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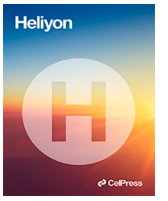

Research article

A novel method for colposcopic shunting in HPV-positive women: Quantitative detection of HPV E7 oncoprotein

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ABSTRACT

The objective of the study was to evaluate the clinical application potential of quantitatively detecting human papillomavirus (HPV) E7 oncoprotein in HPV-positive women, with the goal of detecting potential high-grade cervical squamous intraepithelial lesions (HSIL) and cervical cancer improving the accuracy of colposcopic shunting in these patients.HPV-positive women (N = 611) were selected for quantitatively detecting HPV E7 protein levels by magnetic particle-based chemiluminescence immunoassay before colposcopy. Receiver operating characteristic (ROC) curve analysis was performed (n = 400) to determine diagnostic detection thresholds for HPV E7 oncoprotein. ThinPrep cytology test (TCT) and Aptima HPV E6/E7 mRNA analysis were also performed (n = 211). The diagnostic performance of these three diagnostic methods in detecting HSIL and cervical cancer was compared with the gold standard of pathological diagnosis. The area under the ROC curve was 0.724. The diagnostic detection threshold of HPV E7 oncoprotein was ≥10.88 ng/mL. The sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NPV), and Youden index of HPV E7 oncoprotein for the identification of HSIL and cervical cancer were 78.7 %, 77.9 %, 72.2 %, 83.3 %, and 56.6 %, respectively, which were higher than those of TCT and HPV E6/E7 mRNA.The results indicate that quantitative detection of HPV E7 oncoprotein can effectively shunt HPV-positive women and reduce unnecessary colposcopy and biopsy. It can detect potential HSIL and cervical cancer in a timely manner and prevent high-risk patients from missing diagnosis.

1. Introduction

According to the Global Cancer Statistics Report 2020, cervical cancer had the ninth highest incidence and death rate among malignant tumors worldwide [1]. Epidemiology and molecular biology studies have reported that persistent human papillomavirus (HPV) infection is closely related to the development of cervical cancer [2]. However, 70%–80 % of women will be infected with HPV in their lifetime, and autoimmunity will usually clear HPV within two years of infection. A transient HPV infection cannot cause cervical cancer [2]. Only a small percentage of women with persistent HPV infection may develop cervical lesions and eventually develop invasive cervical cancer [3]. This provides a favorable opportunity for early detection and intervention of cervical cancer

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because it takes about 10 years to progress from low-grade to high-grade squamous intraepithelial lesion (HSIL) to cervical cancer [4]. Early detection of cervical lesions is of great value because cervical cancer is curable if detected early. Cervical cancer screening aims to identify high-risk individuals for HSIL and cervical cancer for early intervention [5]. Therefore, adopting a more accurate screening technology and shunting scheme to improve the comprehensive benefits of cervical cancer screening is a clinical concern.

When during persistent HPV infection, the HPV genome gets integrated with host cell chromosomes. The increased levels of E6/E7 oncoprotein are important for inducing the transformation and carcinogenesis of cervical epithelial cells [6]. Therefore, detecting HPV E6/E7 expression products to determine the carcinogenic activity of HPV and the risk of cervical cancer has garnered increasing attention in recent years. Presently, several commercial HPV E6/E7 mRNA assays are available with gradually recognized clinical value. However, only a few quantitative methods can detect HPV E6/E7 oncoprotein. In this study, the levels of HPV E7 oncoprotein in cervical exfoliated cells was detected by magnetic particle-based chemiluminescence immunoassay, and the clinical application value of quantitative detection of HPV E7 oncoprotein in HPV-positive women was determined.

