RESEARCH COMMUNICATION

Life-style and Genital Human Papillomavirus in a Cross-Sectional Survey in Shanxi Province, China

Lily Zou[1], Yan-ping Bao[2,3], Ni Li[2], Min Dai[4], Cong-ping Ma[5], Yong-zhen Zhang[6], Xin-fu Liu[2], Manning Feinleib[7], You-lin Qiao[2*]

Abstract

Background: To determine the age-stratified HPV prevalence rate and the risk factors of life-style associated with HPV infection among women in rural China. Methods: An age-stratified, cross-sectional survey of 941 women between 16-59 years old was conducted in rural China. Carcinogenic HPV infection was determined using Digene's Hybrid Capture II HPV DNA test and interviews of life-style were conducted. Odds ratios (OR) and 95% confidence interval (CI) from the logistic regression models were used to determine the risk factors associated with HPV. Results: Among 941 women, 745 who had sexual intercourse underwent a cervical examination. The prevalence rate of 13 carcinogenic HPV infections among women 20-59 years old was 15.97%. the rate of HPV prevalence in the 25-34 age group was statistically lower than that in 20-24 and 35-59 age groups (X^2=13.3, *P*=0.0013). The OR of bathing every 7-19 days, 20-180 days, less than once every 180 days vs. bathing at least once a week were 1.19, 1.83 and 2.29 respectively and they had a dose-response relationship (Trend Test: *P*=0.003). The OR of women aged 25-34 age group vs. 20-24 age group was 0.40 (0.16 - 0.97) and the OR of bathing once every 180 days or less vs. at least once weekly was 2.22 (1.14 - 4.33) adjusted for the other confounding factors,. Conclusions: The HPV prevalence rate was lowest among child bearing women aged 25-34 year. Also, personal hygiene is significantly associated with the HPV infection in this area, regardless of age.

Keywords: Human papillomavirus (HPV) - bathing - age-stratified - prevalence rate - China

Asian Pacific J Cancer Prev, **12**, 781-786

Introduction

Cervical cancer is one of the leading cancers among women world wide, with an estimated 493,000 new cases and 274,000 deaths occurring every year according to the estimate from the International Agency for Research on Cancer (GLOBOCAN, 2002) (Parkin et al., 2005). It remains the most common cancer among women in many developing countries. Basic and epidemiologic research conducted during the past 15-20 years have provided overwhelming evidence to the extent that Human papillomavirus (HPV) has been established as a necessary cause of cervical cancer and its precursors, including cervical intraepithelial neoplasias grades 2 and 3 (CIN 2-3) (Bosch et al., 1995; Walboomers et al., 1999; Munoz et al., 2003; Castellsague et al., 2006). HPV prophylactic vaccines hold great promise to reduce the global burden of cervical cancer (Harper et al., 2004). Some HPV recombinant vaccine have been approved by the FDA in some developed countries, however in China, the government did not approve the HPV vaccines and still needs the information of the epidemiology of HPV infection to ensure vaccines will be appropriate for different populations and to tailor for specific groups where maximum protection would be needed. In China it is important to prepare the baseline information for the HPV vaccine as well as learn the characteristics of HPV for the development of further preventive methods.

To prepare for the vaccine trial and to find ways to prevent HPV infection and the development of cervical cancer, HPV epidemiology surveys were conducted by the Cancer Institute of the Chinese Academy of Medical Science (CICAMS) and the International Agency for Cancer Research (IARC) in China and the results showed that HPV prevalence varies markedly with age (Dai et al., 2006; Li et al., 2006; Wu et al., 2006). To prevent the HPV infection, it is necessary to determine the high risk factor for HPV infection and the target population of vaccination. The objectives of this paper are to identify the age-stratified HPV infection prevalence rate and the risk factors associated with HPV infection in rural China, with the intent of identifying potential preventive strategies in

*[1]Department of Biology, [7]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA, [2]Department of Cancer Epidemiology, Cancer Institute, Chinese Academy of Medical Sciences, [3]National Institute on Drug Dependence, Peking University, Beijing, China, [4]The International Agency for Research on Cancer, Lyons, France, [5]Yangcheng County Cancer Hospital/Institute, Yangcheng, Shanxi, [6]Shanxi Provincial Cancer Hospital/Institute, Taiyuan, China *For correspondence : qiaoy@public.bta.net.cn*

rural China.

