

RESEARCH ARTICLE

Genotype Distribution and Behavioral Risk Factor Analysis of Human Papillomavirus Infection in Uyghur Women

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Abstract

We investigated the distribution of HPV genotypes in Uyghur women in Xinjiang region of China, and behavioral factors which could predispose them to HPV infection. In this cross-sectional study, women aged 15-59 years were recruited by cluster sampling method in Yutian region in 2009. Liquid-based cytology samples were analyzed centrally for HPV genotype with a linear array detector. Univariate and multivariate logistic regression analyses were performed to identify behavioral risk factors for HPV infection. A total of 883 Uyghur women were recruited successfully. The prevalence of high-risk HPV and low-risk HPV were 7.25% and 1.58%, respectively; the most common HPVs were HPV16, 51, 31, 39 and 58. We found that age of first sexual intercourse was a strong predictor for HPV infection (odds ratio of 4.01 for ≤ 15 years versus ≥ 25). Having sexual partners ≥ 3 was the second predictor (OR 3.69, 95% CI 2.24-7.16). Cleaning the vagina after sex showed an increased risk of HPV infection (OR 2.72; 95% CI 1.98-5.13); Using the condom showed protective factors for HPV infection (OR 0.36; 95% CI 0.12-0.53). HPV16, 51, 31, 39 and 58 were the priority types; the age of first sexual intercourse was identified as a major risk factor for HPV infection. Other notable risks were number of sexual partners and cleaning the vagina after sex. Changing these behavioral risk factors could help to reduce the occurrence of cervical cancer in this population.

Keywords: Human papillomavirus - genotypes distribution - risk factors - Uyghur women

Asian Pac J Cancer Prev, 14 (10), 5861-5865

Introduction

Cervical cancer harm to women's health seriously, It's become the second most common cause of cancer-related deaths in the world (Sharma et al., 2013). In China, there are nearly 80,000 new cases were reported in 2008, which accounting for 14.2% of the world's new cervical cancer cases (Arbyn et al., 2011). The morbidity rate and mortality rate of cervical cancer in Uyghur women are significantly higher than the other ethnic group in Xinjiang- an multi ethnic gathering area, this two data were 459/100000-590/100000 and 15.78/100000, respectively (Guzhalinuer et al., 2007). The infection of High-risk human papillomavirus is now recognized as the main pathogenic factors of cervical cancer (Murphy et al., 2012; Rana et al., 2013). Infection with certain genotypes of HPV has been linked with cervical cancer development. In china vaccines were developed to combat HPV infection, consequently, the most prevalence HPV types should be determined in each region (Arbyn et al., 2010; Faust et al., 2013). The Uyghur women who live in desert regions have the unique lifestyles and religion has the highest prevalence of cervical cancer in China. Thus made the screen program become urgent, an accurate

assessment of the regional distribution of HPV genotypes are extremely important for both prevention of cervical cancer and for public hygiene management (Guan et al., 2012; Nam et al., 2013).

As a part of The Chinese women's urogenital HPV infection and cervical neoplasia type lesions epidemiological study, which were supported by the Cancer Foundation of China and Medical Center of Cleveland. We collected data from Uyghur women who live in Yutian region- a typical desert region in Xinjiang by cluster sampling method. The objective of the present analysis was to determine the distribution of HPV genotypes in Uyghur women, and investigate and behavioral risk factors that might predispose women to HPV infection.

Materials and Methods

Subject

The study was a population-based, observational, cross-sectional study conducted between March 2006 and February 2009, based on the voluntarily participating Uyghur women in Yutian County, Xingjian, China. The study has been approved and registered by Ethics

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Table 1. Demographic Data of Study Sample