2. Methods

2.1. Clinical data

This study included 611 HPV-positive women aged 19–70 years who visited the colposcopy outpatient clinic of Tianjin Central Obstetrics and Gynecology Hospital from September 2019 to January 2022. After obtaining informed consent, clinical and epidemiological information was collected, and cervical exfoliated cell samples were obtained. Cervical exfoliated cells were evaluated to

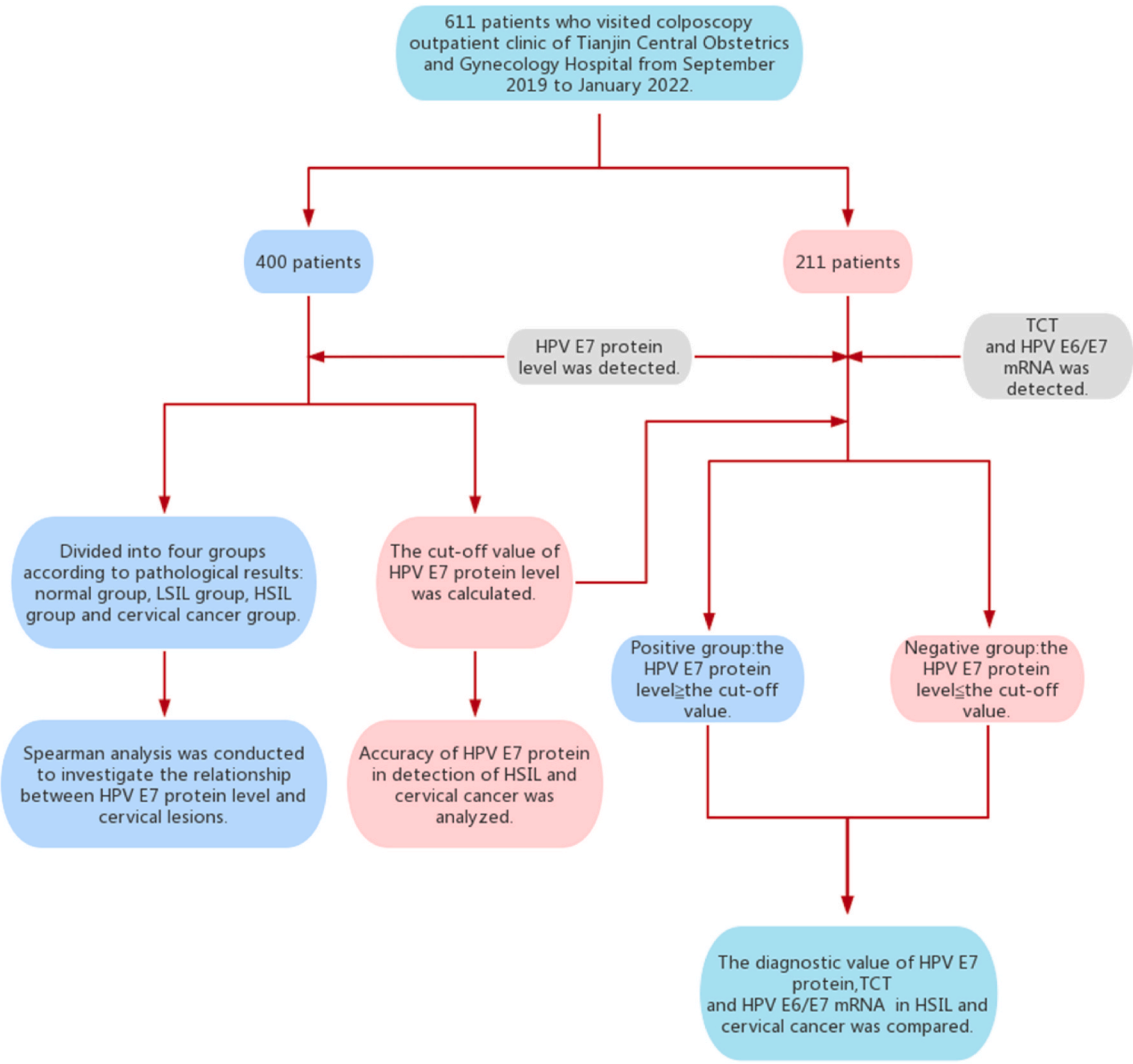

Fig. 1. Study flowchart.

detect the levels of HPV E7 oncoprotein. In addition, all patients underwent colposcopy and cervical biopsy, with pathological diagnosis as the diagnostic gold standard.

