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Original Article

Analysis on predictive effect and accuracy of HPVL1 protein and human papilloma virus dna load in cervical lesions

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Abstract: Objective: We aimed to discuss the predictive effect and accuracy of HPVPL1 protein and human papilloma virus (HPV) DNA load in patients with cervical lesions. Methods: A total of, 103 patients with cervical lesions in our hospital were selected as subjects of study and included in an observation group (OG) and 24 patients with chronic inflammation (CI) and healthy physical examination who received treatment in the same period were included in the control group (CG). A definite diagnosis was made in all patients through histocytological examination. The HC2 technology was used for the measurement of HPV DNA load in patients; and immunohistochemistry (IHC) was used for the measurement of HPVPL1 protein in cervical exfoliated cells (CEC) in the two groups. The histocytological diagnosis results were taken as the gold standard to compare the predictive effect of HPVPL1 protein and HPV DNA load in cervical diseases. Results: A definite diagnosis of cervical lesions was made in 127 patients through histocytological examination. Thereinto, there were 103 patients with cervical lesions, including 27 with CIN1, 32 with CIN2 and 24 with CIN3. There were 24 patients with CI in the CG. The negative rate (NR) of HPV DNA load in the OG was lower than that in the CG ($P<0.05$). There were statistical differences in NR of CIN Stage I, CIN Stage II, CIN Stage III and HPV DNA load in cervical cancer (CC) in the OG ($P<0.05$). The positive rate (PR) of HPVPL1 protein in CEC in the OG was higher than that in the CG ($P<0.05$). The sensitivity and specificity of HPVPL1 combined with HPV DNA load in cervical lesions were higher than those of single HPVPL1 or HPV DNA load ($P<0.05$). Conclusion: The expression of HPVPL1 protein in CEC showed a descending trend in patients with cervical lesions while the HPV DNA load showed a rising trend, so the joint detection through the two methods could predict cervical diseases and is worthy of promotion and application.

Keywords: Cervical exfoliated cell, HPVPL1 protein, cervical lesion, HPV DNA load, predictive effect, histocytology

Introduction

CC is a common malignant tumor in found clinical practice. About 530,000 global patients suffer from CC every year and over 85.0% of them are from developing countries. Every year, about 275,000 patients die of CC [1, 2]. Previous research showed [3] that HPV infection was the inducing factor of CC and CC incidence could be reduced through early detection and diagnosis. A large amount of research showed that the persistent HPV infection was the necessary condition for CC and precancerous lesions. Besides, HPV16 and HPV18 were the most common ones in 14 types of HPV and more than 70.0% of CC cases were caused by HPV16/18 infection. According to WHO Guidance, it is suggested that HPV detection shall be used as the method for basic examination of CC. But the detection specificity is relatively low in the measurement of HPV DNA load and the cost of joint detection through cervical cytology is relatively high in clinical practice, so it is hard to promote and apply it in primary hospitals.

HPVPL1 protein, approximately accounts for over 90.0% of capsid protein, is recognized as an important target for immune response to attack of HPV virus and virus infection on host cells. With a high conservative property, it is mainly encoded by late transcriptional gene L1 protein and is the main genus-specific antigen [4]. Now, the main capsid protein HPVL1 in HPV, is a biomarker found recently, plays a crucial

role in predicting HPV infection. Foreign scholars indicated [5] that if the human body was able to fight HPV virus infection depended more on whether the rapid and powerful cellular immune response and humoral immune response were formed in locally infected sites. The compound of HPVPL1 protein and T cells, etc. can form an immune antibody to achieve the effect of immune elimination, but there is little research on its predictive value in cervical lesions [6, 7].

So in this study, patients with cervical lesions, CI and healthy physical examination were selected as subjects to discuss the predictive effect and accuracy of HPVPL1 protein in CEC and HPV DNA load in patients with cervical lesions, and the report is shown below.

