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ORIGINAL RESEARCH

E7 as a Promising Biomarker for Monitoring the Progression of Cervical Lesions in Patients with HPV 16/18 Infections

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ABSTRACT

Objective • To investigate the expression of E7 protein and its relationship with the progression and prognosis of cervical pre-cancerous lesions in patients with human papillomavirus (HPV) 16/18 infections.

Methods • A total of 211 patients with positive HPV 16/18 were included in this study. Patients were categorized into three groups based on colposcopy results: NILM (Negative for Intraepithelial Lesion or Malignancy), LSIL (Low-Grade Squamous Intraepithelial Lesion), and HSIL (High-Grade Squamous Intraepithelial Lesion). E7 protein levels were quantified using Immunochromatographic Assay and compared using ANOVA. Cervical E7 protein levels were assessed before and one year after cervical cone biopsy in the HSIL group.

Results • Among HPV 16/18-positive patients with normal Cervical Thinprep Cytologic Test (TCT) results, E7 protein content exhibited abnormal and significant values ($P = .001$). Mean E7 protein levels for the NILM, LSIL, and HSIL groups were 44.52 ng/mL, 114.60 ng/mL, and 389.20 ng/mL, respectively, and showed statistical significance ($P = .000$). In the HSIL group, E7 protein levels in HPV-negative patients were significantly lower one year after cervical cone biopsy compared to before ($P = .001$). However, HPV-positive patients displayed no significant alteration in E7 protein levels before and after biopsy ($P = .08$).

Conclusions • E7 protein levels in detached cervical cells are closely associated with the severity and prognosis of cervical pre-cancerous lesions, suggesting their potential role as a biomarker for monitoring cervical lesion development. (*Altern Ther Health Med.* 2023;29(8):310-314).

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INTRODUCTION

Cervical cancer ranks among the most prevalent gynecological malignancies globally, affecting women across continents. Persistent infection with high-risk human papillomaviruses (HR-HPVs) is the primary causative factor in over 99% of cervical cancer cases, which plays a pivotal role in the progression from pre-cancerous lesions to full-fledged cervical cancer.[1] The driving forces behind HPV-mediated transformation lie within the oncoproteins E5, E6, and E7. Working collectively, these proteins extend cell-cycle progression, delay differentiation, and hinder apoptosis within the host keratinocyte cell, thereby establishing an environment conducive to viral replication.[2]

E7, an oncogene of the human papillomavirus (HPV), exhibits a direct correlation with cervical intraepithelial neoplasia (CIN) and the onset of cervical cancer.[3] Notably, the E7 protein is pivotal as the principal transforming agent among HR-HPVs, and its persistent expression is a crucial requirement for the progression of carcinogenesis.[3] This significance is further emphasized by the observation of striking amino acid sequence conservation in the E7 protein across high-grade cervical neoplasia and cervical cancer specimens, as revealed through the sequencing of viral genomes.[4]

E7's most extensively characterized function involves the dysregulation of the G1/S-phase transition, promoting heightened proliferation through disrupting E2F transcription factor activity.[1] In addition to its role in driving proliferation,

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emerging evidence suggests that E7 might also contribute to the impairment of cellular differentiation.[5] Quantitative assessment of E7 protein levels within cervical smear cells serves multiple purposes: (1) it holds potential as an adjunctive tool for cervical tumor cytology diagnosis; (2) it may aid in evaluating lesion severity and progression, and (3) it stands as a prospective novel biological marker for cervical cancer lesions.

This study explores the significance of the E7 protein concerning the development and prognosis of cervical lesions by comparing variations in E7 protein levels within cervical detached cells across different stages of patients afflicted with HPV 16/18-infected lesions.

MATERIALS AND METHODS

Study Design and Patients Selection

A total of 211 patients infected with HPV types 16 and/or 18 were enrolled between December 2019 and December 2020. All participants were non-pregnant women with a sexual history and no reported chronic diseases, infectious diseases, or other malignancies. This follow-up study excluded individuals undergoing cervical destructive treatment or receiving local and/or systemic medical interventions. Before the examination, patients abstained from sexual activity for 48 hours and refrained from vaginal douching or medication. The age range of the participants was 25 to 50 years, with a median age of 40.2 years. This study adhered to the principles outlined in the Declaration of Helsinki and received the approval of the Ethics Committee of The Third Central Clinical College of Tianjin Medical University. Informed consent was obtained from all participants, and the study's objectives and implications were properly explained to them.