The inclusion criteria were as follows: 1) Women with prior sexual life history. 2) No sexual activity or vaginal infection within 7 days before sampling. 3) No vaginal/cervical manipulation or medication performed within 7 days before sampling. 4) No history of cervical surgery. 5) No previous history of cervical lesions or cervical cancer. The exclusion criteria were as follows: 1) Cervical exfoliated cells could not be adequately collected. 2)Women during pregnancy, puerperium and menstruation. 3)The clinical data were incomplete.

A total of 611 women were divided into two parts, of which 400 women were divided into the following four groups based on the pathological results: 1) Normal group: the pathological result was regular or chronic cervicitis, 2) Low-grade cervical squamous intraepithelial lesions (LSIL) group: the pathological result was LSIL (including CIN1 and part of CIN2), 3) HSIL group: the pathological result was HSIL (including CIN3 and part of CIN2), 4) Cervical cancer group: the pathological results were cervical cancer (including cervical squamous cell carcinoma and adenocarcinoma). Spearman correlation analysis was used to determine the correlation between HPV E7 oncoprotein levels and cervical lesions. The diagnostic accuracy of HPV E7 oncoprotein level for HSIL and cervical cancer was determined by receiver operating characteristic (ROC) curve, and the diagnostic threshold was determined.The remaining 211 women were divided into the following two groups according to pathology: 1) The control group: the pathology was normal cervix and LSIL and 2) The observation group: the pathology was HSIL and cervical cancer. According to the cut-off value, the remaining 211 cases were divided into two groups. 1) Positive group: the HPV E7 oncoprotein levels $\geqq$ cut-off value, and 2) Negative group: the HPV E7 oncoprotein levels $\leqq$ cut-off value. ThinPrep cytology test (TCT) and HPV E6/E7 mRNA was detected in these 211 women. The SEN, SPE, PPV, NPV Youden index and Kappa value of these three methods in detecting HSIL and cervical cancer were calculated and compared with pathological examination as the gold standard(Fig. 1).

The Ethics Committee approved this study at Tianjin Central Obstetrics and Gynecology Hospital (no. 2019KY102), and all patients signed informed consent and participated in the study voluntarily.

2.2. Preparation and preservation of samples

2.2.1. TCT testing

The mucus and secretions on the cervical surface were wiped with a dry cotton ball. The exfoliated cells in the cervical transition region were collected with a sterile cervical brush and kept in a vial containing cell preservation solution (Hologic, USA) for TCT examination. Cytological slides were prepared using the ThinPrep2000 system. The samples were pretreated with digestive fluid (glacial acetic acid: cleaning fluid was 1:9), programmed with ThinPrep machine (Hologic, USA), and fixed staining. Results were reported using the Bethesda Cervical Cytology Reporting System (TBS). They were divided as negative for intraepithelial lesion or malignancy (NILM), atypical squamous cell of undetermined significance (ASCUS), ASC-H (atypical squamous cells cannot exclude HSIL), LSIL, HSIL, and atypical glandular cell (AGC).

2.2.2. Aptima HPV E6/E7 mRNA testing

The remaining samples after TCT detection were used for Aptima HPV mRNA detection. The Optima HPV mRNA detection method developed by Hologic was used for E6/E7 mRNA detection. This method can detect E6/E7 mRNA of 14 types of high-risk HPV (hr-HPV). The 14 types of hr-HPV were as follows: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This method has two steps for detection. The first step is to detect 14 types of hr-HPV E6/E7 mRNA. If the expression is positive, the second step is to perform the 16 and 18/45 two-type detection, and positive type 16 is highly suggestive of cervical squamous cell carcinoma risk. Positive type 18/45 is highly suggestive of cervical adenocarcinoma risk. This kit adopted the automatic Tigris DTS system based on the test results' interpretation of the signal-cut-off ratio (S/CO value). A S/CO value greater than or equal to L was regarded as a positive mRNA test. Otherwise, it was negative.

