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Clinical Significance of Detection of Human Papilloma Virus DNA and E6/E7 mRNA for Cervical Cancer Patients

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ABSTRACT

This study aimed to explore the clinical significance of detection of human papillomavirus (HPV) DNA and E6/E7 mRNA for cervical cancer patients. For this purpose, a total of 300 patients with cervical lesions who performed the colposcopy examination for the cervix between January 2018 and January 2020 were divided into three groups according to the level of cervical intraepithelial neoplasia (CIN): Low-level group (Group A, n = 101), high-level group (Group B, n = 149), cervical cancer group (Group C, n = 50) and gynecological inflammation group (Group D, n = 60). Tissue samples were collected from subjects above to perform the immunohistochemistry for E6/E7 protein and detection of HPV DNA and HPV E6/E7 mRNA. The results showed that HPV DNA copies and the positive rates of protein, DNA and mRNA of HPV E6/E7 in Groups A, B and C were significantly higher than those in Group D (all $P<0.05$), while no significant difference was identified in the comparison of the positive rate and copies of DNA among Group A, B and C ($P> 0.05$); in the Group A, B and C, patients had a higher positive rate of E6/E7 mRNA than those in the Group D ($P<0.05$), while in the Group B and C, the positive rate and copies of mRNA of HPV E6/E7 and HPV E6/E7 proteins were all higher than those in the Group A ($P<0.05$). In addition, the specificity of HPV E6/E7 mRNA was inferior to HPV DNA in the detection of the low-level intraepithelial neoplasia, but it performed better in the detection of the high-level intraepithelial neoplasia and cervical cancer. In general, HPV E6/E7 mRNA shows promising value in the detection of the development and progression of cervical cancer.

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Introduction

Cervical cancer, with the tremendous changes in the lifestyle and diet structure in recent years, has witnessed an increase in the prevalence in China, so it is quite important to optimize the medical technique for early diagnosis (1). Clinical findings reveal that a long-term pre-cancerous pathological change is required for the development of cervical cancer, and, thus, strengthening the screening is conducive to reducing the death caused by cervical cancer (2). This disease is very important and is one of the causes of disputes and dissatisfaction of couples, which also causes divorce (3). Previously, cytologic tests dominated the screening of cervical cancer, while increasing epidemical evidence has shown the correlation between cervical cancer, pre-cancerous lesion and the continuous infection of the human papilloma virus (HPV) (4). Thus, cytologic test in combination with the molecular test of HPV DNA is increasingly preferred in current clinical practice (5, 6). However, this method is limited to detecting the pathogens but fails to reflect the cancerous changes of cells precisely (7). As is known to all, cancerous changes in the cervix are induced by the overexpression of E6/E7, which is believed to be caused by the integration of HPV DNA to the chromosome of the host (8-10). Meanwhile, HPV E6/E7 overexpression, by increasing the activity of MMP-2 and MMP-9, can promote the migration of cervical cancer cells (11, 12). As such, we aimed to perform the detection of HPV E6/E7 mRNA for patients with cervical lesions, so as to provide a reference for the early diagnosis of cervical cancer.

Materials and methods

General data

A total of 300 patients with cervical lesions who performed the colposcope examination for the cervix

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between January 2018 and January 2020 were divided into three groups according to the level of cervical intraepithelial neoplasia (CIN): Low-level group (Group A, n = 101), high-level group (Group B, n = 149), cervical cancer group (Group C, n = 50) and gynecological inflammation group (Group D, n = 60). Inclusion criteria: 1) Patients with a history of intercourse before admission; 2) Patients not in pregnancy; 3) Patients with no history of physical treatment for cervix; 4) Patients who agreed to participate in this study after they were informed of the content of study. Patients with a history of pelvic chemotherapy or poor compliance to treatment were excluded from this study. Among all subjects, the average age was (47.12±5.82) years old, and patients had an average disease course of (4.87±1.19) months. Comparison of the matching factors among four groups showed no significant difference (P>0.05), suggesting that the data of patients were comparable. This study had been approved by the Ethical Committee of Cancer Hospital of China Medical University Liaoning Cancer Hospital & Institute.

Detection of high-risk HPV E6/E7 mRNA

Kits and relevant apparatus for detection were provided by Kodia (Xinxiang) Biotech Co., Ltd, including the 96-well plate and Cervix Homeostasis Kit, and the kit and apparatus for detecting HPV DNA were provided by Digene in the US. All patients underwent the cytological test by using the Liquid-based Thin-layer Cytological Kit for Cervical Lesions via biopsy and the pathological tests. Cervical epithelial cells were collected and then centrifuged at 3000 rpm, with the supernatant being discarded. Sediment was then dissolved in the TRI lysis buffer and protease K for 120 min, during which sediment was shaken every 30 min. The lysate was placed in the 96-well plate, with 2 replicate wells and 2 Positive-control wells, followed by the determination of optical density. For mRNA, a capture probe was used to collect the mRNA which was later magnified and incubated with the substrate and catalyst. Following that, magnified mRNA was then hybridized with the ALP-labeled probe to generate the light signals. RNA copies were then read by the corresponding software, and samples with RNA copies > 0 were taken as a positive result, while those with 10000 RNA copies were taken as high risk.

