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Clinical diagnostic value of free body of reduced iron protoporphyrin in uterus epithelial cells on cervical carcinoma and precancerous lesion

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Abstract. – OBJECTIVE: Cervical cancer is a common malignant tumor in women with increased incidence and younger onset age. As a curable tumor, timely diagnosis and early intervention are critical. Based on the golden standard of cervical tissues pathology examination, we investigated the value of free body of reduced iron protoporphyrin (FH) in uterus epithelial cells for the diagnosis of cervical cancer and precancerous lesions, aiming to provide novel methods for early screening of cervical cancer.

PATIENTS AND METHODS: A total of 574 women who were screened for cervical cancer according to golden standard of pathology as the reference, were recruited for the analysis of authenticity, reliability, and predictive values of FH. The diagnostic value of FH on cervical carcinoma and precancerous lesion diagnosis was further analyzed.

RESULTS: 340 individuals had normal cervical or benign lesion by pathology examination, while 155 people had precancerous lesion, among which 79 cases presented early infiltration and infiltrative cancer. In FH screening, 361 and 213 people had negative and positive results, respectively. No significant differences in the results were observed between the two methods in screening cancer and precancerous lesion ($p>0.05$). FH showed 93.55% sensitivity and 81.94% specificity in diagnosing precancerous lesion, while the sensitivity and specificity for cervical cancer diagnosis were 93.53% and 81.01%, respectively.

CONCLUSIONS: FH assay was demonstrated to have advantages of high diagnostic value for cervical cancer and precancerous lesion, and might be used for early screening.

Key Words:
Free body of reduced iron protoporphyrin (FH), Cervical carcinoma, Precancerous lesion, Clinical diagnosis.

Introduction

Cervical cancer is a malignant tumor that invades female reproductive system with high incidence. In developing countries and regions, it is characterized as a major health concern for women[1]. In China, more than 130 thousand women were newly diagnosed with cervical carcinoma. The recent trend of aging onset, however, changes and tends to occur in women with around 30 years old[2,3]. The duration from precancerous lesion to infiltrative cervical carcinoma is generally 10 years. The screening of cancer and early diagnosis largely facilitates the treatment of the disease, and thus reduces the incidence and mortality of cervical cancer[4]. Therefore, WHO recommends large-scale screening for cervical cancer in women. Classical Papanicolaou smear presents profiles of low cost with easy manipulation, thus has been widely applied worldwide in recent decades. However, due to only 49% sensitivity and 50.5% false negative rate, misdiagnosis frequently occurs. ThinPrep Cytologic Test (TCT) smear shows remarkably higher quality than Papanicolaou smear, with much faster examination speed and lower false negative rate (12.8%). However, TCT requires relatively expensive instrument and high examining costs, and the application of TCT in under-developed area is largely limited[5,6]. A multitude of detection methods have been developed for the diagnosis of human papillomavirus (HPV) recently,[7] in which HCII is one method mostly applied, with relatively higher sensitivity and specificity. Single positive results of HPV, however, cannot verify the cervical cancer oc-

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currence, and should be accompanied with definite diagnosis by cytology and histo-pathology. Vaginoscope can observe and reveal micro-lesion in cervical and lower reproductive tracts, providing precise location guiding biopsy. The intra-cervical tube lesion, however, is unable to be detected by vaginoscope. Also, screening methods for cervical cancer may vary according to the social economic status of the areas[8]. Those methods with easy manipulation, lower cost and high efficiency are of utmost necessity in clinics for large-scale screening of cervical cancer. Free body of reduced iron protoporphyrin (FH) has been found to cause instability of cervical epithelial cells, leading to conformational changes of certain proteins. The detachment of FH substances in cellular proteins causes elevated level of free FH in cervical epithelial cells and in cervical secretory substances[9]. The test of FH substances in cervical secretion, therefore, can be used to screen cervical cancer and precancerous lesion. This study compared the results of FH assay and pathology examination of cervical cancer tissues, and evaluated the clinical diagnostic value of FH assay for cervical cancer and precancerous lesion.

