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The role of FH detection combined with HPV screening on the diagnostic significance of cervical cancer and precancerous lesions

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Abstract. – OBJECTIVE: Cervical cancer is a common type in gynecologic malignant tumor that accounts for the leading morbidity only after breast cancer in female. This study aims to determine the diagnostic significance of uterine epithelial cells stability free body of iron protoporphyrin reduced state (FH) detection combined human papillomavirus (HPV) screening for cervical cancer.

PATIENTS AND METHODS: A total of 800 patients were enrolled. FH test and HPV genotyping detection were performed. The cases with abnormal results were treated by colposcopy. Pathological detection was treated as the golden standard. Coincidence rate, sensitivity, and specificity were calculated.

RESULTS: The positive rate of FH and high-risk HPV rate were 11.5% and 16.5%, respectively. The coincidence rate of single FH detection positive and negative was 76.08% and 85.71%, respectively. FH detection exhibited sensitivity of 74.26% and specificity of 61.22%. The coincidence rate of single positive and negative HPV test was 53.03% and 88.58%, respectively. HPV screening presented sensitivity of 77.25% and specificity of 65.37%. The coincidence rate of positive and negative combined detection was 92.11% and 100%, respectively. The sensitivity and specificity of combined detection was 96.32% and 81.22%, respectively, which was significantly higher than that of single detection group ($p < 0.05$).

CONCLUSIONS: FH detection combined with HPV screening is efficient in the diagnosis of cervical cancer and precancerous lesion, which is better than single use of FH detection. Our data highlight the importance of FH with HPV screening for the clinical application.

Key Words:

Uterine epithelial cell stability FH test, HPV, Cervical cancer, Precancerous lesion, Diagnosis.

Introduction

Cervical cancer represents the second leading incidence rate in gynecological tumor after breast cancer[1,2]. It is particularly common in developing countries and economic less-developed region. In spite of the development of diagnosis and therapy, the morbidity and mortality remain high. Cervical cancer thus poses a severe threat to the world especially in developing countries[3,4]. Also, the morbidity and mortality are significantly higher in China than that in developed countries[5,6]. Cervical cancer causes serious economic burden to patients and global economy. The unsatisfactory screening and prevention methods postpone early treatment intervention, and the cervical cancer is still difficult to effectively control[7]. At present, exfoliative cytology is most widely used in cervical cancer screening. Because of sampling limitation and poor picture quality, the actual detection rate of cervical lesions was still low[8]. Thinprepcytoloaic test (TCT) is one of the most commonly used cervical screening methods, while the step is cumbersome and the cost is comparably high[9]. As an important and necessary factor of cervical disease, HPV infection is involved to determine the risk level of cervical cancer. High-risk HPV can be detected in more than 99% cases of cervical cancer (including squamous carcinoma and adenocarcinoma)[8,9]. High-risk HPV infects the body through the skin or mucous membrane and often spreads through sexual activity or contact[10,11]. HPV screening is, therefore, of great significance in cervical cancer detection[12]. Uterine epithelial cell stability FH test analyzes free ferrous protoporphyrin by colorimetric method through staining uterus epithelial cell upon

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redox reaction between FH and specific substance[13,14]. It has been extensively employed for cervical cancer screening[15,16]. In this study, we aim to verify the effect of combined detection of FH and HPV screening in the diagnosis of cervical cancer and precancerous lesion.

Patients and Methods

Subject Selection

A total of 800 patients in The Wenzhou People's Hospital (Zhejiang, China) between June 2015 and October 2017 were enrolled with mean age at 25.1 ± 6.6 (22-62) years old (Table I). No patients received cervical cancer treatment within 1 year. All the enrolled subjects had sexual life, anti-inflammatory, while received no treatment, including surgery, chemotherapy, or radiotherapy. Exclusion criteria included pregnancy, surgery history, previous radiotherapy or chemotherapy treatment, combined with other diseases, such as infectious diseases, reproductive tract acute inflammation, severe cervical erosion, malignant tumor, cervical ulcers, cervical polyp, hysterectomy, ametria, and menstruating, or patients unwilling or unable to cooperate follow-up[5]. This work was approved by the Ethics Committee at The Wenzhou People's Hospital (Zhejiang, China) and all patients had provided informed consent.

