRNA detection is better than the existing LBC detection method. We prefer to use HR-HPV E6/E7 mRNA detection rather than LBC detection, especially for women under 30 years old. Our findings show that HR-HPV E6/E7 mRNA detection may serve as a more useful test for the screening of cervical cancer, the risk assessment of HPV infection progression, evaluating patient prognosis and monitoring follow-up in patients with cervical cancer. This method provides a valuable tool for triage during cervical cancer screening [16, 17].

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

JMC and BRX—designed the research study. AHZ—performed the research. ZFZ and YFZ—analyzed the data. AHZ and HHW—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Approval of the ethics committee of The Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University ([2020]KY158-01) was obtained for the use of all samples. The 308 subjects all provided signed and informed consent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: A Cancer Journal for Clinicians. 2020; 70: 7–30.

[2] Zhao FH, Lewkowitz AK, Hu SY, Chen F, Li LY, Zhang QM, *et al*. Prevalence of human papillomavirus and cervical intraepithelial

neoplasia in China: a pooled analysis of 17 population-based studies. International Journal of Cancer. 2012; 131: 2929–2938.

[3] Baussano I, Elfström KM, Lazzarato F, Gillio-Tos A, De Marco L, Carozzi F, *et al*. Type-specific human papillomavirus biological features: validated model-based estimates. PLOS ONE. 2013; 8: e81171.

[4] Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, *et al*. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Journal of Lower Genital Tract Disease. 2013; 17: S1–S27.

[5] Burmeister CA, Khan SF, Schäfer G, Mbatani N, Adams T, Moodley J, *et al*. Cervical cancer therapies: current challenges and future perspectives. Tumour Virus Research. 2022; 13: 200238.

[6] Zhou Y. Investigation of the clinical application value of HR-HPV DNA combined with liquid based cytology in colposcopy of cervical cancer. Contrast Media & Molecular Imaging. 2022; 2022: 5054507.

[7] Arbyn M, Simon M, de Sanjosé S, Clarke MA, Poljak M, Rezhake R, *et al*. Accuracy and effectiveness of HPV mRNA testing in cervical cancer screening: a systematic review and meta-analysis. The Lancet Oncology. 2022; 23: 950–960.

[8] Rijkaart DC, Heideman DA, Coupe VM, Brink AA, Verheijen RH, Skomedal H, *et al*. High-risk human papillomavirus (hrHPV) E6/E7 mRNA testing by PreTect HPV-proofer for detection of cervical high-grade intraepithelial neoplasia and cancer among hrHPV DNA-positive women with normal cytology. Journal of Clinical Microbiology. 2012; 50: 2390–2396.

[9] Nikolic N, Basica B, Mandic A, Surla N, Gusman V, Medic D, *et al*. E6/E7 mRNA expression of the most prevalent high-risk HPV genotypes in cervical samples from Serbian women. Diagnostics. 2023; 13: 917.

[10] Bruno MT, Ferrara M, Fava V, Rapisarda A, Coco A. HPV genotype determination and E6/E7 mRNA detection for management of HPV positive women. Virology Journal. 2018; 15: 52.

[11] Usyk M, Zolnik CP, Castle PE, Porras C, Herrero R, Gradissimo A, *et al*. Cervicovaginal microbiome and natural history of HPV in a longitudinal study. PLOS Pathogens. 2020; 16: e1008376.

[12] Li B, Guo R, Lai T, Qiao L, Fu H. The application of PAX1 methylation detection and HPV E6/E7 mRNA detection in cervical cancer screening. The Journal of Obstetrics and Gynaecology Research. 2021; 47: 2720–2728.

[13] Cesur Ö, Nicol C, Groves H, Mankouri J, Blair GE, Stonehouse NJ. The subcellular localisation of the human papillomavirus (HPV) 16 E7 protein in cervical cancer cells and its perturbation by RNA aptamers. Viruses. 2015; 7: 3443–3461.

[14] Sharma B, Lakhanpal V, Singh K, Oberoi L, Bedi PK, Devi P. Evaluation of HPV E6/E7 mRNA detection in clinically suspected cases of cervical cancer with abnormal cytology: time to upgrade the screening protocols. Journal of Laboratory Physicians. 2022; 14: 336–342.

[15] Yang YM, Wang SJ, Wang FY, Chen R, Xiao Q, Kang N, *et al*. Preliminary study of the use of E6/E7mRNA detection in screening and triage management of HR-HPV infection during pregnancy. Annals of Translational Medicine. 2021; 9: 1428.

[16] Bruno MT, Ferrara M, Fava V, Barrasso G, Panella MM. A prospective study of women with ASCUS or LSIL pap smears at baseline and HPV E6/E7 mRNA positive: a 3-year follow-up. Epidemiology and Infection. 2018; 146: 612–618.

[17] Derbie A, Mekonnen D, Woldeamanuel Y, Van Ostade X, Abebe T. HPV E6/E7 mRNA test for the detection of high grade cervical intraepithelial neoplasia (CIN2+): a systematic review. Infectious Agents and Cancer. 2020; 15: 9.

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