Sample Collection

Cervical Thinprep Cytologic Test (TCT) results were ascertained using third-generation, liquid-based thin layer cell production methodology. Cervical detached cells were collected using a soft brush, gently rotated 2-3 times at the external opening of the cervix. Subsequently, these collected cells were frozen at -20°C before E7 protein quantification. In the case of patients categorized within the highly squamous intraepithelial lesion group, a subsequent procedure involving brushing cervical detached cells for HPV and E7 protein determination was conducted one-year post-Loop Electrosurgical Excision Procedure (LEEP).

Colposcopy, Cervical Biopsy, and Pathological Evaluation Procedures

All patients underwent comprehensive colposcopy and cervical biopsy examinations. Colposcopies were expertly conducted by skilled colposcopists, who performed multipoint biopsies of any identified abnormal areas, guided by white acetate and iodine testing. A systematic randomized-point biopsy approach was employed when no evident lesions were present. For cases where colposcopy yielded unsatisfactory results, an EndoCervical Curettage (ECC) procedure was conducted.[6]

Pathological interpretations of all findings were carried out by a seasoned pathologist affiliated with the Department of Pathology at the Third Central Hospital of Tianjin. In situations where achieving a definitive pathological diagnosis proved challenging, two additional senior pathologists offered their insights, with a minimum requirement of two out of three opinions in agreement. The final pathological diagnoses stemming from the cervical biopsies were carefully documented. Based on the outcomes of the biopsy pathology assessments, patients were categorized into three distinct groups: (1) NILM: Negative for Intraepithelial Lesion or Malignancy; (2) LSIL: Low-Grade Squamous Intraepithelial Lesions; and (3) HSIL: High-Grade Squamous Intraepithelial Lesions. All patients classified within the HSIL group underwent LEEP cervical cone biopsy, conducted under the supervision of senior medical professionals at our institution.

E7 Protein Quantification Method

The measurement of E7 protein was executed utilizing the HPV E7 Protein Quantitative Detection Kit (Immunochromatographic Assay - ICA) from Shanghai Yuping Biotechnology Co. Ltd., Shanghai, China. Cervical detached cell samples underwent the following procedure: (1) Samples were thawed to room temperature, followed by the addition of 500 μL of lysate. After vortexing for one minute, the samples were lysed for 10 minutes and then centrifuged for 10 minutes (at 10 000 rpm). The supernatant was subsequently collected for further analysis.

(2) Samples were carefully transferred from the required test slips while maintained at 4°C, followed by a 15-minute equilibration period at room temperature; (3) In each well of a well-plate, 100 μL of supernatant was introduced, and the sample was allowed to stabilize for an additional 15 minutes at room temperature; (4) Fluorescence measurement was performed, and a T/C value was recorded; (5) The HPV 16/18 E7 protein concentration within the samples was then calculated through reference to a standard curve.

Statistical Analysis

The statistical analysis was conducted using IBM Statistical Product and Service Solutions (SPSS) version 21.0 (IBM, New York, USA). All measurements are reported as means ± standard deviation ($\bar{x} \pm s$). Age comparisons between groups were assessed using a t test, while group rates and composition ratios were evaluated using a chi-squared (χ^2) test. To compare E7 protein levels within each group, a one-way ANOVA followed by a Student-Newman-Keuls (SNK-q) post hoc test was performed. Furthermore, the comparison of E7 protein levels before and after biopsy was executed using a t test. A significance threshold of $P < .05$ was considered statistically significant.

RESULTS

Clinicopathological Profile of Patients

Our study involved collecting data from 211 patients diagnosed with cervical HPV 16/18 infection based on the

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outcomes derived from cervical biopsies. The patient population was categorized into three distinct study groups: 92 individuals within the NILM group, 56 within the LSIL group, and 63 within the HSIL group. The mean ages for these respective groups were 41.9, 40.8, and 39.9 years. Statistical analysis revealed no significant age disparities among the groups ($F = 0.603$, $P = .548$). Positive diagnoses were documented as follows: HSIL at 29.8% (63/211), LSIL at 26.5% (56/211), and a cumulative frequency of cervical lesions at 56.4% (119/211); see Table 1.