2.2.3. HPV E7 oncoprotein testing

At the junction of the squamous and columnar epithelium of the patient's cervix, the dedicated cervical brush was rotated clockwise at least 5 times to collect the exfoliated cervical cells. Brush heads were stored in labeled dry-frozen tubes for later use. Samples were sent to the laboratory within 2 h for testing. HPV E7 oncoprotein magnetic particle-based chemiluminescence immunoassay and automatic chemiluminescence instrument developed by Fremid Biotechnology Co., LTD were used for detection. The HPV E7 oncoprotein was first bound to FITC-labeled monoclonal antibodies and then bound to magnetic particles linked with *anti*-FITC antibodies after incubation for a certain period. An external magnetic field was used to separate the immune complex from other substances. After obtaining the complex, AP-labeled monoclonal antibodies were added, and again under an external magnetic field, the immune-formed complex was separated from other substances. Then, the enzymatic chemiluminescence substrates were added. Moreover, the luminescence intensity of the reaction was detected after excitation.

2.2.4. Colposcopy and pathological examination

The lesions were sampled and detected by colposcopy per the operation specifications of colposcopy. When colposcopy showed no lesions, a multi-point biopsy sampling was performed at 3, 6, 9, and 12 points at the junction of the cervical squamous column and endocervical curettage simultaneously. All pathological specimens were reviewed and diagnosed by professional pathologists after hematoxylin and eosin staining. The highest pathologic grade was the final diagnosis if different sites had different diagnosis results.

2.3. Statistical analysis

SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Analysis of variance was used to compare the differences in E7 oncoprotein levels among the groups. Spearman correlation analysis was performed to investigate the relationship between HPV E7 levels and the lesions. The ROC curve was used to analyze the diagnostic accuracy of HPV E7 detection. The SEN, SPE, PPV, NPV and Kappa value were calculated to evaluate the diagnostic value of the HPV E7 oncoprotein detection method in this study. The level of significance was set at $P < 0.05$.

3. Results

3.1. Relationship between HPV E7 oncoprotein and cervical lesions

A total of 400 women who visited the colposcopy clinic were divided into four groups according to pathology as follows: 1) normal group (n = 176), 2) LSIL group (n = 101), 3) HSIL group (n = 113), and 4) cervical carcinoma group (n = 10). HPV16 E7 oncoprotein concentration was significantly different among the four groups ($P < 0.001$) (Table 1). The Spearman correlation analysis of HPV E7 oncoprotein levels in the cervical lesions of different degrees showed that the HPV E7 oncoprotein levels were positively correlated with the degree of the cervical lesions, and the higher the degree of the cervical lesion, the higher the HPV E7 oncoprotein levels ($R = 0.121$, $P < 0.01$) (Table 1).

3.2. Accuracy of the HPV E7 oncoprotein in detecting HSIL and cervical cancer

The 400 women were divided into two groups according to pathology: 1) The control group: the pathology was normal cervix and LSIL (n = 277) and 2) The observation group: the pathology was HSIL and cervical cancer (n = 123). The ROC curve was obtained to evaluate the accuracy of HPV E7 oncoprotein in detecting HSIL and cervical cancer (Fig. 2). The area under the ROC curve (AUC) was 0.724 (confidence interval 0.683–0.766; Table 2). The diagnostic detection threshold of HPV E7 oncoprotein was ≥10.88 ng/mL.

3.3. Diagnostic performance of HPV E7 oncoprotein in detecting HSIL and cervical cancer

A total of 211 women were divided into two groups based on HPV E7 oncoprotein levels: 1) Positive group: HPV E7 oncoprotein levels ≥10.88 ng/mL (n = 97) and 2) Negative group: HPV E7 <10.88 ng/mL (n = 114). Normal cervix and LSIL were pathologically negative, whereas HSIL and cervical cancer were pathologically positive. The value of HPV E7 oncoprotein in detecting cervical lesions was evaluated using pathological diagnosis as a gold standard. The SEN, SPE, PPV, NPV and Youden index of HPV E7 oncoprotein were 78.7 %, 77.9 %, 72.2 %, 83.3 %, and 56.6 %, respectively (Table 3). The Kappa value of HPV E7 oncoprotein in detecting HSIL and cervical cancer was 0.558.($P < 0.01$).

Table 4Consistency test of E7 oncoprotein levels detection and pathological diagnosis.

