RELATIONSHIP BETWEEN THE EXPRESSION OF HPV E6/E7 AND HTERC GENES AND THE PROGRESSION OF CERVICAL LESIONS AND THE SIGNIFICANCE OF COMBINED DETECTION IN EARLY SCREENING OF CERVICAL CANCER

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ABSTRACT

Objective: *To explore the relationship between the expression of human papillomavirus E6/E7 (HPV E6/E7) and human telomere (hTERC) genes and the progression of cervical lesions and the significance of combined detection in early screening of cervical cancer.*

Methods: *125 patients who underwent TCT examination in our hospital from June 2017 to February 2019 were selected for the study and were divided into normal group (NC) 25 cases, low-grade squamous intraepithelial neoplasia (CIN1) 33 cases, medium-grade squamous intraepithelial neoplasia (CIN2) 31 cases and high-grade squamous intraepithelial neoplasia (CIN3) 26 cases according to the results of pathological blood examination. 10 cases of invasive carcinoma group (ICC). According to modern cervical cytology (TBS classification), 25 cases were divided into NC group, 35 cases in ASCUS group (atypical squamous epithelial cells without definite diagnostic significance), 15 cases in LSIL group, 11 cases in ASCH group (atypical squamous epithelial cells including highly squamous epithelial lesions), 6 cases in AGC group, 28 cases in HSIL group, and 28 cases in ASCH group. There were 5 cases of squamous cell carcinoma (SCC). The cervical exfoliated cells were collected by TCT and the positive expression rate of HPV E6/E7 was detected by nucleic acid hybridization. The positive amplification of hTERC gene was detected by FISH. To establish ROC curve and analyze the significance of HPV E6/E7 and hTERC gene detection alone or in combination in early screening of cervical cancer.*

Results: *The results showed that the positive expression of HPV E6/E7 and hTERC genes in NC group, ASCUS group, LSIL group, ASCH group, AGC group, HSIL group and SCC group increased, and the higher the grade of cytological lesions, the higher the positive expression of HPV E6/E7 and hTERC genes (P<0.05). The positive expression of HPV E6/E7 and hTERC genes in different histological classifications of NC, CIN1, CIN2, CIN3 and ICC groups showed an upward trend (P<0.05). With the increase of histological lesions, the positive expression rates of HPV E6/E7 and hTERC genes increased significantly, and the positive expression rates of HPV E6/E7 and hTERC genes in ICC group were as high as 100%. The results showed that the sensitivity and specificity of HPV E6/E7, hTERC and HPV E6/E7 + hTERC were 73.08%, 66.67%, 61.13%, 92.55%, 82.39%, 96.42%, respectively. The sensitivity and specificity of combined detection of HPV E6/E7 and hTERC genes were significantly higher than that of single detection.*

Conclusion: *The expression of HPV E6/E7 and hTERC genes increased significantly with the increase of the degree of cervical cancer lesion. The above indices can reflect the development of cervical cancer to some extent, but the combined detection of HPV E6/E7 and hTERC has the highest significance in early screening of cervical cancer.*

Keywords: *HPV E6/E7 mRNA, HTERC, progress of cervical lesions, joint detection, cervical cancer, early screening.*

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Forewords

Cervical cancer was one of the malignant tumors that seriously threaten women's life and health, and its incidence was second only to breast cancer. With the progress of medical science and technology, the prevention and screening of cervical cancer had been significantly accelerated, and the mortality rate of patients has decreased significantly, but the new cases were still more than 100,000 every year. Therefore, it was very important to find indicators with high sensitivity and specificity[(1)]. According to the association of human papillomavirus (HPV) with precancerous lesions or cervical cancer, the study found that persistent infection with high-risk HPV was the primary cause of cervical cancer, persistent

HPV E6/E7 mRNA transcription was a key cause of cervical cancer[2, 3]. HPV virus E6 protein and E7 protein played key roles in the replication process of host cells, and they could interact with many protein molecules to cause infinite proliferation and malignant transformation of cells[4]. At present, thinprepcytologic test (TCT) combined with HPV DNA was mainly used for cervical intraepithelial lesions, but HPV DNA detection had certain limitations. Zhang et al.[5] found that HPV E6/E7 mRNA detection was more specific than HPV DNA. It was reported that most of the human telomerase mRNA component (hTERC) genes on chromosome 3 are amplified abnormally in the process of tumor transformation in cervical epithelial cells.

