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Application of Cervista® human papilloma virus high-risk test in cervical cancer screening of Xinjiang Uyghur women

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Summary

Objective: This study aims to evaluate the diagnostic value of the Cervista® human papilloma virus (HPV) high-risk (HR) test in cervical cancer and precancerous lesion screening in Xinjiang Uyghur women. *Materials and Methods:* Three hundred and seventy three Uyghur women from Bachu County underwent the Cervista® HPV HR test, ThinPrep cytologic test, and cervical biopsy under a colposcope. Then the relationship between the infection rate of high-risk human papilloma virus (HR-HPV), the cytological results, and the histological results was analyzed. *Results:* With increasing cytological and pathological classification, the HR-HPV infection rate also increased and reached 100% in patients with cervical cancer. The highest proportion of the pathologically positive group consisted of patients with group A9 HPV infection as compared to patients infected with group A5/A6 or A7 HPV. *Conclusion:* HR-HPV is closely correlated with cervical cancer and precancerous lesions. Group A9 HPV has a high predictive value in cervical disease screening in Uyghur women. When cytological examination shows atypical squamous cells of undetermined significance (ASCUS), the Cervista® HPV HR test can provide a sensitive differentiation method.

Key words: Cervical cancer; Cervista HPV HR test; ThinPrep cytologic test.

Introduction

Nowadays, cervical cancer is the only cancer that has the potential to be destroyed. Persistent infection of high-risk human papilloma virus (HR-HPV) has been recognized as a major risk factor for cervical cancer [1]. The clear pathogenic factors of cervical cancer and the slow development of the disease offer a valuable opportunity for mass screening and treatment. A large number of clinical practices show that cervical precancerous lesions can be found by screening and that they can be blocked before the occurrence of cervical cancer. The ThinPrep cytologic test (TCT) combined with HPV detection is recommended as the first screening method. However, cytological examinations should be performed by experimental cytologists and include continuous quality control, which is difficult in regions without adequate medical resources. HPV detection for cervical cancer and precancerous lesions has high sensitivity and good repeatability, stability, and objectivity, which is suitable for cervical cancer screening in regions without adequate medical resources. The effectiveness of cervical cancer screening depends on the high sensitivity of HPV DNA detection methods.

For women, in terms of incidence, cervical cancer ranks second among malignant tumors worldwide. Particularly in developing countries and regions, it ranks first [2, 3]. The incidence of cervical cancer and death in Xinjiang is the highest in Xinjiang in China. In Xinjiang, especially in Southern Xinjiang, cervical cancer is the leading cause of death in Uyghur women. Its prevalence and mortality are much higher in Uyghur women than in women of other ethnicities living in the same region, and the onset age is also lower than that in other ethnic populations. Among all the minorities in China, Uyghur women have the highest mortality due to cervical cancer [4]. Thus, an economical and effective screening method for cervical cancer is of great significance for the rural areas of the Xinjiang Uyghur Autonomous Region. The cervical cancer screening program in Bachu County was supported by the National Natural Science Foundation of China and the Chinese Cancer Foundation. This program was in cooperation with the Maternal and Child Health Hospital of Bachu County. In this program, a series of commonly used cervical cancer screening technologies were systemically evaluated. The Cervista® HPV HR test was used in this study for screening HR-HPV in Uyghur women in the Bachu County of Xinjiang in order to explore its diagnostic value in detecting cervical cancer and precancerous lesions in Uyghur women. Additionally, the test could provide a scientific basis for the differential diagnosis of patients with a cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS).

Materials and Methods

Subjects

Among the 5045 Uyghur women who attended the screening program in Bachu County, any woman who tested positive for the care HPV test, TCT, or acetate/iodine staining was recalled for colposcope examination and cervical biopsy of suspicious lesions.

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Among the patients who underwent a colposcope examination, 373 patients were randomly selected to undergo the Cervista® HPV HR DNA test. The patients were 20 to 65 years old, with a mean age of 39.25 ± 8.65 years. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Xinjiang Medical University. Written informed consent was obtained from all participants.

Diagnosis using the TCT

Diagnosis using TCT results was based on the new Bethesda system classification standards recommended by the National Cancer Institute in 2001. According to the severity of cell atypia, the specimens could be classified as normal cells, inflammation, or ASCUS, including the atypical squamous cells of highly squamous intraepithelial lesions (ASCH), low-grade squamous intraepithelial lesions (LSIL), highly squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (SCC).

Cervista® HPV HR test

The 14 kinds of HR-HPV that could be detected by the Cervista® HPV HR test, could be divided into the following three groups: group A5/A6 (HPV51, HPV56, and HPV66), group A7 (HPV18, HPV39, HPV45, HPV59, and HPV68), and group A9 (HPV16, HPV31, HPV33, HPV35, HPV52, and HPV58). DNA extraction and the Cervista® HPV HR test were carried out according to the manufacturer's instructions.