3.4. Comparison of the diagnostic performance of HPV E7 oncoprotein, E6/E7 mRNA, and TCT in the early diagnosis of cervical lesions

The 211 women were divided into two groups according to the result of Aptima HPV E6/E7 mRNA: 1) Positive group: the result of HPV E6/E7 mRNA was positive (n = 92) and 2) Negative group: the result of HPV E6/E7 mRNA was negative (n = 119). Furthermore, the 211 patients were divided into two groups based on TCT results: 1) Positive group: ASCUS, ASC-H, LSIL, HSIL, and cervical cancer (n = 108) and 2) Negative group: NILM (n = 103). The dignostic values of HPV E6/E7 mRNA and TCT in detecting cervical lesions were evaluated using pathological diagnosis as a gold standard. The SEN, SPE, PPV, NPV and Youden index of HPV E6/E7 mRNA in detecting HSIL and cervical cancer were 72.2 %, 69.4 %, 63.7 %, 77.1 %, and 41.6 %, respectively. The Kappa value of HPV E6/E7 mRNA in detecting HSIL and cervical cancer was 0.409 ($P < 0.01$). The SEN, SPE, PPV, NPV and Youden index of TCT were 64.7 %, 61.9 %, 53.4 %,72.2 %, and 26.6 %, respectively. The Kappa value of TCT in detecting HSIL and cervical cancer was 0.257 ($P < 0.01$). The SEN, SPE, PPV, NPV and Youden index of HPV E7 oncoprotein in detecting HSIL/cervical cancer were higer than those of HPV E6/E7 mRNA and TCT (Table 4).

Table 1
Comparison of HPV E7 oncoprotein levels in different degrees of cervical lesions.

Group	N	HPV16 E7 Oncoprotein*(ng/mL)
normal cervix	176	4.21 (0.49–16.91)
LSIL	101	5.69 (2.56–25.19)
HSIL	113	26.93(18.05–164.31)
cervical carcinoma	10	41.27 (21.36–387.67)
P Value	<0.001	
Correlation Coefficient**	0.121	

* Median of HPV 16 E7 oncoprotein concentration, with 25th–75th percentile in parentheses.
** Correlation is significant at the 0.01 level (2-tailed).

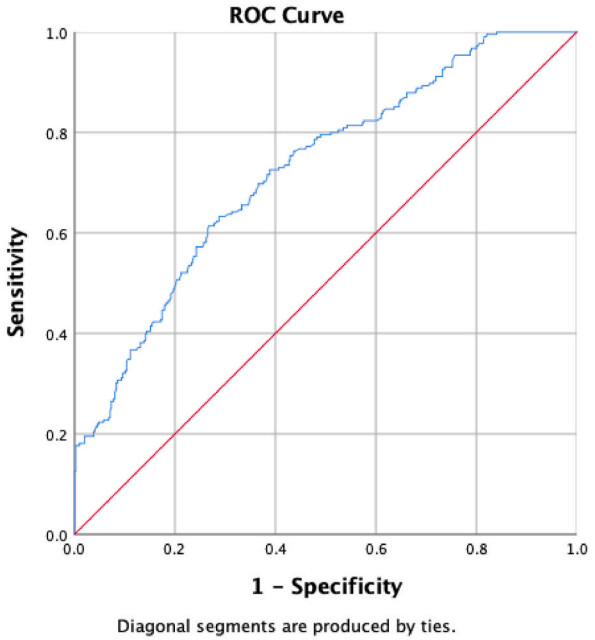

Fig. 2. ROC curve of HPV E7 oncoprotein levels for detection of HSIL and cervical cancer.

Table 2
Accuracy of E7 oncoprotein levels in the detection of HSIL and cervical cancer.

Area	Std. Error	Asymptotic Sig.[b]	Asymptotic 95 % Confidence Interval	
			Lower Boundary	Upper Boundary
0.724	0.021	0.000	0.683	0.766

a.Under the nonparametric assumption.
[b] Null hypothesis: true area = 0.5.

Table 3
Diagnostic performance of E7 oncoprotein levels in HSIL and cervical cancer.