E7 Protein Levels and Their Association with Cervical Lesions

Among patients who tested positive for HPV 16/18, the incidence of cervical lesions (LSIL and HSIL) detected via transvaginal cervical biopsies amounted to 78.48% (62/79). Patients exhibiting normal and abnormal TCT results displayed a statistically significant difference in E7 protein content ($t = 4.17$, $P = .001$).

In the entirety of HPV 16/18-positive patients, E7 protein levels within cervical detached cells were assessed before undergoing colposcopy. The E7 protein results for the three categorized groups are outlined in Table 1. The 95% confidence intervals for E7 protein concentrations in these groups were as follows: [37.056, 51.998], [96.544, 132.655], and [341.177, 437.215]. E7 protein levels exhibited statistically significant variations across the three groups ($P = .000$), as depicted in Figure 1.

HPV and E7 Protein Alterations One Year After LEEP in HSIL Patients

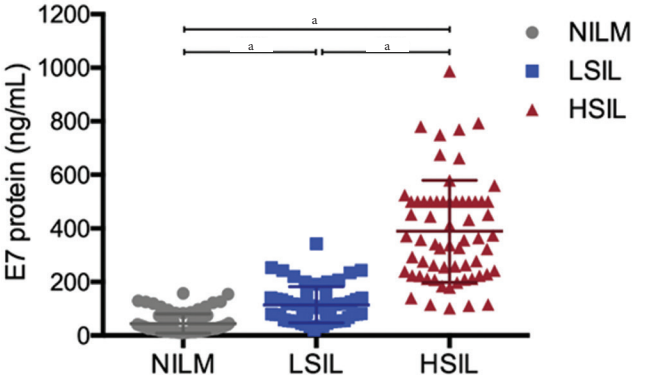
Among the 63 patients diagnosed with HSIL who underwent LEEP, twenty individuals exhibited high-risk HPV positivity during the one-year follow-up assessment, resulting in an incidence rate of 33.3% post-operation. However, while there was a noticeable trend, the changes in E7 protein levels before and after the operation did not achieve statistical significance ($P = 0.08$). Conversely, in patients testing negative for HPV, the E7 protein levels following the operation were significantly reduced compared to their pre-operative levels ($P = .001$); see Table 2.

Table 1. A comparison of age and E7 protein content between the three groups.

Groups	NILM	LSIL	HSIL	F	*P* value
n	92	56	63		
Age (Years)	41.9 ± 11.82	40.8 ± 11.25	39.9 ± 11.22	0.603	.548
E7 (ng/mL)	44.52 ± 36.05	114.60 ± 67.42	389.20 ± 190.67	184.19	.000

Note: E7 protein levels in the three groups were statistically significant ($P = .000$). No statistically significant differences in age were present between the groups.

Figure 1. E7 protein levels in the three groups.



[a]$P = .000$

Note: E7 protein levels in the HSIL group were significantly higher than in the other two groups.

Table 2. E7 protein levels in HPV positive and negative patients before and after LEEP in the HSIL group.

HSIL Group	E7 Protein Before LEEP	E7 Protein 1 Year After LEEP	*P* value
HPV Negative (ng/mL) (n = 40)	310.3 ± 198.7	148.0 ± 128.3	.001
HPV Positive (ng/mL) (n = 20)	362.3 ± 203.5	313.4 ± 153.8	.08

Note: In HPV-negative patients, E7 protein levels after the operation were significantly lower than before the operation ($P = .001$)

DISCUSSION

The appearance of E7 as an early protein generated after HPV infection holds considerable sway by initiating substantial genomic modifications that ultimately lead to cellular mutations. This process serves as a driving force in cervical cancer progression.[7] E7 plays a central role as a notable protein connected with HPV infection, occupying a crucial position as the primary driver responsible for cervical cancer initiation and subsequent progression.[8]