Materials and Methods

Study subjects and enrollment

The survey was done in Yangcheng County of Shanxi Province where the cervical cancer mortality rate (52/100,000) in 1992 was much higher than the national average mortality rate (4.3/100,000) Shanxi province is also a region of high HPV infection especially among middle-aged women; the rate of HPV prevalence in the women aged 30-50 years was 27.4% (Belinson et al., 2003; Shen et al., 2003; Zhao et al., 2006).

The cluster random sampling was used to select 8 villages from a target commune for an age-stratified sample of 1000 women aged 16-59-years old (150 women in each of the 5-year age groups for ages 16-24 years, and 100 women in each group for ages 25-59) in Shanxi Province. Informed consents were obtained from all women aged 18-59 and from their guardians (father or mother) if they were under 18 years old.

Survey procedure

Informed consent and interviews were conducted at home or at screening clinics in an individual room without the presence of other patients or relatives. Eligible women were interviewed about risk factors for HPV infection and cervical neoplasia. Personnel to administer the questionnaires were specially trained by epidemiologists the week prior to the start of the survey. Following their interview, study participants underwent gynecological examinations for the collection of cervical cellular material performed by specially trained nurses and doctors.

Of 941 women, 745 sexually active and non-pregnant women underwent gynecological examinations. For the HC-II HPV DNA detection and liquid-based cytology (LBC) tests, cervical samples of exfoliated cervical cells from the endo- and ectocervix were collected only from married or previously married women. The cytobrush containing cervical cellular material was placed in the STM media provided by Digene Corp. and the specimen was shipped to CICAMS for processing. The Bethesda system was used to categorize the cytology results.

Following specimen collection, the gynecologists performed visual inspection with Acid (VIA) and visual inspection with Logol`s iodine (VILI) and digital colposcopy blindly. Each woman who was positive for each test of VIA, VILI and colposcopy underwent directed biopsy and ECC. The women with normal colposcopy but having either liquid-based cytology of low-grade squamous intraepithelial lesion (LSIL) or more, or atypical squamous cells of undetermined significance (ASCUS) and being HPV positive had the 2,4,8,10 random biopsy and ECC. The pathologic slides were confirmed by a senior pathologist of the CICAMS and then reviewed by experts of the IARC.

HPV DNA Test

After liquid-based cytology, samples were checked for HPV DNA analysis using the second-generation Hybrid CaptureTM (HC) systems (Digene, Inc., Gaithersburg, MD), the only HPV test currently approved by the US FDA. The HC system is a nucleic acid hybridization assay with signal amplification for the qualitative detection of DNA of high-risk , cancer-associated HPV types (13 high risk strains of HPV including: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) in cervical specimens. It cannot determine the specific HPV type present. A value of 1.0 pg HPV DNA or more was used as the cut-off for positive HPV.

Statistical analysis

A total of 13 types of high-risk HPV and aged-specified prevalence rates were calculated based on the 745 women. The descriptive statistics including means or percent were performed to generalize the baseline information of 941 women. Then the odds ratios (OR) of risk factors for HPV DNA detection and 95% confidence interval (CI) were computed by means of unconditional logistic regression. Based on the results of univariate statistics, Multiple Logistic Regression was used to control for the other confounding factors. All analysis was done using the software SAS8.0. P-values lower than 0.05 deemed significant.

Ethical considerations

The Institutional Review Board of the CICAMS and of the IARC approved this study.

Results

Screening compliance

There were 2157 qualified women with registered permanent residences in the 8 target villages. Among them, there were 1963 eligible women who knew about the screening. The rest were out of town (n=36), did not know about the screening (n=157), or mentally incapacitated (n=1). Among the 1963 eligible women, 1273 women were willing to participate in the screening and the population compliance rate for screening was 64.8%; the other 690 women did not take part in the screening. Among the 1273 women, 870 took part in the screening during May 2004, and the rest of the 403 women were excluded due to the size limitations of the study. The anticipated 150 women for each 5-year age group were enlisted for

Table 1. Demographic Information of 941 Women of Yangcheng County, Shanxi Province, China

Variable	Mean±S.D.[a]	Range	No. of Women
Age at Screening	36.2±13.2	15-59 [b]	941
Age of First Sexual Activity	19.8±1.75	15-26	782[c]
BMI Index	24.3±3.57	16.9-38.1	941
Number of Live Births	2.0±0.90	0-6	764[c]
Age at first Menstrual Period	15.05±2.09	10-23	940[d]

[a] S.D., standard deviation; [b]2 subjects were 15 years old; [c] Only 764 subjects had given birth previously; [d] A 16-year old subject had not yet had her first menstrual period and was excluded.