Patients and Methods

Patients

A total of 574 women who received cervical cancer screening in the First Affiliated Hospital of University of South China from January 2014 to January 2016 was recruited. Patients' age was from 18 to 66 years (average age = 34.78 ± 7.52 years). All patients had sexual activity, with 0-6 parity (average = 2.15 ± 0.74). Those women at gestation, lactation period, with cervical surgery, uterectomy or radiotherapy history, with virginal bleeding including cervical polyp, severe cervical erosion or hysteromyoma were excluded. This study has been approved by the Ethical Committee of the First Affiliated Hospital of the University of South China, Hengyang, Hunan, China. All participants have signed consent forms with full knowledge of objective and significance of this study.

Sample Collection

Patients were laid in lithotomy position for collecting samples of cervical tissues. Samples were rinsed in storage buffer and cervical cells were eluted for further analysis.

FH Assay

FH assay for uterus epithelial cell stability was performed with commercial diagnostic kit (Meilun Bio, Dalian, China). Those cells without staining were negative, while weak positive (+) was identified as light blue color, positive result (++) was shown in red, and dark blue followed by dark yellow/brown-red color was deduced as strong positive (+++). Overall positive results were identified with color staining.

Histo-Pathology Assay

Cervical tissues were collected, prepared in paraffin sections for hematoxylin and eosin (H&E) staining. Pathology diagnosis was conducted with light field microscope (Olympus, Tokyo, Japan). Following WHO diagnostic guideline (2003 version), the course of disease was sub-divided into normal, benign lesion, CIN1, CIN2, CIN3, early phase infiltrative cancer, and infiltrative cancer. CIN1 and higher grade were considered as positive pathology results.

Statistical Analysis

SPSS18.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Measurement data were presented as mean ± standard deviation (SD) while enumeration data were presented as N number of percentage. Negative result was set as 0 while positive results were set as 1 in χ^2-test. A significant difference was defined as $p<0.05$.

Results

Pathology test Result for Cervical Tissues

A total of 340 cases (out of 574) showed normal or benign lesions in cervical tissues. There were 31, 54 and 70 patients at stage CIN1, CIN2 and CIN3, respectively, while 79 patients had early phase infiltrative lesion or infiltrative cancer (Table I).

Stable FH screening result of Uterus Epithelial cells

In the stable FH screening result of uterus epithelial cells, there were 361 cases with negative results, 46 with weak positive, 71 with positive and 96 with strong positive cases (Table II).

Comparison Between two Methods in Detecting Precancerous Lesion

Among all 340 cases determined as negative results by histopathology test, 318 individuals

Table I. Test result for cervical tissue pathology.

	N	Percentage (%)
Normal	340	59.23
CIN1	31	5.40
CIN2	54	9.41
CIN3	70	12.20
Infiltrative cancer	79	13.76

Table II. Screening of stable FH in uterus epithelial.

	N	Percentage (%)
Negative	361	62.90
Strong positive	46	8.01
Positive	71	12.37
Strong positive	96	16.72

Table III. Analysis of FH test in detecting pre-cancerous lesion of cervical.

	Histopathology test		
FH screening	0 (Normal)	1 (Pre-cancerous lesion)	Total
0 (Negative)	318	28	346
1 (Positive)	22	127	149
Total	340	155	495

showed negative expression of FH. Precancerous lesions were found in 155 patients, and 127 of them were confirmed positive in FH assay. No significant difference existed between the two methods (χ^2=0.720, p=0.396, Table III)

Comparison Between the two Methods in Detecting Cervical Cancer

The result in histo-pathology test showed 79 patients with precancerous lesion, while in the detection of FH, the number of positive cases was 64. Statistical analysis demonstrated no significant difference between the two methods (χ^2=0.973, p=0.324, Table IV).

Diagnostic Value of FH in Detecting Cervical Cancer and Precancerous lesion

The sensitivity and specificity of FH in diagnosing precancerous lesion were 93.53% and 81.94%, respectively, with a Kappa value of 0.76. The sensitivity and specificity of FH in diagnosing cervical cancer, on the other hand, were 93.53% and 81.01%, respectively, with a Kappa value of 0.72 (Table V).

