Risk factors for cervical cancer in China: a case-control study

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Summary

Objectives: To determine the prevalence of human papillomavirus types and investigate the risk factors for cervical cancer in Hubei, China. *Methods:* We conducted a case-control study to investigate risk factors. *Results:* HPV DNA was detected in 94.55% of patients with cervical carcinoma, and 23.64% of control subjects. The most common HPV type in cervical cancer was HPV type 16 (81.82%), followed by HPV 58 (6.36%). HPV infected patients have a higher risk of developing cervical carcinoma, which is 75.79 times more than non-infected people. The other risks were age at first intercourse ($p = 0.017$) and number of live births ($p = 0.032$). A history of previous cytologic screening was associated with a substantial reduction in risk ($p = 0.001$). *Conclusions*: The three principal reasons that Hubei has a high rate of women developing cervical carcinoma are HPV infection, age at first sexual intercourse and number of live births. Cervical cytology screening provides efficacious protection.

Key words: Cervical cancer; Risk factors; Case-control study; HPV.

Introduction

Cervical cancer is the second commonest type of cancer in females worldwide. Incidence rates of this disease varies from about 10 cases per 100,000 women per year in many developed countries to more than 40 per 100,000 in some developing countries [1]. Of the half million cases of cervical cancer estimated annually in the world, nearly 80% occur in developing countries [2].

China has one of the highest incidence rates of cervical cancer with 135,000 new cases detected every year, approximately one-fourth of those in the world [3]. However, there are quite different frequencies in various zones of China. Wufeng (Hubei province) in the central region of China, is one of the highest prevalence areas of cervical carcinoma (1,073.34 per 100,000). Beijing (2.54 per 100,000) and Shanghai (3.80 per 100,000) have the lowest incidence of cervical cancer. The rate in Hubei is 422 times that in Beijing [4]. The high incidence of cervical carcinoma may reflect a poor screening program [5] and differences in the human papillomavirus (HPV) infecting the Chinese population. In addition, HPV infection appears to be a necessary but not a sufficient cause of cervical cancer. The role of specific viral host or environmental factors for progression from infection to invasive disease has not been clarified.

The incidence of cervical carcinoma is relative to some risk factors, including three aspects. The first is biological factors, such as HPV infection [6]. The second is behavioral risk factors, such as early sexual intercourse, multiple sex partners, more pregnancies, more births, early age at first birth [7], low social economic status [8], smoking [9], malnutrition [10], and long-term use of oral prophylactics [11]. The third factor is genetic susceptibility, and familial aggregation has been observed in recent years [12].

To better assess the role of HPV infection and other risk factors in the development of cervical carcinoma, we carried out an analysis of data from case-control studies of cervical carcinoma in the Hubei province, China. Our aim was to determine the prevalence of human papillomavirus types in cervical carcinoma and investigate risk factors for this disease in Hubei. It would be helpful to know the specific types of HPV operating as cervical carcinogens in Hubei regions and to know whether it is necessary to tail interventions to the specific needs of particular areas. The definition of risk factors can help identify groups at particularly higher risk and can lead to new preventive strategies.

Materials and Methods

Study population

Recruitment of study subjects was conducted from 2003 through 2004. Case subjects were women with cervical cancer that was newly diagnosed and histologically confirmed at Zhongnan Hospital, Wuhan University in Hubei. The subjects had not received previous treatment for cervical cancer, and all were in sufficiently good physical and mental condition to provide reliable answers. Histologic slides were reviewed by two expert pathologists. Control subjects were women without cervical cancer who were selected from the same hospital. These women had no history of treatment with conization or hysterectomy. In addition, as was required for the case subjects, control subjects had to be in sufficiently good physical and mental condition to provide reliable answers. Other reasons for ineligibility of control subjects included diagnoses of cancer and tobacco-related disease.

The 110 patients were compared with 110 healthy women (ratio of 1:1). The condition of comparison was that both patients and healthy women lived in the same area and had similar ages (with differential no more than 2 years). The two groups were residents of Hubei province.