The growth of cancer cells relies on the continual presence of a certain protein known as an oncoprotein. Xia et al.[9] demonstrated that within just 48 to 76 hours after the body starts producing E6/E7 proteins from specific genes called oncogenes in individuals with a high-risk HPV infection, changes linked to tumor development become evident. The E7 protein induces excessive cell proliferation by interfering with the pRb pathway, leading to cervical intraepithelial neoplasia and cervical cancer progression.[8-9]

The E7 protein is crucial in several aspects of cervical cancer cell behavior, including growth, proliferation, migration, and cell cycle regulation. This protein's influence is strictly connected with the malignancy-related actions of tumor cells. Initially, when HPV-DNA is complete, the expression of the E7 protein remains minimal. However, when the HPV-DNA E2 gene is disrupted, the E7 protein experiences heightened expression.[7] The activities of the E7 protein led to cellular nuclei becoming less stable, and this cumulative impact could potentially contribute to varying degrees of cervical cancer. Therefore, E2 and E7 in pre-cancerous lesions provide a theoretical basis for evaluating and prognosis cervical cancer lesions.[1]

The influence of the high-risk HPV E7 protein on cellular cycle checkpoints constitutes a significant component of the HPV-induced oncogenic mechanism and carries potential as a biomarker for tracking the development of pre-

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cancerous lesions and cervical cancer. Furthermore, it presents a promising avenue for monitoring these cancer types directly, representing a potential breakthrough in overcoming these challenges.

Biomarkers for cervical cancer are essential to identify HPV infections that might progress to high-grade lesions. The key driver of cervical carcinogenesis is the insertion of HPV sequences into the host genome, particularly the abnormal expression of E6 and E7 oncogenes.[10] A study by Pan et al.[11] revealed that among the current cervical cancer screening indicators, the sensitivity of high-risk HPV screening alone is 95.65%, with a specificity of 15.31%. Notably, the specificity of HPV16/18 screening increased to 74.6%. When combining TCT with high-risk HPV screening, the specificity and sensitivity were 71.29% and 79.34%, respectively.

Our findings on HPV and TCT screening for cervical lesions align with previous research. Moreover, patients with abnormal TCT showed a significant increase in E7 protein levels, indicating that the combination of cervical TCT and HPV screening markedly enhances the detection rate of cervical lesions. The HPV assay demonstrated high sensitivity but low specificity, making it unable to differentiate between transient and persistent infections. Screening methods for cervical cancer, including cervical HPV-DNA, TCT, and HPV-mRNA, proved effective in assessing the severity of cervical lesions.[12]

A previous study revealed that the positive expression rates of HPV16 E7 protein in the normal group, CIN I-II and CIN III, were 30.0%, 63.3%, and 76.7%, respectively. Moreover, as the degree of cervical lesions increased, the expression and intensity of the E7 protein gradually increased ($P < .05$), indicating its potential importance in the early stages of HPV carcinogenesis and early CIN development.[13]

In a study by Zhang et al.,[12] the HPV E6/E7 protein assay demonstrated sensitivities of 65.5% and 96.6% for CIN II+, while specificities were 38.2% and 5.9%, respectively. These findings suggest that the E7 protein could serve as a valuable marker for screening cervical lesions. Among all HPV 16/18 positive patients included in this study, the mean E7 protein content was 44.52 ng/mL within the NILM group, 114.60 ng/mL within the LSIL group, and 389.20 ng/mL within the HSIL group. As cervical lesions progressed, the E7 protein levels exhibited significant statistical differences across the three groups, underscoring the E7 protein's role as a biological indicator associated with the severity of cervical lesions.[12,13]

Previous research has indicated that HPV E6/E7 mRNA detection enhances the detection rate of cervical lesions, but when it comes to cervical cancer, both specificity and sensitivity remain unchanged.[14] However, E6/E7 protein detection exhibited superior sensitivity compared to TCT and greater specificity compared to HPV-DNA testing.[15] A prior study also demonstrated that of 111 women who underwent cervical cone biopsy, 84.7% of those with CIN II and higher tested positive for E6/E7.[16] Colposcopy for high-risk HPV-positive patients is time-consuming and costly, while cervical biopsies are invasive. In regions with limited access to colposcopy, high-risk HPV-positive patients might face challenges in receiving this procedure. Given that this study identified a "false negative" rate of 43.18% (57/132) for TCT, quantifying E7 protein can be considered a valuable supplement to cytology.