Variables	N	%
Age		
≤24	147	16.65
25-34	251	28.43
35-44	251	28.43
44-59	234	26.5
Annual income (¥)		
≤1000	794	90.64
1000-5000	77	8.79
≥5000	5	0.57
Age of menarche		
≤15	572	65.15
>15	306	34.85
Number of marriages		
1	590	66.97
2	205	23.27
≥3	86	9.76
Age of First marriage		
≤15	845	95.91
15-25	32	3.63
≥25	4	0.45
The number of first live births		
0	25	3.11
1	154	19.15
≥2	625	77.74
The age of initial childbirth		
≤20	649	80.12
20-30	159	19.63
≥30	2	0.25
The age of first sex		
≤20	844	96.24
≥20	33	3.76
The total number of sexual partners		
1	529	62.02
2	173	20.28
≥3	151	17.7
Cleaning the vulva and vagina before Sex		
N	646	74.51
Y	221	25.49
Cleaning the vulva and vagina after sex		
N	31	3.56
Y	840	96.44

Committee of China Cancer Foundation in January 2006, the Ethics committee approved relating screening, and data collection of these subjects, all subjects signed written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki.

The participants were included using the following selection criteria: sexual life, aged between 16 to 59 years, using cluster sampling method, they were sub grouped by every 5 years, the average of number of each group was about 125. The virgins, pregnant women and women with the history of cervical cancer, cervical lesions, and abortion within 3 months, women who had double uterus, congenital absence uterus, had undergone a cervical conization, hysterectomy or chemotherapy history; women who had mental illness or other serious systemic diseases were excluded.

Research Process

The participants signed informed consent after fully informed. The screening program and epidemiological survey about the risk factors of demographic, socio-economics, behavioral habits and other aspects were collected by a trained Uyghur investigator in closed

environments. Visual screening and cervical HPV sampling were collected by experienced gynecological clinical physician. Survey results with missing items, omissions, logic errors, and unreasonable were excluded for analysis; the participants with the positive results of cytology, HPV testing and positive for clinical symptom screening on gross were recalled to do the colposcopy and cervical biopsy. The findings of cytology, the gross screening, and colposcopy were followed by the general guidelines have been reported.

HPV detection and typing methods

After included, the participants were asking avoiding intercourse, vaginal drugs using and flushing vagina for 24 hours, then sampled by a specially trained gynecologist with a special sampling brush at the cervical rotation with 3-5 laps, the collected cervical exfoliated cells were analyzed by centralized laboratory for HPV genotyping. Genotyping was performed using the linear array detector. We used the polymerase chain reaction (PCR) and nucleic acid hybridization detected 37 kinds of HPV DNA type: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MMg); 81, 82 (MM4); 83 (MM7), 84 (MMS), 1539 and CP6108. The cervical cells were denaturalized and dissolved by high temperature to release HPV DNA, and then determined the nucleic acid sequence of HPV L1 genomic regions by the primer, which could amplify 37 kinds HPV genotypes Including 13 high-risk types (16, 15, 31, 33, 35, 39, 45, 51, 52, 56, 55, 59 and 65); Amplification product of biotin-label matched probe and giant oligonucleotide probe were hybridized, then flushed out the unbinding portions, and binding portions by peroxidase enzymes. The results are read though comparing the blue stripe at the location of the probe with the standard card.

Data process and statistical analysis

Double-pass entry of all data is used FOXPRO 6.0 and all statistical analyses were performed using SPSS16.0 statistical package for Windows. Univariate logistic regression analysis was conducted to evaluate the association between HPV infection and potential risk factors.

The potential risk factors evaluated include age, smoking habits, total number of sexual partners, age at first sexual intercourse; first age of childbirth; cleaning the vulva and vagina before having sex; cleaning the vulva and vagina after having sex; the use of condoms. The relative risk estimation (odds ratio [OR] with 95% confidence interval [CI]) is presented for each explanatory variable. The multivariate logistic regression model between HPV infection and the explanatory variables was adjusted for the variables used in the study design and for the variables identified as statistically significant in the univariate analysis. $P < 0.05$ was considered statistically significant.