Detection of HPV DNA

PCR reverse dot blot was carried out to test the epithelial cell samples in the cervix, and HPV test and genotyping were conducted as per the instruction of kits.

E6/E7 detection

Immunohistochemistry streptavidin-peroxidase (IHC-SP) method was adopted to detect the antigens in the paraffinized biopsy samples of cervical tissues, and the samples with the brown-stained substances were taken as the positive results.

Indicators

We recorded and calculated the results of detection of HPV DNA, DNA copies, HPV E6/E7 mRNA and mRNA copies and the protein expression of HPV E6/E7, and the sensitivity and specificity of indicators in evaluating the cervical lesion in 3 groups.

Statistical analysis

All data were processed in SPSS 21.0 software (SPSS, Inc, Chicago, IL, USA). Measurement data were expressed in form of mean ± standard deviation (mean ± SD). Differences among groups were testified by using the chi-square test, while the intergroup multiple comparisons were carried out via the Dunnett's T3 method. Enumeration data were compared by using the chi-square test. For multiple comparisons, α=0.05 was set as the inspection level, and for pairwise comparison, Bonferroni correction was adopted, with α'=0. 0083. P < 0.05 suggested that the difference had statistical significance.

Results and discussion

Comparison of the results of HPV DNA detection and DNA copies among groups

HPV DNA copies and the positive rates in Groups A, B and C were significantly higher than those in Group D (all P<0.05), while no significant difference was identified in the comparison of the positive rate and copies of DNA among Group A, B and C (P> 0.05) (Figure 1).

Comparison of the results of HPV E6/E7 mRNA detection and mRNA copies among groups

In Groups A, B and C, patients had a higher positive rate of HPV E6/E7 mRNA than those in

Group D (P<0.05), while in Group B and C, the positive rate and copies of mRNA of HPV E6/E7 and HPV E6/E7 proteins were all higher than those in the Group A (P<0.05) (Figure 2).

Comparison of the protein expression of HPV E6/E7 among groups

In Groups A, B and C, patients had a higher positive rate of E6/E7 protein than those in Group D (P<0.05), and the highest positive rate was found in Group C (P<0.05) (Figure 3).

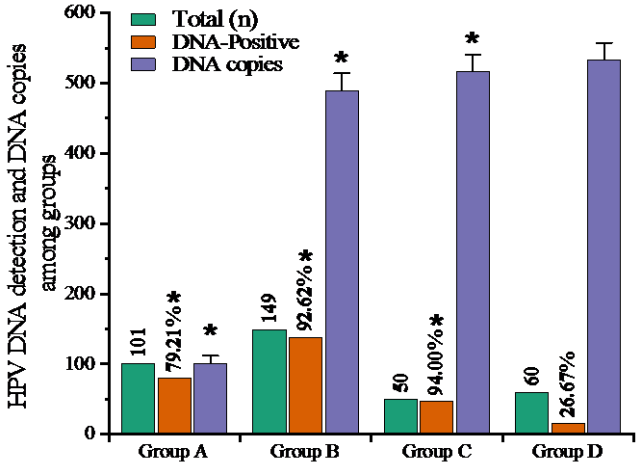

Figure 1. Comparison of the results of HPV DNA detection and DNA copies among groups; *P<0.05 vs. the Group D (Inflammation group)

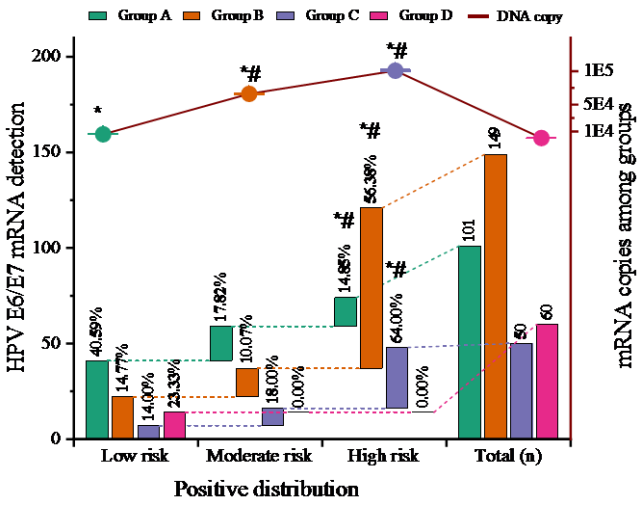

Figure 2. Comparison of the results of HPV E6/E7 mRNA detection and mRNA copies among groups; *P<0.05 vs. the Group D (Inflammation group); #P<0.05 vs. the Group A (Low-level group)