In this study, few patients tested positive for high-risk HPV but negative for TCT. E7 protein quantification presents an opportunity to prevent instances of overlooked cervical lesion diagnoses. In accordance with the 95% confidence interval established for cervical LSIL within this investigation, HPV 16/18 positive patients should undergo further cervical biopsy if the E7 protein value exceeds 96.54 ng/mL. Conversely, when the E7 protein level falls below 96.54 ng/mL, biopsies should be considered in the presence of TCT abnormalities, and a watchful approach may be taken if TCT results are normal. Notably, the implementation of cervical E7 protein content measurement can potentially lead to a reduction in the frequency of colposcopy examinations for HPV16/18 patients.

Prolonged follow-up is necessary following cone biopsy in HSIL patients with positive HPV-DNA to monitor the progression and severity of cervical lesions. However, the prospect of extended follow-up colposcopy may impose a substantial burden. Viral oncogenic proteins transcribed from E6/E7mRNA, which originate from high-risk HPV infections, play a pivotal role in cervical carcinogenesis. Remarkably, E6/E7 mRNA detection demonstrates superior diagnostic efficacy in predicting treatment failure when compared to HPV-DNA testing.[17]

Shi et al.[18] demonstrated that while the sensitivity of the E6/E7 protein assay was somewhat lower than HC2 detection (71.3% vs. 96.6%, respectively), the assay significantly enhanced specificity (67.6% vs. 5.9%, respectively). Hence, the combined assessment of HPV E6/E7 protein expression with an E6/E7 mRNA assay offers a promising avenue to further enhance the diagnostic potential as a novel biomarker for cervical cancer.[8]

In this study, the positive rate of high-risk HPV among patients with HSIL one year after biopsy stood at 33.3%. Notably, E7 protein levels experienced a significant reduction ($P = .08$) in patients who tested negative for HPV. Conversely, the decrease in E7 protein levels post-biopsy in high-risk HPV-positive patients was not statistically significant compared to pre-biopsy levels. This finding suggests that the quantitative measurement of E7 protein holds promise as an indicator for monitoring the progression of cervical lesions. Moreover, to assess E7 protein levels, immediate freezing of cervical detached cells is necessary, which might present challenges for clinical application. This novel approach represents a pioneering method for quantitatively determining E7 protein within cervical exfoliated cells.

Our findings suggest that the E7 protein is a critical factor influencing the initiation and progression of cervical cancer. Additionally, E7 protein levels within cervical detached cells hold promise as a potential and viable biomarker for cervical cancer. Moreover, our findings emphasize the correlation

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between E7 and the severity of cervical pre-cancerous lesions and their subsequent prognosis.

Study Limitations

A few limitations should be acknowledged in this study. First, our sample size was relatively small, which might restrict the generalizability of our findings. Additionally, the single-centre nature of the study could introduce potential bias and limit the diversity of patient populations. Furthermore, the follow-up duration was relatively short, preventing a comprehensive assessment of long-term outcomes. The reliance on specific assays and methodologies might have influenced the accuracy and comparability of our results. Despite these limitations, our study provides valuable insights into the potential role of E7 protein in cervical lesions and serves as a foundation for future research endeavors. Future studies, including multiple hospitals with a larger sample, are required to better understand the role of E7 protein in cervical lesions.

CONCLUSION

In conclusion, this study sheds light on the pivotal role of the E7 protein in the progression of cervical lesions in individuals with HPV 16/18 infections. The findings underscore the significance of E7 as a potential biomarker for monitoring the development of cervical lesions, particularly when combined with established screening methods like TCT and high-risk HPV detection. The study's insights contribute to a deeper understanding of the intricate molecular mechanisms underlying cervical carcinogenesis, offering valuable implications for the early detection and management of cervical cancer. Further exploration of E7's role could pave the way for enhanced diagnostic strategies and therapeutic interventions in cervical health.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHORS' CONTRIBUTIONS

HW and YW designed the study and performed the experiments, Y Shi and Y Sun collected the data, Y Shi, Y Sun, and AZ analyzed the data, and HW and YW prepared the manuscript. All authors read and approved the final manuscript.

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