Pathological grading

Cervical biopsies could be diagnosed histologically as chronic cervicitis, cervical intraepithelial neoplasia grade I (CIN I), CIN grade II (CIN II), CIN grade III (CIN III), and cervical SCC (cervical cancer I and infiltrating carcinoma).

Statistical analysis

All results were analyzed using SPSS 18.0 software. The positive rates of HR-HPV in the different grades of cervical lesions were compared using the Chi-square and rank sum tests. A value of $\alpha = 0.05$ was used as the test standard, and $p < 0.05$ was considered statistically significant.

Results

Cytological diagnosis by TCT

Among the 373 samples, there were 265 normal specimens (71.0%), 25 cases of ASCUS (6.7%), ten cases of ASCH (2.7%), 52 cases of LSIL (13.9%), and 21 cases of HSIL (5.6%). A total of 108 patients (29%) displayed ASCUS or further cytological changes.

Relationship between HR-HPV infection and the cytological grades

Among the 265 cytologically normal samples, there were 210 (79.2%) HR-HPV-positive cases. Among the 25 cases of ASCUS, 21 (84%) were HR-HPV positive, while among the patients with ASCH, nine (90%) were HR-HPV positive. In patients with LSIL and HSIL, the HR-HPV infection rates were 100%. The HR-HPV infection rate in the samples with different cytological grades showed a statistically significant difference ($c^2 = 20.958$, $p < 0.05$; Table 1). The HR-HPV infection rate increased with the cytological grades.

Table 1. — *HR HPV infection rates in different cytological grades of samples.*

Cytological diagnosis	Cases (n)	HR HPV-positive (cases)	Positive rate of HR HPV
Normal	265	210	79.2%
ASCUS	25	21	84.0%
ASCH	10	9	90.0%
HSIL	21	21	100.0%
LSIL	52	52	100.0%
Total	373	315	84.5%

Table 2. — *The infection rates of group A9, A5/A6, and A7 HR HPV in the different grades of cytological abnormalities.*

Groups	A5/6 (cases)	A7 (cases)	A9 (cases)	Total (cases)
ASCH	1	1	8	10
ASCUS	8	1	16	25
HSIL	7	2	19	28
LSIL	12	4	42	58
Normal	74	55	130	259
Total	102	63	215	380

Relationship between cytological abnormalities and the infection rates of different HR-HPV groups

According to the Cervista® HPV HR test results, there were 102 cases of group A5/A6 HPV infection (26.8%), 63 cases of group A7 HPV infection (16.6%), and 215 cases of group A9 HPV infection (56.6%). The infection rate of group A9 HPV was prominently higher than that of the other two groups, including single infection and coinfection with two or three groups. The infection rates of group A9, A5/A6, and A7 HR-HPV in the ASCUS, LSIL, and HSIL cases showed a statistically significant difference ($c^2 = 19.544$, $p < 19.544$; Table 2).

Association of HR-HPV infection rates with pathological grades

Based on the histological results, 373 patients were classified into the chronic cervicitis (n = 247), CIN I (n = 68), CIN II (n = 26), CIN III (n = 22), and cervical cancer (n = 10) groups. The positive rate of HR-HPV in the chronic cervicitis group was 78.1% (193/247), significantly lower than the rates in the CIN I, CIN II, CIN III, and cervical cancer groups, respectively (95.6% [65/68], 100.0% [26/26], 100% [22/22], and 100% [10/10], respectively; [$p < 0.05$]; Table 3). The HPV infection rate increased with the increase in the pathological grade. Among the 315 HR-HPV-positive cases, chronic cervicitis, CIN I, CIN II, CIN III, and cervical cancer accounted for 61.3% (193/315), 20.6% (65/315), 8.3% (26/315), 7.0% (22/315), and 3.2% (10/315) of cases, respectively. The HR-HPV-positive cases included single infection and mixed infection. The infection rates for group A5/A6, group A7, and group A9 HR-HPV were 26.8%, 16.6%, and 26.8%, respec-

Table 3. — *HR-HPV infection in patients with different pathologic grades.*

Histologic grades	Number of cases	HR-HPV positive	Positive rate
Cervical cancer	10	10	100.0%
CIN III	22	22	100.0%
CIN II	26	26	100.0%
CIN I	68	65	95.6%
Chronic cervicitis	247	193	78.1%
Total	373	315	84.5%

Table 4. — *The distribution of HR-HPV positive cases in different pathologic grades.*

Groups	A5/6 (cases)	A7 (cases)	A9 (cases)	HPV-positive (cases)	HPV-negative (cases)
Chronic cervicitis	73	45	118	236	54
CIN I	18	13	46	77	3
CIN II	5	3	22	30	0
CIN III	5	2	20	27	0
Cervical cancer	1	0	9	10	0
Total	102	63	215	380	57

tively. In the chronic cervicitis, CIN I, CIN II, CIN III, and cervical cancer groups, the infection rate of group A9 HR-HPV was markedly higher than the infection rates of the other groups ($c^2 = 16.288$, $p < 0.05$; Table 4).