Material and methods

Clinical materials

In total, 103 patients suffering from cervical lesions from May, 2017 to August, 2019 were selected as subjects of study and included in the OG, with the age range of (25-64) years old and an average age of (46.79±5.71) years old; and a disease course of (1-6) months and an average disease course of (3.15±0.61) months. Thereinto, 83 patients suffered from cervical intraepithelial neoplasias (CIN) and 20 patients suffered from squamous cell carcinoma (SCC). Twenty-four CI patients who received treatment in the same period were included in the CG, with an age range of (26-65) years old and an average age of (47.21±5.73) years old; and a disease course of (1-7) months and an average disease course of (3.16±0.63) months. Inclusion criteria: (1) All patients meet the diagnostic criteria of cervical lesions [8] and were diagnosed definitely through cervical cytological examination. (2) All patients, with tolerability, were able to finish the detection of HPVPL1 protein in CEC and HPV DNA load. Exclusion criteria: This study excluded (1) patients complicated with mental disorders or during pregnancy or with a history of pregnancy; (2) those definitely diagnosed with endometrial cancer, vulvar cancer, carcinoma of fallopian tube and other malignant tumors; and (3) those treated with chemoradiotherapy and immuno-biological cancer therapy before examination. This study was approved by the Ethics Committee of Xuzhou Central Hospital. The research subjects and their families were informed and they signed an informed consent.

Methods

(1) Pathological examination. The definite diagnosis was made in all patients through histocytological examination. After admission, the cervix of two groups was fully exposed by vaginal speculum and then a sterile cotton ball was used to clean cervical secretion. After specimen collection, the slices were made routinely and diagnosed under light microscope. CEC/vaginal exfoliated cells were diagnosed based on the standards set by American Cancer Society and the pathological diagnosis was performed in high-risk or abnormal patients [9]. (2) Measurement of HPV DNA load. The signal amplification of antibody capture and chemiluminescence signal detection was used to finish the measurement of HPV DNA load in patients. The optical signals generated by specimens were measured through microplate interpretative instrument to obtain the relative light unit and taken as the result of HPV DNA load with the standard ratio of positive control. Negative: measured value <1.00 and positive ≥1.00. The higher the ratio, the higher the HPV DNA load. The HPV DNA load was divided into mild (1.00-10.00), moderate (10.00-100.00), and severe (≥100.00) [10, 11]. (3) Measurement of HPVPL1 protein in CEC. IHC method was used to measure the HPVPL1 protein in CEC in two groups, with the specific methods shown below. ① Specimen collection. After the cervix brush was inserted into the cervix of all patients and rotated for 5 cycles, the brush head was put into the little bottle containing Cytorich preservation solution to collect the epithelial cells exfoliated from the surface of cervix. The brush head was taken out and covered up properly, and then was sent for inspection after making corresponding labels. ② Detection methods. The specimens collected above were taken out for paraffin embedding and slicing. After conventional dewaxing and antigen retrieval, the hydrogen peroxide was used to remove the activity of endogenous peroxidase. Then, HPV L1 capsid antigen was added after the slices were sealed with serum. The antibody concentration was 1:200. The secondary antibodies were added after the reaction continued overnight. Upon the completion of reaction, the slides were cleaned for conventional color development through DAB. Then hematoxylin

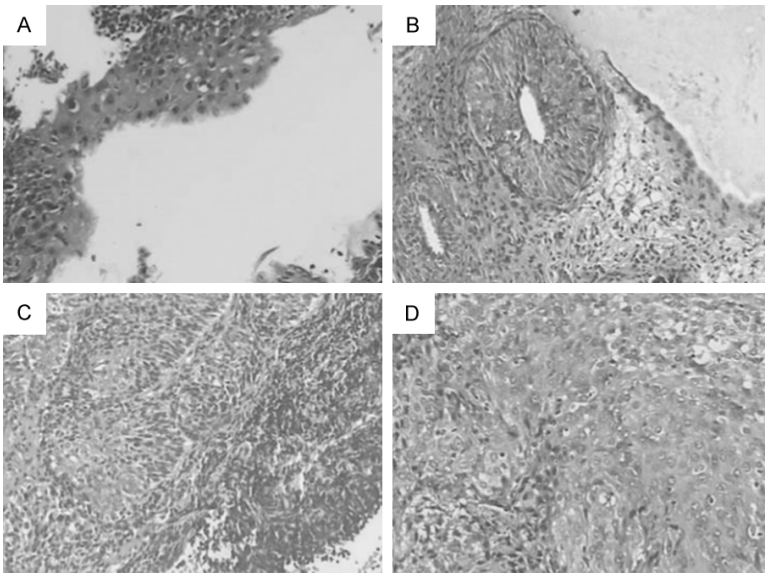

Figure 1. Pathological examination results of all patients (×400). Notes: (A) is the IHC results of CINI; (B) is the IHC results of CINII; (C) is the IHC results of CINIII; and (D) is the IHC results of CC.