Table 2. The Age-stratified HPV Prevalence Rate of 745 Women in Yangcheng County, Shanxi Province, China

Age-group		Women	Pos	HPV (%)		X^2 (*P*)
				Prevalence	95% CI[b]	
20-24		52[a]	10	19.3	8.52-29.9	0.33 (0.56)[c]
	From 8 villages	40	7	17.0	5.72-29.3	
	From other villages	12	3	25.0	0.50-49.5	
25-34		194	15	7.73	3.97-11.5	3.70 (0.05)[d]
	25-29	96	11	11.6	5.09-17.8	
	30-34	98	4	4.08	0.16-8.00	
35-59		499	94	18.4	15.4-22.3	1.18 (0.88)[e]
	35-39	100	18	18.0	10.5-25.5	
	40-44	98	20	20.1	12.4-28.4	
	45-49	99	20	20.0	12.3-28.1	
	50-54	103	16	15.3	8.54-22.5	
	55-59	99	20	20.0	12.3-28.1	
Total		745	119	15.97	13.3-18.6	13.3 (0.001)[f]

[a] including one woman 19 years old; [b] 95% Confidence interval; [c] There was no significant between the HPV rates of women from 8 target villages and other villages; [d] There was no significant difference between the HPV infection rates 25-29 and 30-34; [e] There was no significant difference among the HPV infection rates 35-59; [f] There was significant difference among the HPV infection rates 20-24, 25-34 and 35-59.

Table 3. The Odds Ratios (ORs) of Lifestyle and Other Risk Factors for HPV Infection (n=745)

Variables		No. of Women	HPV Positive (%)	OR (95% CI[a])
Age group	20-24	52	10 (19.2)	2.77 (1.56-4.91)
	25-34	194	15 (7.73)	1.0(ref)
	35-59	499	94 (18.8)	2.84 (1.19-6.77)
The Age of First Sexual Activity	>=20	346	50 (14.5)	1.0(ref)
	<=19	399	69 (17.3)	1.24 (0.83-1.84)
Total number of sexual partners during	1	616	96 (15.6)	1.0(ref)
lifetime	2-3	111	18 (16.3)	1.05 (0.61-1.82)
	>=4	18	5 (27.8)	2.08 (0.73-5.98)
The Number of Sexual Partners During	1	668	103 (15.4)	1.0(ref)
Past 5 Years	>=2	77	16 (20.8)	1.41 (0.80-2.59)
Days between Baths	<7	222	27 (12.2)	1.0(ref)
	7-19	283	40 (14.1)	1.19 (0.70-2.01)
	20-180	157	32 (20.4)	1.85 (1.06-3.23)
	>=181	83	20 (24.1)	2.29 (1.20-4.37)
P-value for Trend: 0.003				
Monthly Communal Bath Attendance	>=2 times	228	28 (12.3)	1.0(ref)
	<=1 times	487	88 (18.1)	1.58 (1.00-2.49)
Days between Baths of Partner	<7	140	15 (10.7)	1.0(ref)
	>=7	604	104 (17.2)	1.73 (0.98-3.08)
Condom Usage	No	731	118 (16.1)	1.0(ref)
	Yes	14	1 (7.14)	0.40 (0.05-3.08)
Use Birth Control Pill	No	722	113 (15.7)	1.0(ref)
	Yes	23	6 (26.1)	1.9 (0.73-4.93)
Tuberculosis	No	728	116 (15.9)	1.0(ref)
	Yes	17	4 (17.7)	1.13 (0.32-4.00)
Age of first Menstruation	<16	388	57 (14.7)	1.0(ref)
	>=16	357	62 (17.4)	1.22 (0.82-1.81)
Menopause	Yes	199	38 (19.1)	1.0(ref)
	No	546	81 (14.8)	1.36 (0.89-2.07)
# of Live Births	1-2	550	89 (16.2)	1.0(ref)
	>=3	176	28 (15.9)	0.98 (0.62-1.56)

[a] 95% Confidence interval

the age group 16-24, in which there were less than the anticipated number available. Therefore, the women from other villages in the commune were recruited to reach the targeted number and a total of 71 women participated in the screening for this age group. In total, 941 women participated in the study.