			Pathology		Total
			–	+	
E7 oncoprotein	–	Count	95	19	114
		% Within E7 oncoprotein	83.3 %	16.7 %	100.0 %
		% Within Pathology	77.9 %	21.3 %	54.0 %
		% of Total	45.0 %	9.0 %	54.0 %
	+	Count	27	70	97
		% Within E7 oncoprotein	27.8 %	72.2 %	100.0 %
		% Within Pathology	22.1 %	78.7 %	46.0 %
		% of Total	12.8 %	33.2 %	46.0 %
Total		Count	122	89	211
		% Within E7 oncoprotein	57.8 %	42.2 %	100.0 %
		% Within Pathology	100.0 %	100.0 %	100.0 %
		% of Total	57.8 %	42.2 %	100.0 %

Table 4
Comparison of diagnostic performance between HPV E7 oncoprotein, HPV E6/E7 mRNA, and TCT.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	Kappa Value
HPV E7 oncoprotein	78.7	77.9	72.2	83.3	56.6	0.588
HPV E6/E7 mRNA	72.2	69.4	63.7	77.1	41.6	0.409
TCT	64.7	61.9	53.4	72.2	26.6	0.257

4. Discussion

Many epidemiological and molecular biology studies have confirmed that HPV infection, especially high-risk HPV infection, is closely related to cervical precancerous lesions and cervical cancer [7]. About 1/4 of patients with a long-term HPV infection will develop cervical intraepithelial neoplasia (CIN), and less than 1 % will develop cervical cancer [8]. Advances in molecular biology and virology have shown that the integration of HPV DNA into the host cell genome is a key step in the development and progression of cervical cancer [9]. After HPV invades cervical cells, it is integrated into the host genome in the form of HPV DNA fragments or viruses, resulting in increased expression levels of the HPV E6/E7 oncoprotein, which is closely and directly related to the occurrence and development of cancer [10]. Hr-HPV E6/E7 oncoproteins can be found in almost all HPV-positive cases [11,12]. Early genes E6 and E7 play an essential role in HPV-induced carcinogenesis by interfering with two crucial tumor suppressor genes, p53 and Rb, which regulate normal cell proliferation. The E7 oncoprotein is considered the main transforming oncoprotein of hr-HPV, and its continuous gene expression is critical for tumorigenesis [13,14].

Previous studies on HPV diagnosis and cervical cancer occurrence were mainly performed at the cellular, RNA, or DNA levels and rarely at the protein levels. However, after cervical cells are infected with HPV, the presence of HPV DNA or mRNA is only a relatively low-risk factor for the progression of cervical cancer because the leading cause of cervical cancer is the functional expression of the gene of the hr-HPV E6/E7 oncoprotein [15], and E6/E7 oncoprotein levels reflect the gene expression activity and the severity of cervical lesions [16,17]. Therefore, E6/E7 levels may be more directly related to the risk of cervical cancer.

.Spearman correlation analysis showed that the more severe cervical lesions, the higher the levels of oncoprotein, which wss consistent with previous research results [18,19]. The results suggested that HPV E7 oncoprotein detection could be used as a marker for the early screening of cervical cancer.

In recent years, the introduction of various detection methods for HPV DNA and mRNA has improved the accuracy of cervical cancer screening and has helped avoid unnecessary colposcopy. However, the specificity and sensitivity of these tests vary greatly, and only those with high sensitivity and specificity can be used for population screening [20].

Rezhake et al. [21] reported the type-specific expression of the HPV 16 E6/E7 oncoprotein gene for the first time. The results showed that the E6/E7 oncoprotein was detected in all patients with HSIL, whereas the E6/E7 oncoprotein was detected only in a few patients with LSIL. Some studies have also found that combining E6 and E7 oncoprotein detection can improve detection accuracy [22]. The application of E6/E7 oncoprotein detection in cervical cancer screening can reduce the limitations of the latest TCT and Hybrid Capture 2(HC2)techniques and increase the sensitivity and specificity of screening [23].