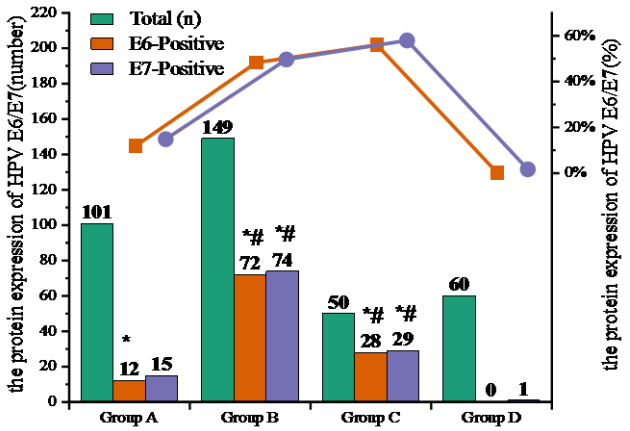

Figure 3. Comparison of the protein expression of HPV E6/E7 among groups; *P<0.05 vs. the Group D (Inflammation group); #P<0.05 vs. the Group A (Low-level group)

Comparison of the diagnose accordance rate of markers for the cervical lesions

Differences in the specificity and sensitivity between HPV mRNA and HPV DNA in diagnosis of the low-level cervical lesion showed no statistical significance (P>0. 05); in the diagnosis of the high-level cervical lesions, HPV DNA had lower specificity than that by HPV mRNA, while HPV mRNA performed better than HPV DNA in sensitivity; as for the diagnosis of cervical cancer, HPV mRNA performed better in the sensitivity and specificity than HPV DNA (Table 1).

Table 1. Comparison of the diagnose accordance rate of markers for the cervical lesions

Group	Specificity				Sensitivity			
	HPV mRNA	HPV DNA	χ^2	*P*	HPV mRNA	HPV DNA	χ^2	*P*
Group A	72.00%	77.61%	0.994	0.402	87.50%	85.48%	1.300	0.692
Group B	79.79%	77.78%	4.110	0.000	83.83%	92.30%	4.849	0.000
Group C	87.50%	75.00%	4.603	0.000	97.05%	85.29%	8.120	0.000

As evidenced by the current survey (13), nearly 99.7% of cervical cancer patients have HPV infection which is considered as the major pathogen for cervical cancer, so it is quite important to strengthen the early screening to improve the prophylaxis and outcomes of cervical cancer (14). At present, diagnosis for cervical cancer is made upon the results of DNA marker, while HPV DNA, as reported by an increasing body of evidence (15), is believed to be an effective supplementary method for the early screening of cervical cancer. Liu *et al.* (16) reported that HPV DNA marker, as the pathogen evidence, can hardly

contribute to the evaluation of the status of cervical infection and the activity of oncogenes. HPV E6/E7 manifests the oncogene activity in cervical cancer, showing the correlation with the disease progression. Besides, high-risk HPV E6/E7 plays a key role in the transformation and maintenance of malignant tumors (17). The mRNA, as the product of HPV E6/E7 gene transcription, can directly reflect the malignant transformation of host cells, as per the literature of Ndiaye *et al*. (18).

In this study, we found that the positive rates of HPV DNA among the low-level, high-level and cervical cancer groups showed no significant differences, and similar results were confirmed in the comparison of HPV DNA copies, suggesting that disease progression does not increase the copies of HPV DNA. Detection of HPV E6/E7 mRNA revealed that the positive rates of HPV E6/E7 mRNA in the high-level and cervical cancer groups were all higher than those in the low-level group, and the pathological grade of the cervical lesion showed a linear correlation with the positive rate and copies of HPV E6/E7 mRNA, indicating that HPV E6/E7 mRNA detection is critical to the diagnosis of cervical cancer. Results of this study also supported that HPV E6/E7 mRNA had a higher sensitivity than HPV DNA in the diagnosis of cervical cancer, while HPV DNA worked better in evaluating the low-level and high-level intraepithelial lesions, similar to the findings of Liu *et al*. (16). As reported, HPV DNA has a sensitivity of 95%, HPV mRNA has a sensitivity of 75% to 91%, but HPV DNA is inferior to HPV mRNA in specificity (40%~47% vs. 56%~97%). Besides, HPV mRNA has a positive likelihood ratio of 2.0 to 5.8 and a negative likelihood rate of 0.16 to 0.29, while HPV DNA has a positive likelihood ratio of 1.1 to 1.9 and a negative likelihood rate of 0.03 to 0.2, suggesting that HPV mRNA-positive result is more prone to cervical cancer, while HPV DNA-negative result indicates no cervical lesions. In addition, the results of this study also indicated that HPV E6/E7 mRNA had lower specificity than HPV DNA in evaluating the low-level cervical intraepithelial lesions, while it performed better in the specificity in evaluating the high-level cervical intraepithelial lesions and cervical cancer. Thus, HPV E6/E7 mRNA is more sensitive in the diagnosis of cervical cancer.

In summary, HPV E6/E7 mRNA shows promising value in the detection of the development and progression of cervical cancer.

Acknowledgements

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Interest conflict

None.

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