Among the 941 participants, 159 (16.9 %) were not married and thus did not undergo the gynecology exam nor answer certain questions concerning sexual or bathing habits in the survey. Finally, 782 women completed the entire questionnaire regarding demographic characteristics, medical history, sexual and reproductive behavior, contraceptive practices and smoking history. Among them, 37 of these subjects chose not to continue on with the physical examination. The reasons included being pregnant (n=14) or menstruating (n=8) or unwilling (n=15). Therefore, a total of 745 women underwent the gynecology exam and specimen collection. Therefore the

Table 4. Adjusted Odds Ratio (OR) of HPV by Multiple Logistic Regression

Factors	No. of Women	No. of HPV Positive (%)	Adjusted OR [b] (95% CI [c])
Age-group [a]			
20-24	52	10 (19.2)	2.45 (1.01-5.97)
25-34	194	15 (7.73)	1.0(ref)
35-59	499	94 (18.8)	2.76 (1.54-4.92)
Number Sexual Partner during the Past 5 years			
1	668	103(15.4)	1.0(ref)
>=2	77	16(20.8)	1.41 (0.77-2.57)
Days between Baths			
<7	222	27 (12.2)	1.0(ref)
7-19	283	40 (14.1)	1.09 (0.64-1.86)
20-180	157	32 (20.4)	1.60 (0.91-2.83)
>=180	83	20 (24.1)	2.25 (1.16-4.40)

[a]fitted model as categorical variable according to their similar risk levels of HPV; [b] Adjusted for the following variables: women's bathing frequency, men's bathing frequency, frequency of communal bathing, number of partners, duration time of sexual relationships, and age at first intercourse; [c] 95% Confidence interval.

compliance rate for gynecological exam among women who had participated in the screening was 97%.

Basic demographic information

Among the 941 surveyed women, 16.9% were unmarried and 93.0% had been married once including 80.3% who were currently married and who were 2.8% widowed. Twenty four (2.6%) women were illiterate and a majority of women had 6-8 or less years of education. A majority of women were farmers. Woman had delivered 2 live births on average, with 1% nulliparous, 23% primiparous and the majority multiparous. Among 941 women, none reported smoking cigarettes currently and 652 women (69.3%) had the secondary smoke exposure. None had a history of cervical cancer or CIN. Their mean age at menarche was 15 years old; the mean Body Mass Index (BMI) was 24.30; the mean age of first sexual activity was 20 years old (Table 1). Sixty nine women (8.8%) had the history of Pap smear and seventeen (1.2%) reported tuberculosis.

Among 782 women with sexual activity, 132 (16.9%) had 2 or more sexual partners and on average the 782 women had 1.3 sex partners. The most common types of contraceptives were intrauterine device (IUD) (51.9%) and tubal litigation (54.8%), 22.1% couples used a combination of these; very few couples used the condom (2.1%) or oral/injective contraceptive (3.1%). About 96% of women went to communal baths in addition to bathing at home. The median number of days between baths was 10 days (excluding the women who were irregular or hardly ever bathed), indicating most women bathed once every 1.5 weeks.

The HPV infection rate

The prevalence rate of 13 high-risk HPV types in 745 eligible women aged 20-59 years old was 15.97%, and the age-stratified HPV prevalence rates were shown in Table 2. There were significant differences among each age group. The women aged 20-24 could be classified into 2 parts according to the recruit ment method and the region: one part of came from the 8 selected target villages, another part were from the other villages, but there was no significant differences between the 2 groups (X^2=0.33, P=0.56). Therefore the two groups of women were combined into one 20-24 age group. HPV infection rates in 20-24, 25-34 and 35-59 age groups were 19.23%, 7.73% and 18.84% respectively, and the HPV prevalence rate among women aged 25-34 was significantly lower than the rate of HPV infection in 20-24 and 35-59 age-groups (X^2=13.3, P=0.0013), and there were no differences between any other age-groups (Table 2).

Risk factors for HPV infection

The risk factors for HPV were identified by unconditional logistic regression model(Table 3). Because the relationship between HPV prevalence rate and age group was not linear, the variable age group (20-24, 25-34 and 35-59) was introduced into the model as a categorical variable. The OR of the 20-24 age group vs. 25-34 age group was 2.77 (95% CI: 1.56-4.91) and the OR of 35-59 age group vs. 25-34 was 2.84 (95% CI:1.19-6.77).