We detected HPV E7 oncoprotein levels in cervical exfoliated cells of 611 HPV-positive women in our group and evaluated the diagnostic efficacy of the detection method by using the ROC curve. According to the sample number of the current test and grouping status, the results showed that HPV E7 oncoprotein detection had good accuracy in differentiating the normal cervix and LSIL from HSIL and cervical carcinoma (AUC = 0.724). This result verified the reliability of the quantitative detection of HPV E7 oncoprotein. Furthermore, the diagnostic detection threshold of HPV E7 oncoprotein was $\geq$10.88 ng/mL, which can be used as the critical value to distinguish the normal cervix and LSIL from HSIL and cervical cancer.

In addition, the detection value of HPV E7 oncoprotein was analyzed and compared with those of the TCT and HPV E6/E7 mRNA in the present study. The SEN, SPE, PPV, NPV and Youden index of HPV E7 oncoprotein were 77.9 %, 78.7 %, 72.2 %, 83.3 %, and 56.6 %, respectively, which were higher than those of HPV E6/E7 mRNA and TCT. This result was consistent with those of previous studies [24,25]. Compared with hr-HPV DNA, hr-HPV E6/E7 mRNA has a lower positive rate in the population and cervical lesions and can only be detected in 52.9 % of hr-HPV-DNA-positive patients [26,27]. The absence of hr-HPV E6/E7 mRNA indicates that the virus is most likely free and the transcriptional regulation of the virus is still effective, suggesting that the spontaneous elimination of infection is likely. With the aggravation of the disease, the uncontrolled transcription of the oncogene leads to increased levels of the E6/E7 oncoprotein, thus initiating the malignant transformation of cells. These results also supported the theory that continuous HPV-related gene expression causes cervical cancer.

The Kappa value of HPV E7 oncoprotein was 0.588 compared to that of pathological examination, which was higher than that of TCT (0.409) and HPV E6/E7 mRNA (0.257). These results suggested that HPV E7 oncoprotein detection was more consistent with pathological results than TCT and HPV E6/E7 mRNA, and HPV E7 oncoprotein has a better diagnostic application value than TCT and HPV E6/E7 mRNA. Hence, HPV E7 oncoprotein detection can be more accurate in cervical cancer screening and can be used as a colposcopic shunting method, further reducing unnecessary colposcopy referral and treatment.

5. Conclusions

The level of HPV E7 protein was positively correlated with the degree of cervical lesions, and the level of HPV E7 protein increased with the aggravation of cervical lesions. Magnetic particle-based chemiluminescence immunoassay can quantitatively detect HPV E7 oncoprotein levels in cervical exfoliated cells. The method is convenient for sampling, simple and quick for detection, and has excellent potential for development in clinical applications. The diagnostic efficacy of HPV E7 oncoprotein detection is higher than that of TCT and APTIMA HPV E6/E7 mRNA in detecting HSIL and cervical cancer. HPV E7 oncoprotein detection may be a better choice for cervical cancer screening because it is non-invasive and further reduces the number of colposcopy referrals, providing more objective evidence and detailed information regarding cervical cancer or precancerous lesions.

Ethics statements

This study was reviewed and approved by the Ethics Committee of Tianjin Central Obstetrics and Gynecology Hospital, with the approval number:no. 2019KY102. All participants provided informed consent to participate in the study.

Consent to publish

Not applicable.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xinmei Wang: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hongyuan Zhang:** Resources, Investigation, Funding acquisition, Data curation. **Leiyi Chen:** Software, Investigation, Data curation. **Juan Xu:** Methodology, Investigation, Data curation. **Pengpeng Qu:** Visualization, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviation

AGC	Atypical glandular cell
ASCUS:	Atypical squamous cell of undetermined significance
ASC-H:	Atypical squamous cells cannot exclude high grade cervical squamous intraepithelial lesions
CIN	Cervical intraepithelial neoplasia
ECC	Endocervical curettage
HC2	Hybrid Capture 2
HPV	Human Papilloma virus
HSIL:	High-grade cervical squamous intraepithelial lesions
LSIL:	Low-grade cervical squamous intraepithelial lesions
NILM	Negative for intraepithelial lesion or malignancy
NPV	Negative predictive value
PPV	Positive predictive value
ROC:	Receiver operating characteristic
SEN	Sensitivity
SPE	Specificity
TCT	ThinPrep cytology test

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