Original Article

A Serological Epidemiological Survey of Antibodies against 4 HPV Subtypes in Uygur Women in Xinjiang

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SUMMARY: This study evaluated the distribution of antibodies against 4 human papillomavirus (HPV) subtypes and their related factors among Uygur women in Xinjiang. A cross-sectional study was conducted from March 2006 to May 2007 involving 883 Uygur women aged 17–54 years living in Yutian County. Demographic indicators, disease history, sexual behavior history, and other parameters were recorded at the interview using a questionnaire. A fluorescence detection method was used to quantify anti-HPV6, -11, -16, and -18 antibodies in venous blood serum. The rate of positive detection of any anti-HPV antibody (anti-HPV6, -11, -16, and -18) in the study population was 13.4%, and the individual positivity rates were 9.5%, 2.6%, 4.3%, and 0.7%, respectively. Peak rates of positivity for the anti-HPV16 antibody were found in women who were 36–40 and 46–50 years old. Seroprevalence of HPV16, which is high-risk for cervical cancer, was associated with the numbers of sexual partners. The rate of infection with high-risk HPV was low among Uygur women from rural areas, although there is a high incidence of cervical cancer in this group. Loyalty to one sexual partner decreased the risk of high-risk HPV infection. This study may provide useful reference data for the prevention and treatment of HPV and cervical cancer and for the application of HPV vaccines.

INTRODUCTION

Human papillomavirus (HPV) infection is strongly associated with cervical cancer, and one study has demonstrated that HPV DNA can be detected in over 90% of cervical cancer patients. Cervical cancer is the 2nd most common cancer among women worldwide, and the leading cause of cancer-related mortality among women in developing countries. Indeed, it is estimated that 80% of cervical cancer patients reside in developing countries (1). China's population of Xinjiang Uygur women also has a high incidence of cervical cancer. It has been reported to range from 459/100,000 to 527/100,000, which is significantly higher than the national incidence (14.6/100,000) (2–4). Overall, cervical cancer is the second leading cause of cancer-related mortality among Chinese women and the leading cause among women in Xinjiang.

There are more than 100 subtypes of HPV, which are divided into high-risk and low-risk based on their propensity to cause cancer (5). Most cervical HPV infections are asymptomatic, latent infections, and are difficult to detect. HPV infection is self-cleaned in more than 90% of cases (6); therefore, only a small proportion of infected individuals develop cervical damage and subsequent cancer. As previous study has demonstrated that HPV DNA cannot be detected in 70% of women after 12 months of HPV infection (7). Thus, the infection rates obtained in previous studies using HPV DNA

detection simply reflect the presence of HPV DNA at a single time point (8–11). Such rates can be spuriously low and do not accurately reflect previous infection and later clearance in the population, or lifetime exposure of the population to HPV. Furthermore, differences in quality of female genital specimens and the timing of the acquisition lead to variable results of the HPV DNA screening among patients (12). Therefore, there exist a number of important limitations to determining population-based HPV infection rates by detection of HPV DNA. Conversely, detection of antibodies against HPV more accurately reflects past exposure and can be used to determine whether population vaccination is necessary (13).

Yutian County is an autonomous Uygur region of Xinjiang with a high incidence of cervical cancer. In this study, we conducted a cross-sectional survey of rural Uygur women living in Yutian County. We detected serum antibodies against HPV and analyzed factors such as previous HPV infection in the study population, in order to provide a reference for prevention and control of HPV infection and cervical cancer.

MATERIALS AND METHODS

Subjects: This study comprised the Xinjiang arm of a multi-center (Beijing, Shanghai, Shanxi, Henan, and Xinjiang) study of HPV infection factors. From March 2006 to May 2007, we used a multi-stage sampling method to recruit 983 Uygur women from rural areas of Yutian County in Xinjiang for a cross-sectional survey. The subjects were divided into 7 age groups: 17–25, 26–30, 31–35, 36–40, 41–45, 46–50, and 51–54 years. The rates of positivity for anti-HPV antibodies were assessed in the different age groups. Inclusion criteria were: women aged 17–54 years; Uygur nationality; a

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rural household registration; and a history of sexual intercourse. Exclusion criteria were pregnancy/pregnancy termination less than 3 months prior to the study; a previous history of cervical cancer or unknown uterine tumor, history of hysterectomy; history of cervical colonization or radiotherapy/chemotherapy; cervical dysplasia; no history of sexual intercourse, and the presence of mental illness or other severe diseases.