The bathing frequency had a strong negative correlation with HPV infection. The trend test yielded a P value of 0.003, which was statistically significant. Compared to those who bathe at least weekly with an OR of 1.0, those who bathe less than once every half year had a higher OR of 2.29 (95% CI: 1.20 - 4.37).

Compared to those who go to communal bathed at least twice a month with an OR of 1.0, those who went to communal baths less than once a month had a higher OR of 1.58 (95% CI: 1.00 - 2.50). Even after adjusting for the total number of sexual partners in the past 5 years, monthly communal bathing attendance still had a strong negative correlation with HPV infection.

The OR of first sexual intercourse at age 19 or under (vs. equal or more than 20) was 1.24 (95% CI: 0.83 - 1.84), the OR of 2-3 lifetime partners and 4 or more (vs. only one partner in lifetime) was 1.05 and 2.08 respectively.

The OR of birth control pill usage (vs. never use) was 1.9, (95% CI: 0.73 - 4.93), condom usage (vs. no use) was 0.4 (95% CI: 0.05 - 3.08), menopause (vs. no menopause) was 1.36 (95% CI: 0.89 - 2.07) and tuberculosis (vs. never) was 1.13 (95% CI: 0.32 - 4.00), however, their p-value rendered them statistically insignificant.

Based on the results of univariate logistic regression analysis, all variables that were possibly associated with HPV infection were entered in a multiple variables logistic regression model. We calculated the adjusted OR in the multivariable LG model by fitted age group as a categorical variable in three groups after adjusting for the confounding factors: the women's bathing frequency, men's bathing frequency, frequency of communal bathing, number of partners, duration time of sexual relationships, and age at first intercourse (Table 4). The adjusted OR of HPV prevalence rate of women aged 20-24 years old and 35-39 years old (vs. of 25-34 age group) was 2.45 (95% CI: 1.01-5.97) and 2.76 (95% CI: 1.54-4.92) respectively. The OR of the HPV rate among women who bathe once a week (vs. once half year) was 2.25 (95% CI: 1.16 - 4.40).

Discussion

The prevalence rate of the 13 carcinogenic HPV types in 20-59 years old in this study was 15.97% and the rate among child-bearing women ages 25-34 years was lower than the other age groups in the 20-59 age range. This survey also showed that personal hygiene is significantly associated with the HPV prevalence, regardless of age. The HPV prevalence rates in different age groups as well as the risk factors of HPV will assist in the determination of the optimal time-point for the HPV vaccination in rural China.

The HPV prevalence rate in 20-59 years old in this study was 15.97% which was lower than rates (23.6%) reported previously in other population-based studies including another study in Shanxi Province (Belinson et al., 2003; Shen et al., 2003; Zhao et al., 2006). Both studies reported high-risk HPV infection using the Hybrid Capture II by the same technicians in the same laboratory, and the difference did not come from the HPV detection method. A possible reason was that the age groups of the target populations in the 2 surveys were different. The target population focused on the group of ages 35-50 in the previous survey (Shen et al., 2003); in this study, a larger age group of women of ages 16-59 were investigated, resulting in the biggest age range studied of all population-based surveys of HPV prevalence in China. Women aged 25-34 years old with the lowest HPV prevalence rate (7.73%) accounted for 26% of target population in this study which reduced the average HPV infection rate. The HPV infection rate in subjects aged 35-59 years was 18.84%, keeping with the rate (19.31%) in women aged 35-50-years old in the previous study (Shen et al., 2003; Zhao et al., 2006). The women in 20-24-years old age groups with higher (19.23%) HPV prevalence rate that had no significant difference with the rate of 35-59-years age group only constitutes for about 7 percent of the total population, which could not balance the reduction induced by the lower rate of 25-34-years age group. The second explanation was that the lower HPV infection was in accord with the lower cervical neoplasia prevalence in this survey.