Results

The demographic data of Uyghur participant women A total of 1000 Uyghur women were invited to participate in the study, 117 of them didn't meet the selection criteria,

Table 2. The Proportion and Type of HPV-positive Women

Variables	N	Percentage (%)
Negative of HPV	805	91.17
Positive of HPV	78	8.83
High risk of HPV	64	7.25
Low risk of HPV	14	1.58
HPV-16	27	3.05
HPV-31	6	0.07
HPV-39	4	0.04
HPV-51	7	0.08
HPV-58	4	0.04

Table 3. Univariate Logistic Regression Analysis of High-risk HPV Infection and Potential Risk Factors

Variables	No. of participants	High-risk HPV positive (%)	OR(95%CI)	P value
Age, y	809			
≤24	147	9(6.12%)	1	0.072
25-34	251	18(7.17%)	1.18(0.52-2.71)	
35-44	251	18(7.17%)	1.18(0.52-2.71)	
45-54	234	19(8.12%)	1.36(0.60-3.08)	
Smoking habit				
Never-smoked	864	62(7.18%)	1	0.068
Smoking	19	2(10.52%)	1.65(0.37-7.32)	
Total number of sexual partners				
1	529	31(5.9%)	1	<0.001
2	173	12(6.94%)	1.45(1.26-4.65)	
≥3	181	21(11.6%)	3.09(2.24-7.16)	
Age of First marriage, y				
≥25	169	11(7.26)	1	<0.001
21-24	230	15(6.52%)	1.89(0.56-4.38)	
16-20	256	20(7.81%)	2.21(0.89-5.32)	
≤15	154	17(11.03%)	4.12(2.13-7.43)	
Age of first childbirth, y				
≥25	169	10(5.91)	1	<0.001
21-24	230	18(7.83%)	1.81(1.01-4.38)	
16-20	256	22(8.59%)	2.21(1.45-6.31)	
≤15	154	14(9.09%)	3.58(2.11-7.56)	
Cleaning the vulva and vagina before Sex life				
No	588	43(7.31%)	1	0.136
Yes	221	21(9.50%)	1.24(0.70-2.21)	
Cleaning the vulva and vagina after Sex life				
No	281	18(6.40%)	1	<0.001
Yes	528	46(8.71%)	3.24(2.13-5.87)	
The use of condoms				
No	632	43(6.80%)	1	<0.001
Yes	225	18(8.00%)	0.26(0.08-0.86)	

and were excluded from the study. In all, 883 women were included in the analysis (Table 1); The mean age of them was 36.3 ± 10.1 years, most women had annual income less than ¥ 1000 ($n=794$, 90.64%), most of them married before 20 years ($n=845$, 95.91%), had initial childbirth before 20 years ($n=649$, 80.12%), and had more than two children($n=625$, 77.74%); And more than one third of women had two married history ($n=291$, 33.03%), had sexual partners over two ($n=324$, 37.89%); 646 (74.51%) women did not clean the vulva and vagina before Sex and 840 (96.44) cleaned the vulva and vagina after sex.

The prevalence of HPV in Uyghur women

The overall prevalence of HPV in our study sample was 8.83% (78/883), in which the prevalence of high-risk

Table 4. High-risk HPV Infection and Potential Risk Factors Multivariate Unconditional Logistic Regression Analysis

Variables	OR	95%CI	P
Age of First marriage, y			
≥25		1	<0.001
21-24	1.65	(0.89-4.38)	
16-20	2.21	(1.51-5.37)	
≤15	4.01	(2.11-5.56)	
Lifetime number of sexual partners			
1	1		<0.001
2	1.45	(1.26-4.65)	
≥3	3.69	(2.24-7.16)	
Cleaning the vagina after sex	2.72	1.98-5.13	0.01
Using condoms	0.36	0.12-0.53	0.01

was 7.25% (64/883), the prevalence of low-risk was 1.58% (14/883); The 5 most prevalent HPV genotypes were HPV16 (3.05%, 27/883), HPV 51 (0.08%, 7/883), HPV 31 (0.07%, 7/883), HPV 39 (0.04%, 4/883) and HPV 58 (0.04%, 4/883). Other types of distribution: HPV 33 in 3 cases (0.03%), HPV 68 type in 3 cases (0.03%), HPV 53 type in 3 cases (0.03%), HPV 56 type in 3 cases (0.03%), HPV 35, 45, 52, 56, 42, 54 type in 2 cases (0.03%); HPV 59, 73, 6, 83, 40, 62 type in 1 case (0.01%). The data are reported in Table 2.