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Data and specimen collection

Study subjects were interviewed at the hospital by use of a standardized questionnaire to elicit information on sexual behavior, reproductive history, contraceptive practice, genital hygiene, history of sexually transmitted diseases, screening history, and various measures of socioeconomic status. Two specially trained female technicians administered the interview to all case and control subjects. An effort was made to keep them blinded to the case or control status of the study subjects.

A pelvic examination was performed on both case and control subjects to obtain biopsy specimens. Samples were transferred into sterile tubes and transported to the laboratory in medium at 4°C. On receipt of tissues in the laboratory, they were cut into small pieces, weighed, and stored as multiple aliquots at -80°C.

The study protocol was cleared by the local ethical committee. Written informed consent was obtained from all subjects - total of 110 eligible case subjects and 110 eligible control subjects. Refusal to participate was the main reason for nonparticipation of control subjects.

Detection and typing of HPV DNA

Cervical specimen DNA was extracted from the tissues using following standard techniques [13]. DNA amplication for HPV detection was performed using primers MY11/MY09 (Primer1: 3-GCA CAG GGT CAG AAC AAT GG-5 and Primer 2: 3-CGT CCA AGG GGA TAT TGA TC-5).

PCR reactions were performed using sterile 0.5-ml RNAse-/DNAse-free tubes and each PCR reaction was made up to a final volume of 50 ul. A typical 50 ul of PCR reaction contained 100 mM Kcl, 20 mM Tris-HCL pH 8.0, 2.0 mM $Mgcl_2$, 2.5 mM of dNTPs, 1.5 units of Taq polymerase (Promega Corp. Madison, WI) 25 pmol of each primer, and 10 ul of sample. After thermal cycling (intitally for 90 sec at 94°C for 1 cycle; then 40 cycle at 55°C for 1 min, 72°C for 1 min, and at 94°C for 1 min; and finally 1 cycle at 72°C for 10 min), 10 ul of the PCR reactions were analyzed by agarose gel electrophoresis. All HPV-negative carcinomas were retested for HPV-using the methods described previously to ensure that a positive result had not been "missed" initially.

HPV DNA sequencing

HPV DNA detection by PCR was further verified by sequencing of the DNA in all samples. Products for sequencing were generated using the sample primers as described for the amplification of HPV DNA. Amplified products were subjected to cycle sequencing PCR with the AB1 PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequencing reactions were then run on the ABI PRISM 310 Genetic Analyzer. Viral sequencing was analyzed by sequencing analysis software and sequencing navigator.

Statistical analysis

To estimate the risk of cervical cancer associated with various HPV types and other risk factors, we calculated the odds ratio (OR) and 95% confidence interval (CI) as approximation of relative risk by using unconditional logistic regression. The software used for statistics was SPSS 11.0 of which the level of significance was $\alpha = 0.05$.

Results

For patients with cervical carcinoma, the mean age was 48.06 ± 24.85 years (range 22-72 years) and for control subjects, it was 48.23 ± 25.23 years (range 23-72 years).

Table 1 presents the mono-factor analysis of risk factors relative to cervical carcinoma in Hubei province, China. The following factors were associated with risk: HPV infection (p = 0.0001), age at first intercourse (p = 0.001), number of live births (p = 0.042), number of lifetime sexual partners (p = 0.003), and education level (p = 0.001). In addition, a history of previous cytologic screening was associated with a substantial reduction in risk (p = 0.0001). However, a history of venereal disease (p = 0.291), number of pregnancies (p = 0.633) and use of hormonal contraceptives (p = 0.381) were not associated with risk.

Table 1. — *Mono-factor analysis risk factors related to cervical carcinoma*.