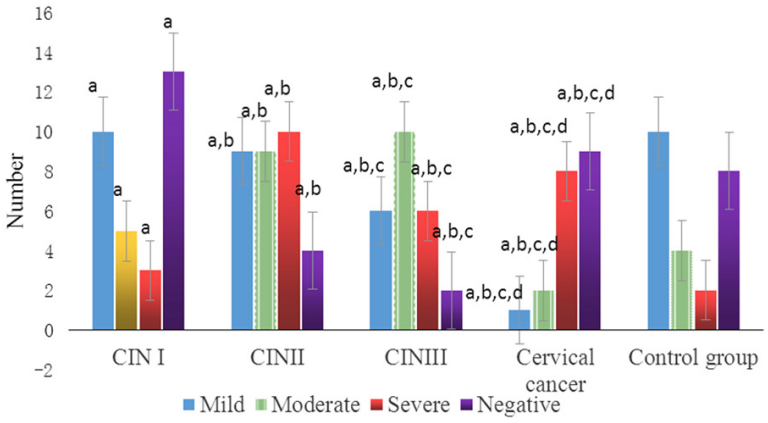

Figure 2. Comparison of HPV DNA load between two groups. Compared with control group, [a]*P*<0.05; compared with CINI, [b]*P*<0.05; compared with CINII, [c]*P*<0.05; compared with CINIII, [d]*P*<0.05.

was used for counterstaining, which was followed by dehydration through conventional gradients of ethyl alcohol. Then, the slices were sealed with neutral balsam and put under the inverted microscope to observe histogenesis. ③ Judgment methods. Positive: Pathological cells were reddish brown in cell nucleus. Negative: No cells that were stained red were found. The positive expression rates of two groups were calculated and compared and the diagnose accordance rates of two detection methods were recorded. The histocytological diagnosis results were taken as the gold standard [12]. (4) Predictive effect. The predictive effect of HPVPL1 protein and HPV DNA load in cervical diseases was calculated, including accuracy, sensitivity, positive predictive value (PPV) and negative predictive value (NPV). ROC curve was made to analyze the predictive efficiency of HPVPL1 protein and HPV DNA load in cervical diseases.

Statistical analysis

SPSS 18.0 software was used for statistical analysis. The enumeration data were represented by n (%) in χ^2 test and the measurement data were represented by ($\bar{x}\pm s$) in *t* test. *P*<0.05 meant that the difference had statistical significance.

Results

Pathological examination results of all patients

In total, 127 patients were definitely diagnosed through pathological examination. From this, there were 103 patients with cervical lesions, including 27 in CIN1, 32 in CIN2 and 24 in CIN3. Besides, there were 24 CI patients in the CG. The IHC results are shown in **Figure 1**.

Comparison of HPV DNA load between two groups

The NR of HPV DNA load in the OG was lower than that in the CG (P<0.05). There was statistical difference in NR of CIN Stage I, CIN Stage II, CIN Stage III and HPV DNA load in CC in the OG (P<0.05). The NR of CIN Stage III and HPV DNA load in CC patients was higher than that of CIN Stage I and CIN Stage II (P<0.05), as shown in **Figure 2**.

Analysis on evaluation of HPV DNA load in different cervical lesions

In the OG, the NR and mild rate of CINI were respectively 37.04% and 33.33%, which were

Table 1. Analysis on evaluation of HPV DNA load in different cervical lesions

Type of cervical lesions	Number of cases	Negative	Mild	Moderate	Severe
CIN1	27	10 (37.04)[a,b]	9 (33.33)[a,b]	5 (18.52)[a,b]	3 (11.11)[a,b]
CIN2	32	10 (31.25)[a]	6 (18.75)[a]	10 (31.25)[a]	6 (18.75)[a]
CIN3	24	2 (8.33)	2 (8.33)	14 (58.33)	6 (25.00)

[a]*P*<0.05 refers to the comparison with CIN3; and [b]*P*<0.05 refers to the comparison with CIN2.