Univariate logistic regression analysis of HPV infection status versus behavioral risk factors

As we can see from table 3, the highest risk occurred in the 45-55 years age group, although this finding was not statistically significant in age groups; In our study, there only 19 women with smoking habit, and only 2 infected the HPV, there was not statistically significant between the smoking and no smoking in HPV infection; For these women first married age ≤ 15, had first child age ≤ 15, had sexual partners ≥ 3 were associated strongly with the prevalence of HPV infection; Cleaning the vulva and vagina after sex life increased the risk of HPV infection (OR 3.24; 95% CI, 2.13-5.87); The use of condoms had a lower risk of HPV infection (OR 0.26; 95% CI, 0.08-0.86). Multivariate logistic regression analysis of HPV infection status versus behavioral risk factors

For these woman, had first marriage age ≤ 15, had sexual partners ≥ 3 remained the significant predictor of HPV infection, and the OR were 4.01 (95% CI, 2.11-5.56) and 3.69 (95% CI, 2.24-7.16), respectively. Women cleaning the vagina after sex showed an increased risk for HPV infection (OR 2.72; 95% CI 1.98-5.13); using the condoms showed protective factors for HPV infection (OR 0.36; 95% CI 0.12-0.53) (Table 4).

Discussion

A meta-analysis results of 157,879 women from 36 countries and regions, showed that HPV infection rate of the global women with normal cytology was 10.4%, which is 8.0% in Asia (de Sanjose et al., 2007; Zou et al., 2011). Pandey et al performed a human papillomavirus screening in north Indian women, and they reported that the HPV incidence in their study was 11.7% (Pandey et al.,

2012). In present study, the prevalence of HPV prevalence of Uyghur women in Xinjiang was 8.84%, high-risk was 7.25%, and low-risk was 1.59%; The results were in agreement with Guzhalinuer's study result (Guzhalinuer et al., 2007), she found that HPV prevalence of Xinjiang Uyghur women was 7.0%, and both showed a lower rate of infection. According to previous research, the results show that the incidence of cervical cancer is high notably occurring at a rate 3-4 times higher in Uyghur women than for Han women in the same area. The mortality of cervical cancer in Uyghur women ranks first among ethnic groups in China. This may be caused by HPV type distribution and behavioral risk factors leading to persistent infection.

This study results show the prevalence of the high-risk of HPV infection of Uyghur women is 82.05%, HPV 16 type is 34.6%. This is the same results as the main type of infection in the literature of HPV16 in cervical cancer in women of southern Xinjiang Uyghur (Guzhalinuer et al., 2007). The 5 most prevalent HPV genotypes were HPV 16, 51, 31, 39 and 58, while HPV18 is not found. HPV16 was the most prevalent genotype detected in the world, followed by HPV 42, 58, 31, 18 and 56. HPV16 was twice as frequent as any other high-risk type in all regions, and the next commonest high-risk types were HPV33 and 56 in Asia (Quek et al., 2013; Wang et al., 2013). In China, HPV16/18 was the predominant type, followed by 58, 31, and 52 (Bao et al., 2008). These data showed that HPV distribute with region and nationalistic difference (Velicer et al., 2009; De Vuyst et al., 2012). Guzhalinuer genotyped the HPV in 100 cervical cancer cases in west Xinjiang province (Guzhalinuer et al., 2007). The result shows that HPV 16, 18 and 68 were the most important HPV type, there exist large different with our study, but our study is population-based and more representative for Uyghur HPV genotype.

However, we did not find the type of HPV18, which is the second prevalent type in the world. HPV18 was associated with the adenocarcinoma (Sanchez-Lander et al., 2012; Siriaunkgul et al., 2012). Yuhua P's study showed that the prevalence of cervical squamous cell carcinoma in Uyghur and Han were 95-99%, and 80-90%, respectively, the difference is statistically significant. This may because population sampling we recruited came from desert area.

