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Roles of high-risk human papilloma virus (HR-HPV) E6/E7mRNA in triaging HPV16/18 cases

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Summary

Objective: This study aimed to investigate the roles of high-risk human papilloma virus (HR-HPV) E6/E7mRNA in triaging patients negative for intraepithelial lesion (NILM) accompanied with HPV16/18 infection. *Materials and Methods:* A total of 514 female patients simultaneously underwent cytological assay, HPV-DNA assay, E6/E7mRNA detection, and colposcopic biopsy were selected. The study endpoint was the histologically confirmed high-grade cervical intraepithelial neoplasia (CIN) II or higher(II+). *Results:* The positive rates of E6/E7mRNA in histopathologically confirmed cervicitis, CIN I, CIN II, CIN III, and cervical cancer were 53.4%, 66.7%, 89.9%, 91.4%, and 100%, respectively. Among the patients that underwent the colposcopic biopsy due to cytological NILM plus HPV16/18 infection, the positive predictive values of HPV16/18 and E6/E7mRNA towards high-grade cervical lesions were 21.5% and 40.4%, respectively, and the comparison between these two factors showed statistically significant difference ($p < 0.05$). The negative predictive value of E6/E7mRNA was 97.8%. *Conclusions:* E6/E7mRNA showed good triaging effects towards the patients with cytological NILM plus HPV16/18 infection and could significantly reduce colposcopy and biopsy.

Key words: Cervical cancer; Screening; E6/E7mRNA; HPV.

Introduction

In 1970s, German virologist ZurHausen firstly proposed the assumption that human papilloma virus (HPV) was closely related with the onset of cervical cancer; a large number of studies had shown that HPV infection, especially persistent high-risk HPV infection, was the main reason of the most cases of cervical precancerous lesions and cervical cancer [1, 2]. HPV-DNA detection could increase the detection rate towards high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer [3], but 80% of high-risk virus infections were transient [4], and most new infections could be extinct in two years [5]. Only 1% of the high-risk virus infected women would gradually develop into cervical cancer [6]. This caused the fact that though the sensitivity of HPV-DNA detection was high, its specificity was relatively low [7]; therefore, it might result in unnecessary colposcopy and biopsy in many women [8], thus increasing patients' economic burdens and psychological burdens. The clinical diagnosis and treatment urgently required one screening method with high specificity and sensitivity towards cervical cancer [9], and the E6/E7mRNA detection is one of the research hotspots. Study had shown that the overexpression of E6/E7 oncoprotein was closely related to the progression risk of cervical diseases [10]; meanwhile, it also had important significance in triaging high-risk cervical virus infections [11]. This study analyzed the expressions of E6/E7mRNA in the patients with cytological negative for intraepithelial lesion (NILM) plus HPV16/18 infection, aiming to investigate its roles and values in triaging high-level cervical lesions.

Materials and Methods

A total of 514 female patients simultaneously underwent cytological assay, HPV-DNA assay, E6/E7mRNA detection, and colposcopic biopsy in Wenzhou People's Hospital from April 2014 to August 2015 and were selected, among which 93 patients with cytological NILM plus HPV16/18 infection. All the study subjects had no history of CIN, cervical cancer, pelvic radiation therapy, total hysterectomy, or present pregnancy.This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Shandong University. Written informed consent was obtained from all participants.

One cervical brush was rotated three to five circles at the cervical squamous columnar junction area; the brush was then fully rinsed in ThinPrep cell preservation solution to maximally collect the cells sampled,.The solution was then sent to the cell lab for programmed processing; the cytological diagnosis was performed by professional gynecological pathologists, and the result determination referred to the modified TBS classification system (2001).

HPV-genotype microarray detection system, PE5700 gene amplifier, HbriMax medical rapid nucleotide hybridization instrument, and HPV amplification genotype detection kit were utilized. DNA extraction kit used the Cape medical rapid nucleotide hybridization instrument as the platform and then high-throughput detected the 21 HPV subtypes (accounting for 95% of HPV infection) on the nucleotide probe-fixed low-density microarray film using the principle of flow-through hybridiza-

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Table 1. — *Relationship between E6/E7mRNA-positive rates and pathological results.*

Index	Cases	E6/E7mRNA-positive rate (%)
Normal/inflammation	245	131 (53.47%)
CIN I	87	58 (66.67%)*
CIN II	89	80 (89.89%)*#
CIN III	81	74 (91.36%)*#
Cervical cancer	12	12 (100%)*#Δ

* Compared with normal or inflammation, significant difference;
# compared with group CIN I, statistically significant difference;
Δ compared with group CIN II, statistically significant difference.

Table 2. — *Relationship between E6/E7mRNA-positive rates and pathological results.*

Item	Number of patients	Positive rate of E6/E7mRNA(%)
CIN II–[a]	332	189 (56.93%)
CIN II+[b]	182	166 (91.21%)
c^2		64.66
P		0.00

[a] CIN II−, including normal cervix, inflammation, and CIN I;
[b] CIN II+, including CIN II, CIN III, and cervical cancer.