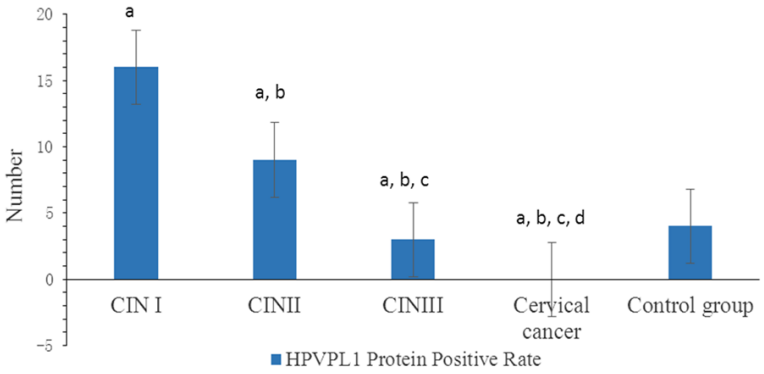

Figure 3. Comparison on PR of HPVPL1 protein in CEC between two groups. Compared with control group, [a]*P*<0.05; compared with CINI, [b]*P*<0.05; compared with CINII, [c]*P*<0.05; compared with CINIII, [d]*P*<0.05.

were respectively 90.53% and 93.75%; and the NPV of them were respectively 46.88% and 58.06%, which indicated that there was no statistical significance (P>0.05). The sensitivity and specificity of HPVPL1 combined with HPV DNA load were respectively 74.76% and 87.50% in cervical lesions, which were higher than 83.50% and 62.50% of single HPVPL1 and 87.38% and 75.00% of single HPV DNA load (P<0.05), as shown in **Table 2** and **Figure 4**.

higher than 31.25% and 18.75% of CIN2 and 8.33% and 18.75% of CIN3 (P<0.05). The moderate rate and severe rate of CINI were respectively 18.52% and 11.11%, which were lower than 31.25% and 18.75% of CIN2 and 31.25% and 18.75% of CIN3 (P<0.05). The NR and mild rate of CIN2 were respectively 31.25% and 18.75%, which were higher than 8.33% and 8.33% of CIN3 (P<0.05). The moderate rate and severe rate of CIN2 were respectively 31.25% and 18.75%, which were lower than 58.33% and 25.00% of CIN3 (P<0.05), as shown in **Table 1**.

Comparison on PR of HPVPL1 protein in CEC between two groups

The PR of HPVPL1 protein in CEC was 16% in the OG, which was higher than 4% in the CG (P<0.05). The PR of HPVPL1 protein in CEC was 16% in CIN1, which was higher than 9% in CINII7, 3% in CINIII and 0% in CC (P<0.05). The PR of HPVPL1 protein in CEC was 9% in CINII, which was higher than 3% in CINIII and 0% in CC (P<0.05), as shown in **Figure 3**.

Predictive efficiency of HPVPL1 protein combined with HPV DNA load in cervical lesions

The predictive accuracy of HPVPL1 and HPV DNA load were respectively 79.53% and 85.04% in cervical lesions; the PPV of them

Discussion

CC is a malignant tumor with a higher incidence found in females. Due to a lack of typicality in clinical symptoms in early stages, the diagnosis rate is low in the early stage [13]. Now, histopathological examination is the main method for CC identification in clinical practice, which is conducive to definite diagnosis, but this examination will bring about a certain risk and trauma and thus lead to poor tolerability and compliance of diagnosis [14]. In this study, 127 patients were definitely diagnosed through pathological examination. From this, there were 103 patients with cervical lesions, including 27 with CIN1, 32 with CIN2 and 24 with CIN3. Besides, there were 24 CI patients in the CG. This implied that the histopathological examination applied to patients with cervical lesions was conducive to definite diagnosis and clinical treatment.

Based on the limitation of pathological examination for cervix, the HPVPL1 protein in CEC and HPV DNA load were used in patients with cervical lesions in this study. The results showed that the NR of HPV DNA load in the OG was lower than that in the CG (P<0.05); there was statistical difference in NR of CIN Stage I, CIN Stage II, CIN Stage III and HPV DNA load in CC (P<0.05); and the PR of HPVPL1 protein in

Table 2. Predictive efficiency of HPVPL1 protein combined with HPV DNA load in cervical lesions

Detection method	Accuracy	Sensitivity	Specificity	PPV	NPV
HPV DNA load	85.04 (108/127)	87.38 (90/103)	75.00 (18/24)	93.75 (90/96)	58.06 (18/31)
HPVPL1 protein	79.53 (101/127)	83.50 (86.103)	62.50 (15/24)	90.53 (86/95)	46.88 (15/32)
Joint detection	77.17 (98/127)	74.76 (77/103)	87.50 (21/24)	96.52 (77/80)	44.68 (21/47)
F	1.291	5.793	7.391	0.647	0.771
P	0.591	0.000	0.000	0.413	0.491