Discussion

HPV infection is highly associated with the occurrence and development of CIN and cervical cancer. It has been proven that the HPV infection rate is almost 100% in the cervical tissues of cervical cancer patients. Currently, there are many methods for HPV detection, among which hybrid capture 2 (HC2) HPV DNA detection was the first method to be approved by the Food and Drug Administration. The HC2 HPV DNA test can detect 13 types of HR-HPV. However, it has some limitations, such as false negatives due to a low level of virus infection or uncorrected sampling, potential cross-contamination, cross-reaction of the HR type probe with the low-risk type probe, high cost, complex operation, large time requirements, and so on. The Cervista® HPV HR DNA test is a new HPV detection method that has also been approved by the Food and Drug Administration recently. It uses Invader chemistry, a signal amplification method for detecting special nucleic acid sequences, rather than the polymerase chain reaction, so that the amplification of erroneous signals can be avoided. Two synchronous isothermal reactions run at the same time, which can improve the specificity and anti-interference capability. Cervista® is the only method with an internal control, which can reduce false-negative results caused by insufficient sample DNA, and has no cross-reaction with low-risk HPV types, which reduces the chance of false positives. Cervista® detects the L1 and E6/7 regions of HPV simultaneously, significantly improving the specificity, and avoiding the false-negative results of methods that only detect the L1 region. It has been reported that there is no significant difference between the HPV-positive rates in the HC2 and Cervista® HPV HR assays, when a population with no intraepithelial lesions or malignant lesions is screened [5-8]. The Cervista® HPV HR test was approved for clinical use by China's State Food and Drug Administration in November 2011. Researchers compared the Cervista® HPV HR test with gene sequencing for HPV detection and got good consistency [9].

Worldwide, the distribution and genotype of HPV differs from region to region and nationality to nationality. Currently, HPV16 is the main type of HPV found in cervical tissues worldwide, followed by HPV18. HPV16/18, HPV31, 33, 35, 45, 52, and 58 are the other subtypes found in cervical cancer [10, 11]. Chinese women have much higher HPV16 infection than in other countries, accounting for 79.6% of all cervical cancers. However, the positive rate of other important HPV types differs in different regions [12, 13]. The distribution of HPV infection also differs according to gender [14]. It has been reported that HPV16 is the most common type of infection in Tibetan and Yao women [15, 16], but the other types have a different distribution in Tibetan and Yao women. For instance, HPV33 and HPV58 rank second and third, respectively, in Tibetan women, but in Yao women, HPV52 and HPV58 are the second and third most common infections, respectively. Previous findings regarding cervical cancer showed that HPV16 is the most common infection type in Uyghur women, followed by HPV58 [17]. This study showed that the HR-HPV infection rate increased with the increasing grade of cytological diagnosis and reached 100% in cervical cancer, further confirming the important role of HR-HPV in the development of cervical cancer. The present findings also showed that the infection rate of group A9 HPV was the highest, followed by group A5/A6. The present results were consistent with the results reported [18-22], suggesting that group A9 HR-HPV has high pathogenicity and a high risk of causing cervical cancer. The high infection rate of group A9 HPV in the cervical lesions of Uyghur women may be related to the high infection rates of HPV16 and HPV58 in Uyghur women, which are both in group A9. Thus, attention should be paid to the Uyghur women infected with HR-HPV, especially with the types in group A9, and early intervention should be taken.

ASCUS presents with more obvious cytomorphological changes than reactive changes, but does not reach the level of squamous intraepithelial neoplasia. It can be a benign lesion with active proliferation or a potential malignant lesion [23]. The treatment of ASCUS is always difficult using the standard treatment of cervical lesions, resulting in excessive or insufficient treatment or delayed diagnosis and treatment. Einstein *et al.* pointed out that in women with cytomorphological ASCUS [24], HR-HPV has a sensitivity of 92.8% and negative predictive value of 99.1% for CIN2+ and a sensitiv-

ity of 100% and positive predictive value of 100% for CIN3+. However, its specificity for CIN2 and CIN3 were only 44.2% and 43%, respectively [24]. The Cervista® HPV HR assay showed that group A9 HPV has high pathogenicity, which can be used as a sensitive differential diagnosis method for the cervical cytomorphological examination. For patients with cytomorphological ASCUS, the Cervista® HPV HR test should be performed. If the specimen is group A9-positive, a colposcopy and cervical biopsy should be performed immediately in order to make a clear diagnosis and provide effective treatment in time. Patients without HR-HPV infection can have regular cytological examinations. It does not only save medical resources and reduce the unnecessary cervical injury caused by colposcopy examination, but also reduces the economic and psychological burden of the patients.

Conclusion

In this study, Cervista® HR HPV DNA test is firstly used to screen cervical lesions in Xinjiang Uyghur Women. The authors aimed to discuss predictive value and found A9 group has high level of pathogenicity, which provides a sensitive shunt method for cervical cytology examination. Further study will focus on consistency of Cervista® HR HPV DNA and HC2, and further validate accuracy of the method.

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