Methods: Questionnaire: Trained investigators conducted individual face-to-face interviews with each study subject. Interview questions covered general demographic information, sexual status, and disease history.

Specimen collection: After completion of the questionnaire, medical staff collected 9 ml of venous blood from each subject into a vacutainer. Sample was immediately centrifuged, and 2 ml of serum was collected into each of 2 tubes and frozen in a -40°C freezer. The serum was shipped to our facility on dry ice; one tube of serum was stored in the Cancer Hospital, and the other was sent to Merck Research Laboratories (MRL; Boston, MA, USA) for HPV antibody detection.

Serum HPV antibody detection: Anti-HPV6, -11, -16, and -18 antibodies were detected using liquid chip technology, also known as flow fluorescence technology, as recommended in the literature (14). The detection platform was the Luminex200 flow fluorescence system (Luminex, Austin, TX, USA). The principle of this technology is that yeast-derived virus-like particles (VLP) bound to fluorescent microspheres to form VLP-antigens, to which known subtype-specific phycoerythrin (PE) labeled monoclonal antibodies, and study serum antibodies are competitively bound. The fluorescent signal of the antigen-bound monoclonal antibodies is inversely proportional to the serum antibody titers; thus, serum antibody titers can be obtained by measuring the fluorescent signal. In this study, 4 genotypes could be detected using 1 pore because the assay used yeast-derived VLPs coupled to sets of 4 distinct fluorescent Luminex microspheres. HPV6, -11, -16, and -18 VLPs were combined with Luminex fluorescent microspheres (mediated by N-hydroxysulfosuccinimide enhanced carbodiimide) to form probe molecules. A total of 12 standard control sera from immunocompromised *Cercopithecus aethiops*, 4 control sera, and 16 serum samples were double-sample tested. The 4 control sera included human sera samples containing low, medium, and high HPV antibody titers plus an HPV antibody negative serum sample. The negative sera were derived from HPV antibody negative human serum. The low HPV antibody sample was derived from human serum after natural infection, whereas the sera with medium and high HPV antibody titers were derived from human serum after vaccination. Sera samples were diluted 4-fold, then added to the 96-well plates along with the antibodies to be tested and the fluorescent microspheres incorporating HPV6, -11, -16, and -18 VLPs. The plates were sealed with antimony paper and incubated for 15–25 h. After washing 3 times, 96-well plates were examined by the Luminex200 flow fluorescence system, and the values were quantified using a standard curve. If necessary, the values were corrected according to the dilution factor, and the results were reported in milli Merck units/ml (mMU/ml). The lowest

HPV antibody titer of the PCR-negative/possible negative samples was designated the cut-off value. The cut-off values for the anti-HPV6, -11, -16, and -18 antibody titers were 20, 16, 20, and 24 mMU/ml, respectively. Values higher than the cut-off indicated positive samples, and values lower than the cut-off indicated negative samples. By determining the critical values of HPV antibody titers (8–48 mMU/ml, 4 mMU/ml increments for each time) (14,15), it was possible to determine whether the sample was positive for HPV antibodies.

Prior to the survey, all participants were required to give an informed consent including the purpose of the study, and the interests and potential risks associated with participation. This study was approved by ethics committees at the relevant institutions of the Chinese Academy of Medical Sciences Cancer Institute and the Cleveland Medical Center.

Statistical analysis: FoxPro software (Microsoft, Redmond, WA, USA) was used to establish a database. Original data were entered by 2 data-entry staff, and the entries were proofread for possible errors. SPSS 19.0 software (Chicago, IL, USA) was used to perform statistical analysis of the data. The chi-square test (χ^2) was used for comparison of rates between groups. Univariate and multivariate logistic regressions were also performed. *P* values < 0.05 were considered statistically significant.