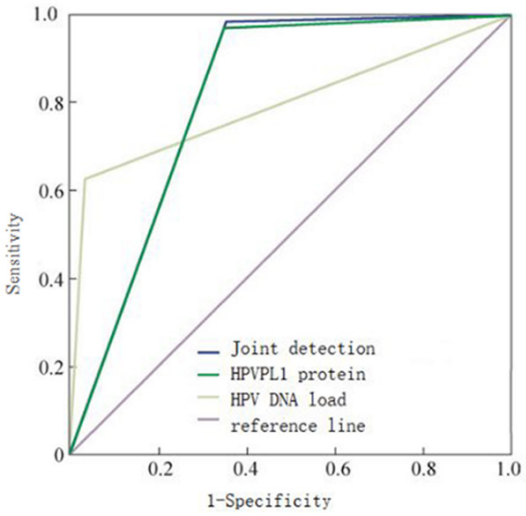

Figure 4. Analysis on ROC curve for prediction of cervical lesions through HPVPL1 protein combined with HPV DNA load.

CEC in the OG was higher than that in the CG ($P<0.05$). This implied that a better predictive effect could be achieved by applying HPVPL1 combined with HPV DNA load to patients with cervical lesions. The capsid protein of HPV virus is composed of 72 capsid proteins, but in clinical practice, there is a controversy about the relation between HPV DNA load and CC. Previous research showed that the high HPV DNA load was negatively correlative with hrHPV clearance rate and positively correlative with CIN2 incidence. With the progress of cervical lesions, the HPV DNA load increased [15]. At present, the detection sensitivity of HPV DNA load is relatively high, so it has been widely used as the screening method of CC. However, the specificity and PPV are relatively low in the clinical application of HPV DNA load [16, 17]. Hence, a higher level of HPV DNA load can predict cervical lesions in a better way and reflect the severity of disease preferably, which is conducive to the guidance of clinical treatment. HPVPL1 protein, accounting for over 90.0% of capsid protein, is the main capsid protein of HPV. Furthermore, L1 protein, with a high conservative property in all types of HPV, is the main genus-specific antigen [6, 12]. As previous research has shown [16, 18], the fight of human body against HPV virus mainly depends on the rapid and powerful cellular immune response formed in infected sites because the HPV virus can cause local organ infection, such as cervix and vagina, etc. Foreign scholars indicated [15, 19] that HPVPL1 protein in CEC could form an immune complex with T cells and MHC I/II and thus achieve the goal of immune elimination. Therefore, due to the lack of HPVPL1 protein in CEC in human body, the immune response cannot be stimulated, which will lead to the escaped detection of lesion cells and increase CC incidence. HPV molecule is double-chain ring DNA molecule, mainly including 6 genes related to DNA replication and cell transformation. Besides, there are 2 genes of capsid protein coded by the virus. Domestic scholars indicated [17] that HPVPL1 protein in CEC and HPV L1DNA could reflect the virus replication in cells to a certain extent and reflect the progress of cervical lesions in a better way [20]. If the HPVPL1 protein in CEC is positive, it implied that the patients have the transient infection of HPV. If there is a lack of HPVPL1 protein in CEC, it implied that the HPV infection exists persistently or patients have developed lesions. In order to further confirm the effect of HPVPL1 and HPV DNA load on patients with cervical lesions, the effects of the two detection methods were analyzed in this study and the results showed that the sensitivity and specificity of HPVPL1 combined with HPV DNA load were higher than those of single HPVPL1 or HPV DNA load in cervical lesions ($P<0.05$). This implied that a good effect could be achieved by applying HPVPL1 and HPV DNA load to patients with cervical lesions, but the two detection methods had different advantages, which helped with definite diagnosis and

provided basis and reference for clinical diagnosis and treatment. So in clinical practice for patients with suspected cervical lesions, the HPV DNA load can be strengthened. For patients with high HPV DNA load, early definite diagnosis can be made by combining the measurement of HPVPL1 protein in CEC [17].

In conclusion, the expression of HPVPL1 protein in CEC showed a descending trend in patients with cervical lesions while the HPV DNA load showed a rising trend, so the joint detection through the two methods could predict cervical diseases and help with clinical diagnosis and treatment. Thus, it is worthy of promotion and application.

Disclosure of conflict of interest

None.

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