Abnormal expression of hTERC gene was the basis of cervical cancer and might also be an early event of cervical cancer formation[6].

However, there were few reports on the combined detection of HPV E6/E7 mRNA and hTERC gene in cervical cancer. This study aimed to investigate the relationship between the expression of HPV E6/E7 mRNA and hTERC gene and the progression of cervical lesions and the significance of combined detection in the early screening of cervical cancer.

Data and methods

Basic information

With the approval of the hospital ethics Committee, 125 patients who underwent TCT examination in our hospital from June 2017 to February 2019 were selected as research objects. According to the results of pathological tissue blood examination, 25 cases were devided into normal control group (NC), 33 cases of cervical intraepithelial neoplasia (CIN1), 31 cases of cervical intraepithelial neoplasia grade2 orworse (CIN2), 26 cases of cervical intraepithelial neoplasia grade3 orworse (CIN3), and 10 cases of invasive carcinoma (Invasive carcinoma). According to modern cervical cytology (TBS classification), 25 patients were divided into NC group, 35 cases of atypical squamous epithelial cells without definite diagnostic significance (ASCUS) group, 11 cases of atypical squamous epithelial cells including highly squamous commercial lesions (ASCH) group, 6 cases of atypical squamous epithelial cells (AGC) group, 28 cases of highly squamous intraepithelial lesions (HSIL) group, and 5 cases of squamous cell carcinoma group (SCC) group.

Inclusion criteria:

• Patients were not treated with radiotherapy, chemotherapy or immunosuppressant before the study;

• Informed consent was signed by patients and their families;

• The patient was not in the menstrual period.

Exclusion criteria:

• Patients with severe liver, kidney or heart dysfunction;

• Patients during lactation or pregnancy;

• The patient had sex or used drugs in the vagina within one week before TCT examination.

There were no significant differences in age and other basic data among all groups (P>0.05).

Main instruments and reagents

Electron microscope (Shanghai Zhengxi Instrument Equipment Co., LTD., No. ZDB-600C); Slicing machine (Guangzhou Weigesi Biotechnology Co., LTD., Model: M3050); Refrigerator (Jinan Chuangxiang Biotechnology Co., LTD., Model: MDF-193); Centrifuge (HerryTech Co.,Ltd., Model: TG18G); Anhydrous ethanol (Sinopharm Chemical Reagent Co., LTD.); Methanol (Anhui Ante Biochemistry Co., LTD.); 0.9% sterile sodium chloride solution (Shanghai Zeye Biotechnology Co., LTD.).

Observation indicators

Cervical exserted cells were collected by TCT method and sent to the laboratory for microscopic examination as soon as possible. The remaining cells were used for HPV E6/E7 mRNA and hTERC gene detection. If they cannot be carried out immediately, they should be stored in a refrigerator at 4°C. Nucleic acid hybridization was used to detect HPV E6/E7 mRNA. Appropriate amount of TCT specimens were put into test tubes and centrifuged to remove the supernatant. The lysate was mixed with ionic water in a ratio of 1:2. After the cells were suspended and dispersed, 5μL protease K was added, and placed at 60 °C for about 60 min, and mixed. Centrifuge was used for centrifugation, and the supernatant was taken to detect HPV E6/E7 mRNA positive expression rate.

FISH was used to detect hTERC gene

Cervical cell slides of 18 healthy subjects were selected from the normal population, and the number of abnormal cells in 150 cells with complete signal was calculated respectively. HTERC gene

amplification was positive if the number of abnormal cells was greater than (abnormal cell mean ±3 SD) ×100%. ROC curve was established to analyze the significance of HPV E6/E7 mRNA and hTERC gene detection alone or in combination in early screening of cervical cancer.

Statistical methods

SPSS20.0 software package was used for statistical data analysis, and independent sample t test was used for measurement data comparison. Statistical data were compared by χ^2 test. Ridit test was used for grade data comparison. ROC curve was established to analyze the significance of HPV E6/E7 mRNA and hTERC gene detection alone or in combination in early screening of cervical cancer. The results were statistically significant with $P<0.05$.