Risk factors	Case group (n = 110) No.	%	Control group (n = 110) No.	%	p value
HPVs					
positive	104	94.55	26	23.64	
negative	6	5.45	84	76.36	0.0001
Age at first intercourse (years)					
> 23	16	14.55	8	7.23	
21-23	32	29.09	24	21.82	
18-20	42	38.18	37	33.64	
< 18	20	18.18	41	37.27	0.001
Lifetime no. of sexual partners					
0-1	67	60.91	87	79.09	
> 2	43	39.09	23	20.91	0.003
No. of live births					
0-1	13	11.82	8	7.27	
2	34	30.91	41	37.27	
3	25	22.73	38	34.55	
> 3	38	34.55	23	20.91	0.042
No. of pregnancies					
0-1	66	60.0	60	54.55	
2-3	26	23.64	27	24.55	
> 4	18	16.36	23	20.91	0.633
Use of hormonal contraceptives (years)					
Never	74	67.27	83	75.45	
1-3	17	15.45	14	12.73	
> 4	19	17.27	13	11.82	0.381
Interval since last Pap smear (years)					
Never	92	83.64	49	44.55	
< 5	5	4.55	31	28.18	
≥ 5	13	11.82	30	27.27	0.001
Education					
Higher	10	9.09	31	28.18	
Primary	77	70.00	65	59.09	
None	23	20.91	14	12.73	0.001
Any veneral disease					
Never	64	58.18	72	65.45	
Once	21	19.09	22	20.00	
More than once	25	22.73	16	14.55	0.291

Table 2 presents the multivariate regression analysis of risk factors associated with cervical carcinoma. The variables that remained associated with risk were HPV infection (p for trend = 0.0001), age at first intercourse (p for

Table 2. — *Multiviate regression analysis risk factors related to cervical carcinoma*.

Risk factors	Case subjects	Control subjects	B	Wald	OR	95% CI for OR Lower	Upper	p value
HPVs								
negative	6	84						
positive	104	26	4.32	51.41	75.79	23.22	247.41	0.0001
Age at first intercourse (years)								
> 23	16	8						
21-23	32	24	-0.09	1.09	0.41	0.08	2.20	
18-20	42	37	0.37	0.23	1.46	0.31	6.79	
< 18	20	41	1.31	2.90	3.71	1.82	16.81	0.017
Lifetime no. of sexual partners								
0-1	67	87						
> 2	43	23	0.01	0.00	1.01	0.32	3.14	0.986
No. of live births								
0-1	13	8						
2	34	41	1.80	3.62	6.05	0.93	38.59	
3	25	38	2.20	4.99	9.06	1.32	62.52	
> 3	38	23	5.20	2.08	16.82	18.10	150.95	0.032
No. of pregnancies								
0-1	66	60						
2-3	26	27	0.51	0.63	1.67	0.47	5.92	
> 4	18	23	1.25	1.96	3.49	0.61	20.10	0.368
Interval since last Pap smear (years)								
Never	92	49						
≥ 5	13	30	1.56	7.29	4.75	1.53	14.7	
< 5	5	31	2.35	8.86	9.52	2.23	49.54	0.001

B: partial regression coefficient; Wald: B/standard error^2; OR: odds ratio.

trend = 0.017), number of live births (p for trend = 0.032), a history of previous cytologic screening (p for trend = 0.001). When comparing women reporting only one live birth, those reporting two had a 6.05-fold increased risk and those reporting three had a 9.06-fold increased risk. Women less than 18 years of age at first intercourse had a 3.71-fold increased risk compared with women who were more than 23 years old. When compared with women who were never screened, those who were screened five or more years before the interview had a 4.75-fold reduction in risk, and those who were screened in the previous five years had a 9.52-fold reduction in risk. HPV-infected patients had a higher risk level of developing cervical carcinoma, being 75.79 times more than non-infected women.

Table 3 summarizes the HPV DNA prevalence in case and control subjects. HPV genotypes including HPV 16, 58, 31, 18, 52, 33, 59, 35, 11, and 6 were detected. The other HPV types were not identified in any specimens. Out of the 110 case subjects, HPV DNA was identified in 104 cases (94.55%), with HPV16 being detected in 90 (81.82%) cases, HPV 58 in seven (6.36%) cases, HPV31 in five (4.55%) cases, HPV 18 in four (3.64%) cases, and the remaining six (5.45%) were HPV negative. Among control subject, the HPV prevalence was 23.64% (26/110). HPV type 11 (9.09%) and 6 (6.36%) were the most common types, followed by types 16 (1.82%), 18 (1.82%) and 35 (1.82%). When the prevalence rates of HPV types detected in case subjects were compared to control subjects only women with HPV 16 and 11 infection showed a statistically significant difference between the two groups (p = 0.001, p = 0.01, respectively). Two or more different HPV types were detected in 12 (10.91%) case subjects and 0 (0.00%) control subjects. The majority of instances of double infections included infections with HPV 16 or HPV 31.