Main Reagents and Instruments

FH staining kit, sampling fingerstall or cervical solution self-inspection, sampling brush, box, collection bottle, staining reagent, forceps, and packing box were purchased from Dofmelo (Qingdao, Shandong, China). HPV genotyping detection kit was bought from Yaneng Biotechnology Co., Ltd (Shenzhen, Guangdong, China). EK-6000 digital electronic colposcope was provided by Yi-Kang (Changzhou, Jiangsu, China). Applied Biosystems 2720 DNA gene amplifier was derived from Thermo Fisher (Waltham, MA, USA).

Table I. General clinical information.

	Cases	%
Age (years old)		
≤ 40	421	52.63
> 40	379	47.37
BMI (kg/m^2)		
≤ 23	456	57.00
> 23	344	43.00
Cervical cancer family history (%)		
With	112	14.00
Without	788	86.00

HPV Screening

The sample was obtained using specific sampling brush from the cervix. The sampling brush was put into the collection bottle containing cell preservation liquid. 18 high-risk types including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, MM4, 83, and 5 low-risk types HPV6, 11, 42, 43, 44 were analyzed. DNA microarray containing polymerase chain reaction (PCR) amplification and DNA reverse dot blot was used to test HPV genotyping. Specific primers designed upon HPV gene were used to amplify the target fragments of 23 HPB genotypes. Next, the fragment was fixed on the membrane and hybrided with genotyping probe to test HPV genotyping.

Uterine Epithelial Cell Stability FH Test

The cervical solution sample was inserted into the cervix through vagina and scrubbed to obtain cells. Next, the sample was stirred in the bottle for mixing. Then, the reagent was added to the test pool from the dropper. Coloration was compared with shade guide within 2 min. Judgement standard: negative (-), no cell staining as good uterine cell stability; probably positive (+/-), pale bluish green as slightly poor uterine cell stability; weak positive (+), light blue as poor uterine cell stability; positive (++), blue as uterine cell unstable; strong positive (+++), deep blue as uterine cell extremely unstable. It was considered as blood mixing when the deep blue changed to dark yellow or red-brown, which was also treated as strongly positive.

Cervical Histopathological Examination Under Colposcopy

Colposcopy was applied for cervical histopathological examination. Conventional iodine test and acetowhite test were performed. Biopsy was taken from 3, 6, 9, and 12 point of the suspicious lesion sites or transformation zones. Endocervical canal curettage was used on transformation zone. The lesion was evaluated as chronic cervicitis, CIN grade I, II, III, and cervical cancer according to the pathology[17].

Statistical Analysis

All data analyses were performed on SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The enumeration data were presented as rate and compared by x^2-test. The measurement data were processed by analysis of variance (ANOVA) with the Tukey's post-hoc test. $p < 0.05$ was depicted as statistical significance.

Results

HPV Screening Results

It was showed that patients from 132 cases suffered from high-risk HPV infection, accounting for 16.5%. There were 50 cases of HPV16, 22 cases of HPV18, 34 cases of HPV52, and 41 cases of HPV33. A total of 31 cases were infected by two types of HPV simultaneously (Figure 1).

Uterine Epithelial Cell Stability FH Test Results

We found 92 cases of positive FH, accounting for 11.5%. The result distribution was listed in Figure 2.

Pathological Examination Results

Colposcopic biopsy was performed on patients with degree II or higher cervical erosion or abnormal cervical screening. Pathological result was treated as the final diagnostic criteria. A total of 242 cases received colposcopic biopsy, including 25 cases in CIN I, 22 cases in CIN II, 21 cases in CIN III, 2 cases of cervical cancer, and, 110 cases of cervical inflammatory lesions, such as condyloma and mucositis (Figure 3).