Discussion

As a common malignant tumor in female reproductive system, both the incidence and mortality of cervical cancer are rapidly increasing in recent years, accompanied with younger age of onset, especially in those under-developed countries and regions[10]. About 500,000 people were newly diagnosed with cervical cancer worldwide, with more than 80% cases occurring in developing countries. China occupies about 28% of all newly found cases (around 130,000 people) each year. In this study, 79 out of 574 females were diagnosed with early phase infiltrative cancer or infiltrative carcinoma (13.76%). Etiology of cervi-

Table V. Diagnostic value of FH in detecting cervical cancer and pre-cancerous lesion.

		Pre-cancerous lesion	Cervical cancer
Reality	Sensitivity (%)	93.53	93.53
	Specificity (%)	81.94	81.01
	Misdiagnosis (%)	18.06	18.99
	Missed diagnosis (%)	6.47	6.47
	Jordan index	75.47	74.54
	Positive likelihood ratio	11.59	12.52
	Positive likelihood ratio	11.59	12.52
	Negative likelihood ratio1.13	1.14	
Reliability	Fitness (%)	69.90	79.47
	Kappa value	0.76	0.72
Predictive value	Positive predictive value (%)	85.23	74.42
	Negative predictive value (%)	91.91	95.50

cal cancer is commonly believed as mainly due to persistent infection by HPV. With elevated viral load and aggravated disease condition, cervical cancer is gradually developed[11-13] from cervical epithelial lesion towards early phase infiltrative cancer within 10 years. Therefore, appropriate screening for detecting precancerous lesion and early phase of cancer, followed by timely intervention and treatment, are great prerequisites to substantially decrease the risk of cervical carcinoma[14]. In the guideline for screening and management of precancerous lesion and early phase cancer stipulated by WHO in 2003, it is highly recommended to perform screening for cervical cancer in females with the age of over 30 years old, and implement treatment is performed immediately after positive results are confirmed. FH substances are widely distributed in human cells and predominately in mitochondria[15]. FH can exert certain physiological functions via binding to specific proteins to form oxygen sensor or peroxidase, which are necessary components in mitochondria[16]. Previous studies showed that the activation of oxygen sensor and hypoxia sensor in tumor cells potentiated the ROS activity[17], changed polarity quantification inside cells, and released FH in hydrophobic nucleus of cellular proteins into free FH. Once released, free FH can cause oxidative stress in cells, destruct tissue/cell stability and dysregulate cell proliferation, differentiation and apoptosis[18,19]. The occurrence of malignant tumor requires multiple steps from benign hyperplasia, precancerous lesion and malignant formation of infiltrative cancer to metastatic lesions[20,21]. During this process, membrane injury was aggravated with severe cell autolysis, leading to more infiltration of FH. Dysregulated metabolism inside cancer cells elevates free FH level during early phase of homeostasis breakage with abnormal hyperplasia. Moreover, free FH level is positively correlated with tumor malignancy. Therefore, malignant tumors can be determined by the oxidation-reduction reaction between one lipophilic substance to penetrate the membrane, with the formation of blue precipitation. The existence and darkness of cellular staining can be used to evaluate malignant tumors. The screening for cervical cancer in this study utilized this principle to detect cervical cancer based on staining of cervical secretory substances. In a total of 574 cases, the result of FH screening identified 361, 46, 71 and 96 individuals as negative, weak positive, positive and strong positive results, respectively. Using histopathology result as the golden standard, we analyzed the screening results of FH in detecting cervical cancer and precancerous lesions. After comparison, no significant difference was found between the two methods. Further analysis regarding clinical diagnostic value of FH indicated higher than 80% of sensitivity and specificity in diagnosing cervical cancer and precancerous lesion, with about 18% misdiagnosis but only 6.47% miss-diagnostic rate. Overall, FH assay presented satisfactory accuracy. The analysis of fitness rate and Kappa value all demonstrated high reliability of FH assay for both precancerous lesion and cervical cancer. Taken together, our data showed that FH assay could be used to screen cervical cancer and precancerous lesion at molecular level, which offset the weakness of cytology methods in screening. Given the characters of non-invasion, easy manipulation, lower technique requirement, no need of special instrument, and lower cost, the application value of FH assay is promising especially in under-developed regions with unequipped instruments. However, those FH-positive patients require further cytology examination for the appropriate intervention and treatment.

Conclusions

FH assay had high value in the diagnosis of cervical cancer and precancerous lesion, and might be a novel approach for the early screening of cervical cancer.

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Conflict of interest

The authors declare no conflicts of interest.

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