Table 3. — *HPV genotype distribution in CIN and cervical cancer biopsies*.

HPV genotype	Total No. = 220	Control subjects No. = 110 (%)	Case subjects No. = 110 (%)
HPV 16	92	2 (1.82)	90 (81.82)
HPV 58	8	1 (0.91)	7 (6.36)
HPV 31	6	1 (0.91)	5 (4.55)
HPV 18	6	2 (1.82)	4 (3.64)
HPV 52	3	0 (0.00)	3 (2.73)
HPV33	2	0 (0.00)	2 (1.82)
HPV 59	3	1 (0.91)	2 (1.82)
HPV 35	3	2 (1.82)	1 (0.91)
HPV 6	8	7 (6.36)	1 (0.91)
HPV11	11	10 (9.09)	1 (0.91)
HPV negative	90	84 (76.36)	6 (5.45)
Single infection	118	26 (23.64)	92 (83.64)
Dual infections	12	0 (0.00)	12 (10.91)

Discussion

The results of this case-control study conducted in Hubei, China confirm the finding of the investigators indicating that HPV DNA is present in the vast majority of cervical cancers (94.55%). The outcome of this research showed that human papillomavirus is not only a high-risk factor of cervical carcinoma, but a principal risk factor, which increased the risk by 75.79 times.

The HPV types identified in this study were, in order of decreasing prevalence, HPV 16, 58, 31, 18 and 52. Other HPV types were detected in no more than 2% of the cervical cancers. The most striking feature of the genotype distribution in the biopsies was the high prevalence rate of HPV 16 infection (81.82%), a proportion that is similar to what has been found in surveys by Wu *et al*. (79.6%) [14] and somewhat higher than prevalence rates of other reports that have shown from 56.2% to 64.1% in parts of Europe and the United States [15, 16]. HPV 58 was the second most common type, accounting for 6.36% of cervical cancers, which is close to the reported prevalence in Guangdong and Jiangxi [14] and lower than the 13.2% reported previously in Shanghai [17] and Taiwan [18]. The prevalence of HPV18 (3.64%) was relatively low in this group, and different from previous reports [15]. The present study showed a high frequency of HPV16/58 infections in cervical cancer in central China. Such HPV typing information is very important for further HPV vaccine designs and applications.

Some research shows that development of cervical carcinoma has something to do with fertility and sexual intercourse factors. The outcome of this article shows that women who were under than 18 years old at first intercourse had a 3.71-fold increased risk compared with women who were more than 23 years old (p for trend = 0.017). When a woman practices sexual intercourse at a very young age, when her genital tract has not yet fully matured, her body is comparatively sensitive to carcino-

genic factors. Thus incidence ratio of cervical carcinoma is significantly higher.

The number of live births is also a high risk factor for cervical carcinoma. When compared with women reporting only one live birth, those reporting two had a 6.05-fold increased risk and those reporting three had a 9.06-fold increased risk (p for trend = 0.032). Multiple pregnancies and fertilities may result in birth injury to the cervix, thus increasing the risk of cervical carcinoma.

In addition, there was a strong protective effect with the interval since last Pap smear. Women who were screened in the previous five years had a 9.52-fold reduction in risk (p for trend = 0.001). Similar associations have been observed in Spain and Colombia, Brazil and the Philippines [19-24]. Thus using Pap smears cervical lesions can be found, patients can be treated in time, and further development of disease may be prevented.

In conclusion, the three principle reasons that Hubei has a high rate of cervical carcinoma are HPV infection, young age at first sexual intercourse and number of live births. Cervical cytology screening provides efficacious protection. The high prevalence of HPV16 and HPV58 in Hubei deserves special attention in future vaccination programs to effectively lessen the burden of cervical cancer in China.

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