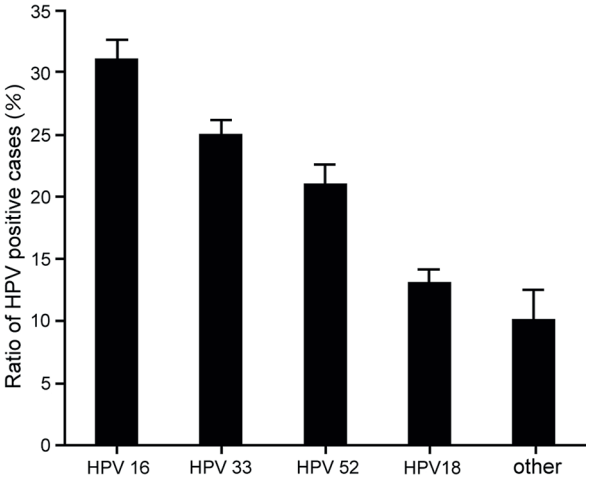

Figure 1. HPV screening results.

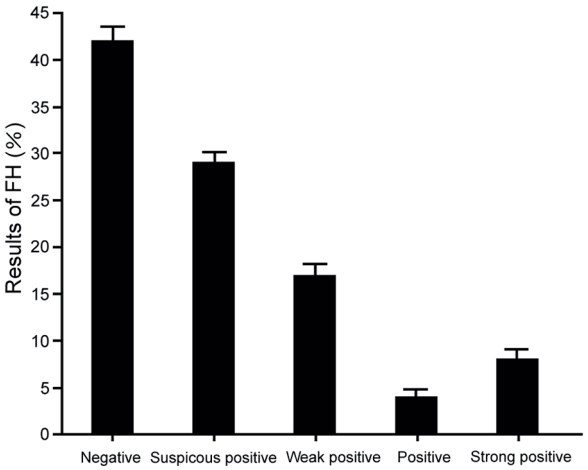

Figure 2. Uterine epithelial cell stability FH test results.

Coincidence Rate Analysis of FH and HPV Single or Combined Detection With Positive Pathological Result

The result of FH and HPV combined detection indicated 76 cases were positive, accounting for 9.5%. Moreover, the positive coincidence rate of HPV single screening was 53.03%, while it was 76.08% in FH detection. The positive coincidence rate of combined detection was 92.11%, which was significantly higher than that in single detection group ($p < 0.05$) (Table II).

Coincidence Rate Analysis of FH and HPV Single or Combined Detection With Negative Pathological Result

The negative coincidence rate of HPV single screening was 85.71%, while it was 88.58% for FH detection. The negative coincidence rate of combined detection was 100%, which was statistically higher than that in single detection group ($p < 0.05$) (Table III).

Sensitivity and Specificity Analyses of FH and HPV Single or Combined Detection

Our data showed that the sensitivity and specificity of FH detection were 74.26% and 61.22%, respectively, while those in HPV screening were 77.25% and 65.37%, respectively. Of note, combined detection showed sensitivity of 96.32% and specificity of 81.22%, which were significantly higher than single detection group ($p < 0.05$).

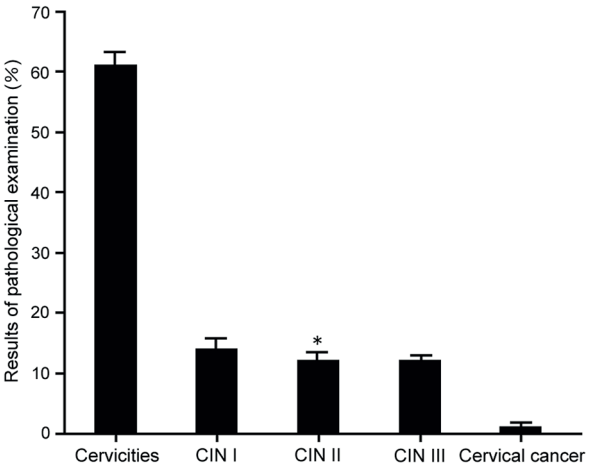

Figure 3. Pathological examination results.