Results

Positive expression of HPV E6/E7 mRNA and hTERC gene in different cytological categories

The results showed that HPV E6/E7 mRNA and hTERC gene expression increased in NC group, ASCUS group, LSIL group, ASCH group, AGC group, HSIL group and SCC group, and the higher the cytological lesion grade, the higher of positive expression of HPV E6/E7 mRNA and hTERC gene ($P<0.05$). See Table 1 and Figure 1.

Group	n	HPV E6/E7 mRNA	hTERC
NC	25	8 (32.00)	2 (8.00)
ASCUS	35	18 (51.43)	6 (17.15)
LSIL	15	8 (53.33)	8 (53.33)
ASCH	11	9 (81.82)	7 (63.64)
AGC	6	6 (100.00)	5 (83.33)
HSIL	28	26 (92.86)	27 (96.43)
SCC	5	5 (100.00)	5 (100.00)

Table 1: Positive expression of HPV E6/E7 mRNA and hTERC gene in different cytological categories (%).

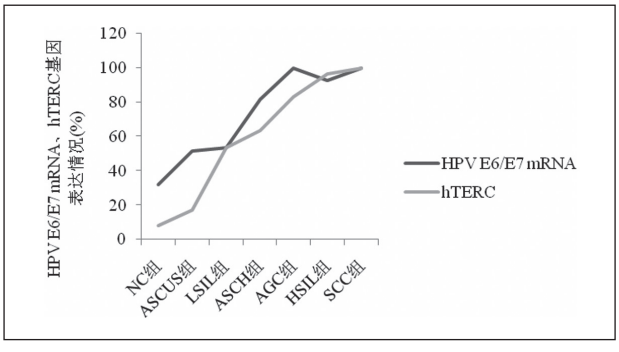

Figure 1: Positive expression of HPV E6/E7 mRNA and hTERC gene in different cytological categories.

Positive expression of HPV E6/E7 mRNA and hTERC gene in different histological categories

The positive expression of HPV E6/E7 mRNA and hTERC gene in different histological classifications of NC group, CIN1 group, CIN2 group, CIN3 group and ICC group showed an upward trend ($P<0.05$). With the increase of histological lesions, the positive expression rate of HPV E6/E7 mRNA and hTERC gene increased significantly, and the positive expression rate of HPV E6/E7 mRNA and hTERC gene in the ICC group was as high as 100%. Shown in Table 2 and Figure 2.

Group	n	HPV E6/E7 mRNA	hTERC
NC	25	8 (32.00)	2 (8.00)
CIN1	33	15 (45.45)	5 (15.15)
CIN2	31	24 (77.42)	20 (64.52)
CIN3	26	23 (88.46)	25 (96.15)
ICC	10	10 (100.00)	10 (100.00)

Table 2: HPV E6/E7 mRNA and hTERC gene positive expression in different histological classifications (%).

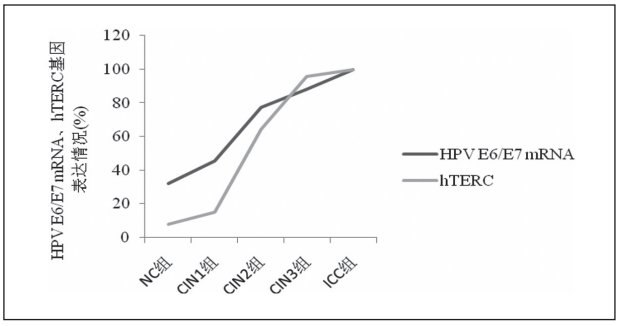

Figure 2: The positive expression of HPV E6/E7 mRNA and hTERC gene in different histological classifications.

Significance of HPV E6/E7 mRNA and hTERC gene detection alone or in combination in the early screening of cervical cancer

The results of the study showed that the sensitivity and specificity of HPV E6/E7 mRNA, hTERC and HPV E6/E7 mRNA+hTERC detection were 73.08% and 66.67%, 61.13% and 92.55%, 82.39% and 96.42%. The sensitivity and specificity of combined detection with HPV E6/E7 mRNA and hTERC gene were significantly higher than that of single detection. See Table 3.