Table 3. — *Relationships between E6/E7mRNA and different cervical lesions in patients with NILM plus HPV16/18 infection.*

Group	Cases	E6/E7mRNA	
		Positive	Negative
CIN II+	20	19	1
CIN II−	73	28	45

tion, including 13 kinds of high-risk subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), five kinds of low-risk subtypes (6, 11, 42, 43, and 44), and three kinds of common subtypes in Chinese populations (53, 66, and CP8304).

The Quanti Virus HPV E6/E7mRNA diagnosis kit was used to detect 14 kinds of HR-HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using the branched DNA technology.

The patients with abnormal colposcopic results then underwent cervical biopsy; the pathological results were diagnosed by two professional pathologists. The diagnostic criteria referred to the WHO classification criteria.

SPSS17.0 statistical software was used for the statistical processing. The counting data were performed using the χ^2 test, with $p < 0.05$ considered as statistically significant. The positive and negative predictive values were calculated using traditional contingency table.

Results

The detection results of E6/E7mRNA in a variety of pathological lesions are shown in Table 1; it could be seen that along with the increased levels of the cervical lesions, the positive rates of E6/E7mRNA gradually increased, among which the intergroup comparison between CIN II and CIN III groups, as well as between CIN III and cervical cancer groups, which showed no statistically significant difference ($p > 0.05$). The comparison of the positive rates of E6/E7mRNA between CIN II− and CIN II+ groups showed statistically significant difference ($p < 0.05$). The data are shown in Tables 1 and 2.

These was a total of 93 patients with cytological NILM plus HPV16/18 infection, including 47 cases with E6/E7mRNA-positive rate and 46 negative cases; 20 cases were pathologically diagnosed as CIN II+. The positive predictive value of HPV16/18 was 21.51% (20/93); the positive predictive value of E6/E7mRNA was 40.43% (19/47). The difference between the two group was statistically significant (21.51%, 40.43%, $\chi^2 = 20.15$, $p < 0.05$); the negative predictive value of E6/E7mRNA was 97.83% (45/46, Table 3).

Discussion

Lu *et al.* have shown that only when the number of the host cell-integrated HR-HPV reached a certain level, the E6/E7 oncogene could be overexpressed, and then high-level lesions even cervical cancer might develop [12]. Therefore, detecting the expression of the E6/E7 oncogene could triage whether the cervical high-risk virus was persistent or transient [13, 14]; according to the central dogma, the expression of the E6 and E7 oncogenes could be realized through detecting the transcription of E6/E7mRNA in the HPV oncogene [15].

The present study aimed to investigate whether the detection of E6/E7mRNA using the branched DNA technology could effectively triage the patients with cytological NILM plus HPV16/18 infection. The results showed that if these patients further underwent the E6/E7mRNA detection triaging, the positive predictive value towards the high-grade cervical lesions could be significantly improved; meanwhile, its negative predictive value was also high, and it could effectively reduce the colposcopy and biopsy, thus alleviating patients' mental and financial burdens.

The results of this study also showed that the positive expression rate of E6/E7mRNA was gradually increased with the increasing of the histopathological levels, consistent with Ratnam *et al.* and Coquillard *et al.* [16, 17]. In particular, the positive rate of E6/E7mRNA in CIN II+ was significantly higher than CIN II−, indicating that the E6/E7mRNA expression was closely related with the cervical lesions, and its high expression could facilitate the occurrence and development of cervical precancerous lesions.

Rijkaart *et al.* [18] found that 8% of the patients with normal cytological results plus cervical high-risk virus infection would eventually develop into high-level cervical lesions (CIN II+). Seventy percent of the patients with cervical cancer would be accompanied with the HPV16 or 18 infection, among which the cervical squamous cell

carcinoma was usually accompanied with the HPV16 infection [19]; therefore, in patients older than 30 years, even if their cytological results were normal, once the HPV16/18 infection was found, the colposcopy and pathological biopsy should also by recommended. The results of this study revealed that among the 93 patients with cytological NILM plus HPV16/18 infection, 20 patients underwent high-grade cervical changes (CIN II+), and the positive predictive value of HPV16/18 towards the high-grade cervical lesions was only 21.51%. If these patients further underwent the E6/E7mRNA triaging, the present authors found that the positive predictive value of E6/E7mRNA in these patients was 40.43%, and the negative predictive value was 97.83%. The positive predictive value of E6/E7mRNA was significantly higher than that of HPV16/18; more importantly, E6/E7mRNA had a high negative predictive value, i.e. if the E6/E7mRNA test result was negative, the patients would rarely develop high-grade cervical lesions, therefore, these patients could be recommended outpatient follow-up. This would greatly reduce the colposcopy and biopsy. Hence, the E6/E7mRNA detection could be an effective triaging approach in patients with cytological NILM plus HPV16/18 infection. The results of this study were similar to those by Perez and Rijkaart *et al.* [11, 20].

Limitations of the present study are the following: first, the case number was small, therefore a larger sample size is required to further confirm the above findings. Second, through the E6/E7mRNA detection, one case of CIN II was misdiagnosed: this patient was 45-years-old and underwent cervical conization. Therefore, if the E6/E7mRNA test results in young CIN II patients with fertility requirements are negative, could they have the follow-up observation? Meanwhile, if the E6/E7mRNA test results in the patients with negative cytological or histopathologic test results are positive, would they be more likely to develop to CIN II+ in future? These queriea require further in-depth studies.

Acknowledgments

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