Discussion

The incidence of cervical cancer keeps rising in recent years due to society development, women's way of lifestyle changes, bad sex habits, work environment and pressure, HPV infection, smoking, poor eating habits, and malnutrition[18]. Cervical cancer is given priority to young adults between 30 and 55 years old. However, presently, it shows a younger trend, which brings huge burden to patient and family[19]. Early screening for cervical cancer becomes the key to clinical prevention and treatment, which effectively reduces the incidence of cervical cancer[20]. As an important and necessary factor of cervical disease, HPV infection is applied to assess the risk level of cervical cancer. High-risk HPV can be detected in more than 99% of cervical cancer (including squamous carcinoma and adenocarcinoma)[21]. HPV can invade from the tiny wound on the epithelium to infect the epithelial basal cells. This kind of small DNA virus can integrate with host continuously, leading to malignant proliferation in epithelial tissue, precancerous lesion, and invasive cancer[22]. High-risk type HPV cervical infection test can be used as an effective method for cervical cancer screening, especially in high-risk groups[23]. This work showed that among 800 subjects, there were 25 cases in CIN I, 22 cases in CIN II, 21 cases in CIN III, 2 cases in cervical cancer. The positive and negative coincidence rate of single HPV test was 53.03% and 88.58%, respectively, the sensitivity and specificity of which was 77.25% and 65.37%, suggesting that HPV screening is to some extent unable to reflect actual cervical lesions. FH exists in uterine epithelial cell protein in combination form under normal circumstances. The protein conformation changes during cell stability disorder caused by pathogenic factors, leading to FH substance fall off from the protein and aggregate in uterine

Table II. Coincidence rate of FH and HPV single or combined detection with positive pathological result.

Method	Positive case	Pathology		Positive coincidence rate	χ^2	*p*
		Cervicitis	Cervical cancer and precancerous lesion			
HPV	132	110	70	53.03%	4.17	< 0.05
FH	92	110	70	76.08%	4.02	< 0.05
HPV+FH	76	110	70	92.11%		

Table III. Coincidence rate of FH and HPV single or combined detection with negative pathological result.

Method	Positive case	Pathology		Positive coincidence rate	χ^2	*p*
		Cervicitis	Cervical cancer and precancerous lesion			
HPV	42	36	6	85.71%	3.21	< 0.05
FH	42	33	9	88.58%	3.74	< 0.05
HPV+FH	110	110	0	100%		

epithelial cells[13]. Intracellular FH stains uterine epithelial cells through redox reaction with a specific material. Uterine epithelial cell stability can be therefore evaluated upon cell staining[14,16]. This study demonstrated that the positive and negative coincidence rate of single FH detection was 76.08% and 85.71%, respectively, with a sensitivity of 74.26% and specificity of 61.22%, revealing that FH detection can assist cervical cancer and precancerous lesion screening to a certain extent. However, it shows no statistical difference compared with HPV single screening. In this scenario, we propose the combined detection of FH and HPV screening. Our data showed a remarkable increase of the positive and negative coincidence rates in combined detection, which was also accompanied with high sensitivity and specificity in cervical cancer screening. This result may provide new insights for the selection of biomarkers besides EMC-6, Beclin1, and Rab5a in the diagnosis of cervical cancer[24].

Conclusions

We demonstrated that FH detection combined HPV screening was efficient in the diagnosis of cervical cancer and precancerous lesion, which lay fundamental leads and offer evitable assistance for further diagnosis and therapy of cervical cancer.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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