The current study suggests that the risk factors for the incidence of cervical cancer include three aspects: behavioral risk factors, biological factors, and genetic predisposition (Guerry et al., 2011; Boccalini et al., 2012; Peralta-Rodriguez et al., 2012). Uyghur women living in southern China's Xinjiang region, and concentrated relatively. They are Muslim communities with lower income and education level, there are obviously different from the Han's lifestyle and habits. This epidemiological study survey aim to find the behavioral factor contributed to the incidence of cervical cancer.

The present study found that there are 2-fold higher risk of infection among women married before 20s old than the ones married after 20 years old, married early increased the risk of cervical cancer significantly (Raychaudhuri et al., 2012). Multivariate analyses result of this study showed that women married before 15-year

had 4.83-fold increased risk of infection compared with women married after 15-year-old. Thus first marriage age was identified as a strong determinant of HPV infection. Most of the literature suggests that age of the initial delivery was risk factors of HPV infection, but in this study the initial age of delivery did not affect the risk of HPV infection, because the majority of Uyghur women in rural areas the age of first marriage is the similar to the age of the first childbirth age, therefore the role of age at first marriage replaced the role of first childbirth Age.

In the multivariate analysis, multiple sexual partners was independent risk factors for HPV infection, women reporting more than 3 sexual partners had a 3.6-fold higher risk fold infection compared with women reporting 1 partner. This result was consistent with the Italian study (Confortini et al., 2010), which showed that women aged 18-24years with 5 or more sexual partners in the previous 3 years had a 6.8-fold increased risk of infection compared who has 1 partner. The Uyghur women is a relatively conservative and have low status in the family, but the Uyghur men had strong sexual desire, and was more trend with sexual confusion, the family relationships was relatively unstable, the multiple sexual partners was caused mainly by women with a history of multiple marriages. On the other hand, multiple sexual partners are a privacy issue, and some of the participants intended to conceal its multiple sexual partners.

Our study found that the poor genitals sanitation were the combined factors of cervical cancer (Cokkinides et al., 2012). We demonstrate that cleaning the vagina after sex was HPV infection risk, (OR: 2.72, 95% CI: 1.98-5.13). This finding was not consistent with present study results. Uyghur women considered that the Muslims must bathe after sex, and semen should be discharged from the vagina. However, the poor health conditions in their home increased the exogenous infection chance. Removal of poor sexual health factors may help reduce HPV infections.

A randomized controlled study performed by Hogewoning (Hogewoning et al., 2003) in Finland found that using condoms can clear HPV infection and promote CIN fallback. In our study, only 8.48% Uyghur women's husband used the condoms, our study still found that using condoms can reduce HPV infection which is a protective factor (OR: 0.36, 95% CI: 0.12-0.53). However, most Uyghur men were the leader of the family, they lack of correct sex education, and refused to use condom because it would affect quality of sex life.

The study is a population-based epidemiological investigation of HPV infection and cervical cancer risk factors with large age span, it was similar to the IARC global multi-center study screening program; The target population of this study was 16-55 year-old Uyghur women, So far, there was not a screening based on Uyghur population in this age span, and thus have a more comprehensive understanding of the status of China's Uyghur HPV infection, and the Uyghur population HPV type distribution; Our study has some limitations, we study the HPV prevalence, related type distribution and risks, but it was lack of multiple HPV infection analysis, and did not follow up the women had positive HPV, this

is the focus in our future research.

In conclusion, our finding shows the HPV prevalence rate in the Uyghur women is relatively low; the 5 most prevalent HPV genotypes were HPV16, HPV 51, HPV 31, HPV 39 and HPV 58. First marriage age was identified as a major risk factor for HPV infection. Other notable risks were total number of sexual partners and cleaning the vagina after sex. These data help to understand the changing of HPV pattern in Uyghur women and the risk factors for HPV infection; and changing behavioral risk factors will help to reduce the occurrence of cervical cancer.

Acknowledgements

We thank the following people to give us support and help during the research and writing: Jerome, Belinson, Youlin Q, Li L, Kaicun Z and thank the support given by Chinese Academy of Medical Science, the women and Children Health center of Yutian County.

This study was supported by the financial support of Chinese women's urogenital HPV infection and cervical neoplasia type lesions epidemiological study, and cooperated by Cancer Foundation of China and Medical Center in Cleveland.

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