Human Papilloma Virus Screening by Hybrid Capture Ⅱ in Chinese Women of Jiangsu Province

QW Wang, PZ Xu, B Zhang, YS Dong, YQ Yang, F Cao, B Yu

ABSTRACT

Objective: *To survey Human Papilloma Virus (HPV) infection in Chinese Women of Jiangsu Province and discuss the relationship between HPV and the biology of cervical cancer.*

Methods: *Two thousand, one hundred and fifty-three sexually active women (including 66 cases of cervical cancer) were selected for high-risk human papilloma virus DNA test with Hybrid Capture II (HCII).*

Results: *The overall HPV prevalence was 32.6% (701/2153) with higher positive rates in cervical carcinoma and Cervical Interstitial Neoplasia (CIN) [93.9% and 54.6%] respectively. For women aged 40−59 years, the overall high-risk HPV prevalence was higher than those of other age groups. Compared with CIN* Ⅰ*, the positivity rate and viral load of HPV DNA in CIN* Ⅲ *is much higher (80.2%* vs *29.9%, 11.89 vs 0.53). Ninety-four per cent (64/66) of patients with Cervical cancer were detected to be HPV positive. There was no significant difference in HPV DNA among each clinical stage and pathologic grade. But the positive rates and the value of HPV DNA were higher in the patients with cervical interstitial incursion. Eighty per cent of patients (20/25) could become negative within six months after operation.*

Conclusions: *High-risk HPV DNA test is effective in screening for cervical diseases. HC*Ⅱ *is an effective method to detect HPV DNA.*

Keywords: Cervical intra-epithelial neoplasia, Cervix neoplasms, DNA,viral, HCⅡ, human papilloma virus

Tamizaje del Virus del Papiloma Humano Mediante Captura Híbrida en Mujeres Chinas de la Provincia de Jiangsu

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RESUMEN

Objetivo: *Investigar la infección por el virus del papiloma humano (VPH) en las mujeres chinas de la Provincia de Jiangsu y analizar la relación entre VPH y la biología del cáncer cervical o del cuello uterino.*

Métodos: *Dos mil ciento cincuenta y tres mujeres sexualmente activas (incluyendo 66 casos de cáncer cervical) fueron seleccionadas para una prueba de ADN con el fin de detectar el virus del papiloma humano de alto riesgo mediante Captura Híbrida 2 (HC2).*

Resultados: *La prevalencia general de VPH fue 32.6% (701/2153), hallándose las tasas positivas más altas en el carcinoma cervical y la neoplasia intersticial cervical (NIC) [93.9% y 54.6%]. Para las mujeres de 40-59 años de edad, la prevalencia general de VPH de alto riesgo fue mayor que para los otros grupos etarios. En comparación con el CIN, la tasa de positividad y la carga viral de ADN del VPH en el CIN es mucho mayor (80.2%* vs *29.9%, 11.89* vs *0.53). Se detectó que noventa y cuatro por ciento (64/66) de las pacientes con cáncer del cuello uterino eran VPH positivas. No hubo ninguna diferencia significativa en el ADN del VPH ADN entre cada fase clínica y el grado patológico. No obstante, tanto las tasas positivas como el valor de VPH ADN fueron más altos en las pacientes con incursión intersticial cervical. Ochenta por ciento de las pacientes (20/25) podrían volverse negativas en seis meses tras la operación.*

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Conclusiones: *La prueba de ADN para la detección del virus del papiloma humano de alto riesgo es un medio efectivo para el tamizaje de las enfermedades cervicales. El HC2 es un método efectivo para detectar el ADN del VPH.*

Palabras claves: Neoplasia intersticial cervical, neoplasias del cuello del útero, ADN, viral, HCII, virus del papiloma humano

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INTRODUCTION

Cervical cancer is becoming the second primary cause of cancer deaths among women around the world, and over 90% of the cases are associated with high-risk human papilloma virus (HR-HPV) infection (1). There are about 100 different kinds of HPV but not all of them cause health problems. Only a few HPV types infect cervical cells that can become cancerous if present for many years (2). Human Papilloma virus types 16 and 18 cause about 70% of cervical cancers and are also known to cause some vulvar and vaginal cancers. Human Papilloma virus is the most common sexually transmitted virus. The likelihood of acquiring a HPV infection over a lifetime has been estimated to be 75% or more, and the human immune system will keep the virus under control or get rid of it completely (3). However, persistent HPV infection could induce cell changes leading to cervical cancer (4−4). The Food and Drug Administration (FDA) recently approved concurrent HPV and 5 Pap smear screening for women aged 30 years and more (6). Human papilloma virus may be found even before there are changes in the cervix. Most cervical cancer cases can be prevented through early detection and treatment of abnormal cell changes before cervical cancer develops.