Index	Positive predictive value	Negative predictive value	Sensitivity	Specificity	Area under the curve
HPV E6/E7 mRNA	89.79	38.32	73.08	66.67	0.638
hTERC	96.85	36.76	61.13	92.55	0.821
HPV E6/E7 mRNA+hTERC	98.93	58.72	82.39	96.42	0.899

Table 3: Significance of HPV E6/E7 mRNA and hTERC gene detection alone or in combination in the early screening of cervical cancer (%).

Discussion

Cervical cancer was the most common malignant tumor in the female reproductive system. Its incidence was second only to breast cancer, ranking second in the world. With the development of society, the incidence of cervical cancer was increasing year by year, and had a younger trends, seriously threaten the quality of life of society and families. Early cervical cancer often had no obvious symptoms. The development of low-grade squamous intraepithelial neoplasia into cervical cancer was a long-term process. Therefore, early detection and diagnosis played an important role in reducing cervical cancer[7, 8]. Studies had found that high-risk HPV cervical intraepithelial neoplasia and cervical cancer are the main causes, but only persistent infection with high-risk HPV can cause cervical intraepithelial neoplasia and cervical cancer[9]. According to reports, HPV can interfere with mitotic spindle function and cytokinesis[10].

HPV gene products could be divided into E region proteins and L region proteins. E6/E7 genes were the early coding genes of HPV, which played an important role in the process of HPV canceration. E6 protein can inhibit the expression of tumor suppressor gene P53 and inhibit cell apoptosis, while E7 protein can bind to retinoblastoma-like protein and make the cell cycle out of control[11]. Some scholars believed that HPV E6/E7 mRNA can be used as an effective indicator to judge the correlation of cervical cancer risk[12]. HPV E6/E7 mRNA can promote the decomposition of retinoblastoma-like protein and P53, leading to the disorder of cell cycle and inducing continuous cell deterioration, ultimately leading to the occurrence of cancer. During the process, the proteins translated by E6 and E7 genes played an important role in the carcinogenesis of the virus. When the body was infected by a virus, E6 and E7 genes were expressed in large quantities, leading to malignant transformation of host cells[13, 14]. Gustinucci et al.[15] found that the sensitivity of HPV E6/E7 mRNA in the diagnosis of grade squamous intraepithelial neoplasia and above are significantly higher than that of HPV DNA.

It had been reported[16] that telomerase activity is closely related to the occurrence and development of tumors. Telomerase was a ribosomal protein that maintained the end of chromosomes, having reverse transcriptase activity and can synthesize DNA repeats of telomeres using its own RNA as a template and add them to the end of chromosomes to maintain the stability of telomere length. Under normal circumstances, only germ and stem cells had high telomerase activity, while most malignant tumor cells show strong telomerase activity[17]. In this study, the positive expression of HPV E6/E7 mRNA and hTERC gene in different histological and cytological categories showed an increasing trend ($P<0.05$), and the positive expression rates of HPV E6/E7 mRNA and hTERC gene were significantly increased with the increase of histological or cytological lesions. These results suggested that the expression of HPV E6/E7 mRNA and hTERC gene can reflect the progression of cervical cancer lesions, which was significantly correlated with the progression of cervical cancer lesions.

The area under ROC curve was an important method to compare and evaluate HPV E6/E7 mRNA, hTERC and HPV E6/E7 mRNA+hTERC for screening early cervical cancer. The sensitivity and specificity of HPV E6/E7 mRNA, hTERC and HPV E6/E7 mRNA+hTERC were 73.08%, 66.67%, 61.13%, 92.55%, 82.39%, 96.42%, respectively. The sensitivity and specificity of combined detection of HPV E6/E7 mRNA and hTERC gene were significantly higher than that of single detection. These results indicated that the combined detection of HPV E6/E7 mRNA and hTERC gene was of great significance for improving the early screening rate of cervical cancer.

In conclusion, the expression of HPV E6/E7 mRNA and hTERC gene increased significantly with the increase of cervical cancer lesions. The above indicators alone or in combination can reflect the development of cervical cancer to a certain extent, but the combined detection of the two has the highest significance in the early screening of cervical cancer.

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