RESULTS

General information: A total of 983 women gave informed consent to participate in the survey, and a final group of 883 women met all inclusion criteria and completed the entire investigation. The women in the study group were an average of 37.5 ± 3.3 years old. Overall, 13.4% of participants tested positive for any anti-HPV antibody. Positivity rates for anti-HPV6, -11, -16, and -18 antibodies were 9.5%, 2.6%, 4.3%, and 0.7%, respectively.

Positivity rates for antibodies against HPV in different age groups: Study subjects were divided into age groups: 17–25, 26–30, 31–35, 36–40, 41–45, 46–50, and 51–54 years. In each age group, anti-HPV6 antibodies were always the most prevalent, followed by anti-HPV16 antibodies. Anti-HPV11 and -18 antibodies were detected at relatively low levels. Positivity rates for antibodies against HPV 6 and -16 fluctuated among the different age groups but were always the highest among women in the 36–40 years age group; these rates declined in the 41–45 years age group but rose again in the 46–50 years age group. In particular, the level of anti-HPV16 antibodies increased significantly in the 46–50 years age group and exhibited a “bimodal” pattern. The observed fluctuations in the levels of HPV11 and -18 antibodies between age groups were relatively insignificant. In general, the positivity rates for anti-HPV6, -16, and -11 antibodies increased gradually with age but gradually decreased after 50 years of age. Conversely, anti-HPV18 antibodies were present at relatively high levels in the lower age groups but gradually decreased with age; however, the levels slightly increased after 36–40 years (Fig. 1).

Univariate analysis: Univariate analysis revealed that the positivity rate for HPV6/11 antibodies among wo-

Fig. 1. (Color online) Positivity rates for anti-HPV6, HPV11, HPV16, and HPV18 antibodies in different age groups.

Table 1. Comparison of positivity rates for HPV6/11 antibodies and HPV16/18 antibodies among groups

Factor	Classification	No. of subjects	HPV6/11 antibody				HPV16/18 antibody			
			No. of positive subjects	Positive rate (%)	χ^2	<i>P</i> value	No. of positive subjects	Positive rate (%)	χ^2	<i>P</i> value
Age (yr)	17-25	147	8	5.44	6.435	0.092	3	2.04	6.052	0.109
	26-35	251	27	10.76			9	3.59		
	36-45	251	34	13.55			15	5.98		
	46-54	234	27	11.54			16	6.84		
Education level (yr)	<6	549	53	9.65	1.126	0.289	21	3.83	2.411	0.121
	≥6	327	39	11.93			20	6.12		
No. of sexual partners	1	529	46	8.70	6.984	0.008	12	2.27	18.263	<0.001
	≥2	324	47	14.51			28	8.64		
Age of 1st sexual intercourse	≥20	69	12	17.39	3.336	0.068	3	4.35	0.000	1.000
	<20	808	83	10.27			40	4.95		
External genital infections	No	547	56	10.24	1.308	0.253	24	4.39	0.162	0.687
	Yes	238	31	13.03			12	5.04		
External genital ulcers	No	573	61	10.65	0.469	0.494	24	4.19	1.542	0.214
	Yes	117	15	12.82			8	6.84		

men with 2 or more sexual partners was significantly higher than the rate among women with only 1 sexual partner ($\chi^2 = 6.984$; $P = 0.008$). This pattern was also observed for HPV16/18 antibodies ($\chi^2 = 18.263$; $P < 0.001$). Stratification of data according to other factors, including age, education level, age of 1st sexual intercourse, presence or absence of external genital infections, and presence or absence of external genital ulcers, showed no significant differences (all $P \geq 0.05$) (Table 1).

Stepwise multivariate analysis: According to the inclusion and exclusion criteria of $P = 0.2$, HPV6/11 and

HPV16/18 antibodies (i.e., positive or negative) were used as dependent variables, and the other variables were used as independent variables to establish two logistic regression equations. Results from univariate analysis were gradually fitted into the 2 logistic regression models. In the equation where HPV6/11 antibodies (i.e., positive or negative) were the dependent variable, none of the factors could be entered into the equation. In the same equation where HPV16/18 antibodies were the dependent variable, the number of sexual partners could be entered into the equation (OR = 3.17; 95% Confidence Interval [CI], 1.35–7.47); however, the