However, HPV cannot be cultured *in vitro*, as such, credible detection of HPV relies strictly on molecular analysis of HPV DNA sequence. Since the late 1980s, researchers started to use nucleic acid probes to detect HPV with good results. One of these, the Hybrid Capture II (HCII, Digene Corporation) is the only HPV test declared by the FDA for *in vitro* diagnostic use. It is a micro plate-based solid phase hybridization assay for the detection of 13 high-risk (16/18/31/33/35/39/45/51/52/56/58/59/68) HPV. Some research showed that the HCII is a rapid method that can be performed in most clinical laboratories (7−8).

In China, a survey showed that about 200 000 women are diagnosed with cervical cancer every year (9). It is very important to carry out cervical cancer screening by HPV and HPV DNA. The latter started in China about 10 years ago. There is a paucity of data on HPV infection in China.

The aim of the present study is to survey HPV infection in Chinese Women of the Jiangsu Province and discuss the relationship between HPV and the biology of cervical cancer.

SUBJECTS AND METHODS

The material of this study comprises 2153 cervical samples collected from women during June 2006 to June 2008 in Changzhou Women and Children Health Hospital. All of the cases (aged 19 to 76 years) were sexually active and willing to undergo HPV testing. After cytology and clinical evaluation, they were classified as follows: 66 cases of cervical cancer, 238 cases of cervical intra-epithelial neoplasia (including CIN I 87, CIN II 65 ,CIN III 86), 1276 cases of cervicitis (including low-grade 276, middle-grade 634 and high-grade 366), 241 cases with other gynaecological problems (including cervical condyloma 95, postcoital bleeding or vaginal bleeding 64), and 332 cases just for routine gynaecological check. The International Federation of Gynaecology and Obstetrics (FIGO) clinical staging system was used to classify the stage of cervical cancer and clinical treatment.

The samples were collected using the cervical swabs of the Hybrid Capture II system (Digene, USA) [7] and then the swabs were immersed in the preservative Medium bottle and stored at 4°C immediately. The sample processing followed the standard protocol of the HCII procedure (Digene, USA) such as cracking - hybrid - capture - signal amplification – analysis. The positive test was considered by the relative light unit (RLU) of the sample that equalled or exceeded the mean of the three positive control values. The value of the HPV DNA was RLU (sample)/RLU (positive control)*1.0 pg/ml. By this way, 13 high-risk (16/18/31/33/35/39/45/51/52/56/58/59/68) HPV can be examined.

We compared differences in proportion for rate of infection by means of the χ^2 statistic, whereas differences in means of HPV DNA variables were compared using Rank Sum test. The value of 0.05 was considered the level for significance.

RESULTS

The rate of HPV infection

Table 1 shows that in 2153 women who came from Jiangsu Province in China, 701 women were infected with HPV, the overall HPV prevalence was 32.6% (701/2153). Furthermore, the patients with cervical cancer and CIN had higher positive rates (93.9% and 54.6%).

After classification by age, it was found that 75% of women infected with HPV were over 30 years old. For women aged 50 − 59 years, the overall high-risk HPV prevalence (46.3%) was higher than those of other age groups (Fig. 1).

Table 2 shows that the level of high-risk HPV DNA increased with severity of cervical diseases. Compared with

Table 1: HPV DNA of 2153 cases

Group	n	Positive cases	Positive rate (%)
Cervical cancer	66	62	93.9
cervical squamous carcinoma	65	61	93.8
cervical adenocarcinoma	1	1	–
CIN	238	130	54.6
CIN Ⅰ	87	26	29.9
CIN Ⅱ	65	35	53.8
CIN Ⅲ	86	69	80.2
Cervicitis	1276	399	31.3
low-grade	276	100	36.2
middle-grade	634	187	29.5
high-grade	366	112	30.6
Other gynaecological symptom	241	76	31.5
cervical condyloma	95	49	51.6
vaginal bleeding	62	13	21.0
others	84	14	16.7
Ordinary gynaecological check	332	34	10.2

CIN Ⅰ, the positivity rate and viral load of HPV DNA in CIN Ⅲ is much higher [80.2% *vs* 29.9%, 11.89 *vs* 0.53] ($p < 0.05$).

Ninety-four per cent (62/66) of patients with cervical cancer were detected to be HPV positive, only four cases were negative (6%). There was no significant difference in HPV DNA in each clinical stage and pathologic grade ($p > 0.05$). But the positive rates for HPV and the value of HPV DNA were higher in the patients who had cervical interstitial incursion ($p < 0.05$) [Table. 3]. After long-term observation in 25 cases, 80% of patients (20/25) became negative within six months after operation, one case became negative in the 10th month, while four cases (16%) remained positive (Table. 4).