Many studies have shown that HPV decreases steadily with age (Burk et al., 1996; Franceschi et al., 2003; Sellors et al., 2003; Ferreccio et al., 2004; Peto et al., 2004). However, our data showed the initial HPV rate was high in the 20-24-years old age group, and then dropped to the lowest point at age 30-34 years, then raised and kept plateau from 35-49 years. There was a slight dip at ages 50-54 years, followed by a slight rise from ages 55-59 years. In China, a majority of women began their sexual activity at 20-24 years old which could explain the highest rate of HPV, and after infection, likely HPV became a latent infection (25-34-years age group). Then the latent HPV could be reactivated in some women when the immune system weakened. Another possibility is that women aged 35-59 years old had increasing marital problems leading to sexual behavior changes and therefore new HPV infections.

Many studies showed that lack of personal hygiene correlates with a higher rate of cervical cancer (Zhang et al., 1989; Kamaluddin, 1999; Hammouda et al., 2004), but not many studies showed the relationship between personal hygiene and HPV infection (Kataja et al., 1993 ; Franceschi et al., 2003). The finding showed that personal hygiene was associated with HPV infection. A possible reason was that bathing was a marker of socio-economic status in the surveyed area where the economic was undeveloped and poor and only 2% could bathing every day and 40% women could bathe once a week. The poor personal hygiene was in accord with the poor nutrition status among women. Bathing infrequently results in increasing viral or bacterial reproduction, which can be curbed by cleaning the lower genital tract on a frequent basis. Previous studies have shown there is also a high prevalence of genital trichomoniasis among women in this area (Zhao et al., 2006). In this rural area of China, there was lack of water. In general, the frequency of bathing in summer was more than the other seasons, especially in winter the bathing infrequency was common. This survey was carried out the spring (neither too hot nor too cold) and questions were based on the average times of bathing once year. The OR values (VS. bathing at least once a week) increased as the length of time between baths increased in a dose response relationship.

A majority of women aged 25-34 years in this rural area were either pregnant or taking care of their children reduce their sexual activity. On the other hand the women aged 25-34years old more frequently bathed and had better personal hygiene which could have contributed to a lower prevalence of HPV infection. As women grew older their frequency of bathing also decreased correspondingly , and the HPV rate increased again. More studies with more participants are needed to confirm the finding of the negative correlation between personal hygiene and HPV infection.

The fact that carcinogenic HPV is transmitted via sexual contact is now well-established. Sexual risk factors such as: more sex partners, early age of first sexual intercourse et al were shown as risk factors of HPV prevalence in some studies (Munoz et al., 1996; Sellors et al., 2002; Jeng et al., 2005; Zhao et al., 2006). Another study found that oral contraceptive is a risk factor because of the increase of HPV infection through more skin contact (Sellors et al., 2003). Condom usage is a possible protective factor because of the barrier function and and decreasing chances of contact with infected skin. However in this manuscript none of those risk factors are associated with HPV infection. The survey area was located in a rural region of mountainous geography with poor travel conditions. The population fluidity was relatively stable, and their marriage characteristics were similar in the area. Firstly, the average number of sexual partner was less and a majority of women had only one sex partner (their husband). The percent of divorce was less and only a few women had married twice or more. Only 17.3% women had two or more sexual partner in their lifetime and only 10.5% women had two or more sexual partner in past five years the this surveyed women in this rural area of China. It is a common and true phenomenon in rural China where a majority of women were conservative and traditional and they thought sexual behavior outside

marriage or having multiple sexual partners was highly stigmatized. In this surveyed population, only small part women had the high risk sexual behavior of transmitting HPV could explain the results. Another reason may be the small samples in the survey.

In summary, the conclusions that can be drawn from this cross-sectional design study were that the prevalence of HPV infection rate in 20-59 years old in this study was 15.97%. HPV infection in the 25-34 age-group was statistically lower than the rate in 20-24 and 35-59 age groups This survey also showed that personal hygiene is significantly associated with the HPV prevalence, regardless of age in Yangcheng County, Shanxi Province. The HPV prevalence rates in different age groups as well as the risk factors assisted the determination of the optimal time point for the HPV vaccination in rural China.

Acknowledgments

We thank Dr Silvia Franceschi from International Agency for Research on Cancer for her advisory for the paper; Dr. Gwendolyn Zahner for the modification of the manuscript; the Gynecologists from the Shanxi Province Cancer Hospital for the gynecological checkup; the pathologists and cytologists from the Cancer Hospital, Chinese Academy of Medical Sciences in Beijing for processing and reviewing the slides and the local health workers from Yangcheng County in Shanxi Province for inviting the women to participate in the project and assisting in the successful completion of the project.

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