Table 2. Multivariate analysis of antibodies and risk factors

Factor	Classification	No. of subjects	HPV6/11 antibody			HPV16/18 antibody		
			No. of positive subjects	Positive rate (%)	OR (95% CI)	No. of positive subjects	Positive rate (%)	OR (95% CI)
Age (yr)	17–25	147	8	5.44	1.00	3	2.04	1.00
	26–35	251	27	10.76	1.59 (0.64–3.96)	9	3.59	3.44 (0.41–28.83)
	36–45	251	34	13.55	2.18 (0.87–5.44)	15	5.98	3.98 (0.48–33.23)
	46–54	234	27	11.54	2.01 (0.77–5.20)	16	6.84	4.95 (0.59–41.31)
Education level (yr)	<6	549	53	9.65	1.00	21	3.83	1.00
	≥6	327	39	11.93	1.38 (0.81–2.34)	20	6.12	1.42 (0.65–3.10)
No. of sexual partners	1	529	46	8.70	1.00	12	2.27	1.00
	≥2	324	47	14.51	1.47 (0.85–2.53)	28	8.64	3.17 (1.35–7.47)
Age of 1st sexual intercourse	≥20	69	12	17.39	1.00	3	4.35	1.00
	<20	808	83	10.27	0.49 (0.22–1.07)	40	4.95	0.98 (0.21–4.52)
External genital infections	No	547	56	10.24	1.00	24	4.39	1.00
	Yes	238	31	13.03	1.74 (0.61–4.96)	12	5.04	0.43 (0.06–3.31)
External genital ulcers	No	573	61	10.65	1.00	24	4.19	1.00
	Yes	117	15	12.82	0.75 (0.24–2.36)	8	6.84	3.79 (0.49–29.25)

other factors, including age, education level, age of first sexual intercourse, external genital infections, and external genital ulcers, could not be entered into the equation (Table 2).

DISCUSSION

Accurate detection of HPV infection is important for the prevention of cervical cancer. Various methods are currently used to detect HPV, including HPV DNA testing, and cytological screening etc. However, detection of HPV DNA reveals the current infection status but cannot demonstrate a history of exposure to HPV, because after HPV infection, more than 90% of the population can clear the virus from the body (16). Only a small proportion of infected individuals experience chronic infection and subsequently develop cervical lesions and cancer. Following infection, anti-HPV antibodies generated by the humoral immune response can be found in the serum within a certain period of time. These antibodies may be present for a long time, even the entire lifetime; therefore, detection of serum HPV antibodies is preferable to HPV DNA for evaluating history of HPV exposure (13). In this study, the positivity rates of serum HPV6, -11, -16, and -18 specific antibodies from the Uygur women in the studied region were 9.5%, 2.6%, 4.3%, and 0.7%, respectively. According to the literature, infection rates of HPV6, -11, -16, and -18 in Uygur women were 1.0%, 0.25%, 9.0%, and 0.25%, respectively, when detected by HPV DNA testing (17). This discrepancy can be partially explained by differences in the detection methods used. Furthermore, not all HPV infections result in the detectable presence of serum antibodies (18). One study showed that HPV antibodies can only be detected in approximately 60% of HPV16-infected women (19). Presumably, with recent infections, there may have been insufficient time for the immune system to produce antibodies against HPV. Moreover, studies have shown that low

HPV DNA-carrying women are less likely to produce serum HPV antibodies than high HPV DNA-carrying women (20). Thus, the data obtained via serum antibody detection in this study may underestimate the actual cumulative exposure to the 4 detected HPV subtypes. Compared with HPV DNA testing, HPV antibody detection is applied less frequently. Indeed, this study is the first study to evaluate cumulative HPV infection among Uygur women using anti-HPV antibody detection. This approach will provide reference data for the future application of HPV vaccines.