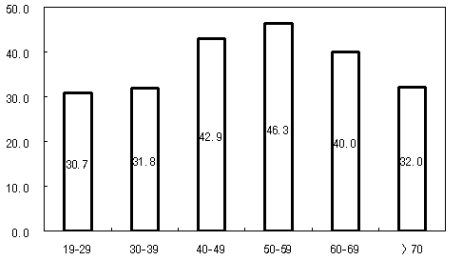

Fig. 1. The high-risk HPV prevalence in each age group

Table 2: The level of high-risk HPV DNA with severity of cervical diseases

Group	n	Median (*M*) (pg/ml)	Inter Quartile Range (IQR)
Cervical cancer			
cervical squamous carcinoma	65	47.99	156.10
cervical adenocarcinoma	1	15.70	–
CIN			
CIN Ⅰ	87	0.53	22.46
CIN Ⅱ	65	2.28	211.67
CIN Ⅲ	86	11.89	264.51

Table 3: HPV DNA of 66 cases of cervical cancer

	n	Positive cases	Positive rate (%)	Median (*M*) (pg/ml)	Inter Quartile Range (IQR)
Clinical stage					
Ⅰ	38	35	92	157.22	1313.56
Ⅱ	28	27	96	70.06	136.56
Pathologic grade					
1	8	8	8/8	28.03	247.37
2	55	51	93	106.91	935.67
3	3	3	3/3	73.54	93.30
Cervical interstitial incursion					
yes	33	33	100	86.71	524.47
no	33	29	88	28.86	72.75

Table 4: The change of HPV DNA in 25 cases after operation

Time	n	Positive cases	Positive rate (%)	Median (*M*) (pg/ml)	Inter Quartile Range (IQR)
Before operation	25	24	96	46.31	147.34
Six months after operation	25	5	20	0.50	1.23

DISCUSSION

Recent research shows that 90% of cervical cancers are associated with high-risk human papilloma virus (HR-HPV) infection (1). In the present study, 10.2% of women who presented for routine gynaecological check were HPV positive, which is higher than that in Hong Kong [7.3%] (10), but lower than that in Taiwan [19.3%] (11). We found that the patients with cervical cancer and CIN had higher positive rates (93.9% and 54.6%) and 75% of infected women were over 30 years old. In China, about 200 000 cases of cervical cancer are diagnosed every year and hence the necessity for cervical cancer screening in China. According to research, HR-HPV DNA examination is a useful technique and it can be used to reduce the incidence of cervical cancer. Therefore, most scholars propose HPV DNA test in women aged 30 years and over. The present study reinforces this; women in the 40−59-year age group have an overall high-risk HPV prevalence of 46.3%. So we suggest that more attention should be paid to women in this group.

The relationship between HPV and cervical cancer has been recognized. Most cases of cervical cancer can be attributable to HPV infection. The most frequent types detected are 16, 18, 45, 31, 33, 52, 58 and 35. In this study, 94% (62/66) of patients with cervical cancer were HPV positive, only four cases were negative. The probable reasons for cervical cancer in HPV negative cases are as follows: (i) HCII system can detect 13 kinds of high-risk (16/18/31/33/35/39/45/51/52/56/58/59/68) HPV and perhaps some types are not detected (ii) the loss of HPV DNA may occur when the tumour tissue is necrotic because of anoxia and (iii) it is most important that enough cervical cells are collected (12). Also we found that there were no significant differences in HPV DNA value among each clinical stage and pathologic grade, while the patients who had cervical interstitial incursion had higher infection rates and HPV DNA value. It suggests that HR-HPV might induce vascular proliferation of cervical cancer, it is propitious for tumour invasion and metastasis by increasing the activity of VEGF. Though the theory about how HPV advances cervical tumour incursion is undecided, the research is of great worth.

A meta-analysis showed that HR-HPV DNA could be used as a predictor of recrudescence or persistent infection of CIN after conization of the cervix (13−14). But whether it can be used to forecast recrudescence of cervical cancer after operation is undecided. We observed 25 cases long term and found that 80% of patients (20/25) can become negative within six months after operation, one case became negative in the 10th month but four cases (16%) remained positive. Whether the four cases have a high risk of recrudescence is still being observed.

In conclusion, high-risk HPV DNA test is effective in screening for cervical diseases and high-risk HPV-related to cervical carcinoma invasion and metastasis. HCII is an effective method to detect HPV DNA.

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