There are over 100 subtypes of HPV, which can be divided into high- and low-risk groups based on the risk of cancer development at the infection site (5). In this study, we analysed HPV infection among Uygur women in Xinjiang by detecting antibodies against HPV6 and -11 (i.e., low-risk HPV) and antibodies against HPV16 and -18 (i.e., high-risk HPV). Positivity rates for antibodies against each of the HPV subtypes reflect the cumulative interaction of past infections and changes in antibody titers. The positivity rates for high-risk HPV16 and HPV18 antibodies were 4.3% and 0.7%, respectively. These rates are not only lower than the rates reported for Han women by Fei et al. (23.2% and 6.5%) (21), but they are also lower than overall rates among women in China, reported by Wang et al. (6.9% and 1.6%) (22), Ji et al. (5.6% and 1.9%) (23), and Smith et al. (6.3% and 2.1%) (24). The positivity rates for low-risk HPV6 and HPV11 antibodies were 9.5% and 2.6%, respectively, both higher than the results reported by Wang et al. (22) and Fang et al. (25). From these results, it appears that Uygur women are more susceptible to low-risk HPV6 and -11 but less susceptible to high-risk HPV16 and -18 when compared with Han women. Sexual intercourse is the factor that is most commonly associated with high-risk HPV infection, and previous studies have demonstrated that HPV infection is associated with a variety of sex-related factors, such as the number of sexual partners, the age of first sexual intercourse,

and the use of condoms. Uygur people follow Islam and hold conservative values; less premarital sex occurs among the women, who are more likely to be loyal to one sexual partner, and some men undergo circumcision. All of these factors prevent spread of high-risk HPV to some extent. In this study, infection with high-risk HPV was found to be correlated with the number of sexual partners. However, no obvious correlations were seen between infection with the 2 low-risk subtypes of HPV and factors relating to sexual behaviour.

HPV16 and -18 are 2 common high-risk subtypes in Asia and China. The positivity rate for anti-HPV16 antibodies in this study was high, but the positivity rates for anti-HPV18 antibodies were low in all age groups. This is indicative of the lower infection rate of HPV18 reported previously in this region (17,26). In addition, the detection result was more or less affected by the antibody titers (20). Our study also reveals that the positivity rate for anti-HPV16 antibodies exhibits an obvious “bimodal” pattern, with 2 peaks in the age groups of 36–40 and 46–50 years. Previous studies performed by Zhao et al. (27) and Xue et al. (28) also reported a “bimodal” pattern of HPV16 infection, although the pattern occurred in younger age groups than the peaks found in this study. The 2 main reasons for this difference might be the delay between antibody production and antigen exposure and the conservative sexual behavior of Uygur women which delays HPV infection. A literature review by de Sanjose et al. found that, in some regions of Africa, Europe, and America, the second infection peak occurs in women older than 45 years (29). High HPV infection rates among elderly women may occur as a result of persistent infection or the recurrence of latent HPV infection. However, the relationships between age and persistent infection with HPV, and the appearance and duration of antibodies are unknown. Possible reasons for these observations are: i) the presence of high-risk sexual behavior factors in elderly women; ii) the recurrence of latent infection because of the sharp change in estrogen levels after menopause, and the immunological changes that occur in elderly women; and iii) a variety of factors, such as ethnic behaviors, habits, and the social environment, which vary among different regions of China. The relationship between HPV infection and age also suggests that appropriate age-based measures should be taken to prevent HPV infection.

There are some limitations to this study. First, the method of sample selection limits the representativeness of the sample which should be considered when extrapolating study results. Second, because the production of anti-HPV antibodies lags behind exposure to HPV antigens, individuals who have experienced recent HPV infection may not be detected by measuring positivity for HPV antibodies. This study aimed to provide a reference for conducting pre-clinical trials of the quadrivalent vaccine for HPV6, -11, -16, and -18, and to identify the baseline prevalence of the 4 anti-HPV antibodies in the population. However, only antibodies against HPV6, -11, -16, and -18 were considered in this study. Further studies on the distribution of all HPV subtypes in women of this region are required.

We conclude the following from the results of this study: the rate of infection with high-risk HPV is low

among Uygur women in rural areas, where the incidence of cervical cancer is high. Loyalty to one sexual partner is able to decrease the incidence of high-risk HPV infection. This study provides useful data for the prevention and treatment of HPV infection and cervical cancer and promote the application of HPV vaccines in the region. Comparative and cohort studies conducted in different areas are required to further explore HPV infection and the incidence of cervical cancer.

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Conflict of interest None to declare.

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