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Original Article

Human papillomavirus infection and its risk factors among women receiving health check-up in Changsha area

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Abstract: Objective: To analyze the epidemiological features of human papilloma virus (HPV) among women who received health check-up in Changsha area. Methods: A total of 9,487 sexually active women were enrolled in this study. During the health check-up they received gynecological examinations, new PAT'S thinprep cytologic test (TCT), and detection of HPV DNA types. Meanwhile, the demographic data, sexual behavior-related information, and awareness of cervical lesions were collected using a self-designed questionnaire. Results: Of these 9,487 women, the overall HPV prevalence was 16.61%, which included the high-risk types (13.51%), low-risk types (0.67%), and intermediate-risk types (2.43%). HPV type 52 was most prevalent (20.43%). As shown by Logistic regression analysis, multiple sexual partners (n>3), frequent sexual activities (>3 times/week), and a family history of malignancy were the major risk factors of HPV infection. Half (50%) of the subjects were not aware that HPV infection is a high-risk factor for cervical cancer, and 44.6% did not know cervical cancer is preventable. Conclusion: The distribution of the HPV genotypes among the female health check-up populations in Changsha area is consistent with the distribution rules across China. Women with multiple sexual partners, frequent sexual activities, and/or a family history of malignancy are more susceptible to HPV infection.

Keywords: Cervical disease, HPV type, risk factors

Introduction

Human papilloma virus (HPV) is a small DNA virus and can cause various lesions ranging from mucosal hyperplasia to malignant tumor. Persistent high-risk HPV infection is a key risk factor for cervical lesions [1], and HPV DNA has been detected in 90% of cervical cancer tissues [2]. Since cervical cancer has a long precancerous stage, timely identification and active treatment during this stage can reduce the development of cervical cancer [3]. Up to now over 100 HPV subtypes have been identified, among which 13 were found to be associated with cervical cancer. The HPV type 16 (HPV16) is the most common type; in fact, HPV16 and 18 together accounted for 71% of HPV infections [4, 5]. Epidemiological studies have shown that the HPV infection rates and their genotype distributions vary among different countries and regions [6-10]. A deeper understanding of the epidemiological characteristics of HPV infection is particularly important for the prevention/treatment of HPV infection and for development of preventive measures of cervical cancer and its precancerous lesions [12]. Furthermore, women's awareness of cervical lesions determines their attitude towards the healthy behaviors for preventing/treating cervical lesions. In our current study, by conducting a survey with large sample size, we explored the incidence, risk factors, and distribution of HPV infection and its distribution among women who received health check-up in Changsha, the capital city of Hunan Province, China, and investigated the awareness of cervical lesions and HPV in this population, with an attempt to provide evidences for the prevention and treatment of cervical cancer in Changsha area.

Subjects and methods

Subjects

In this cross-sectional study, 10,000 sexually active women with different employment back-

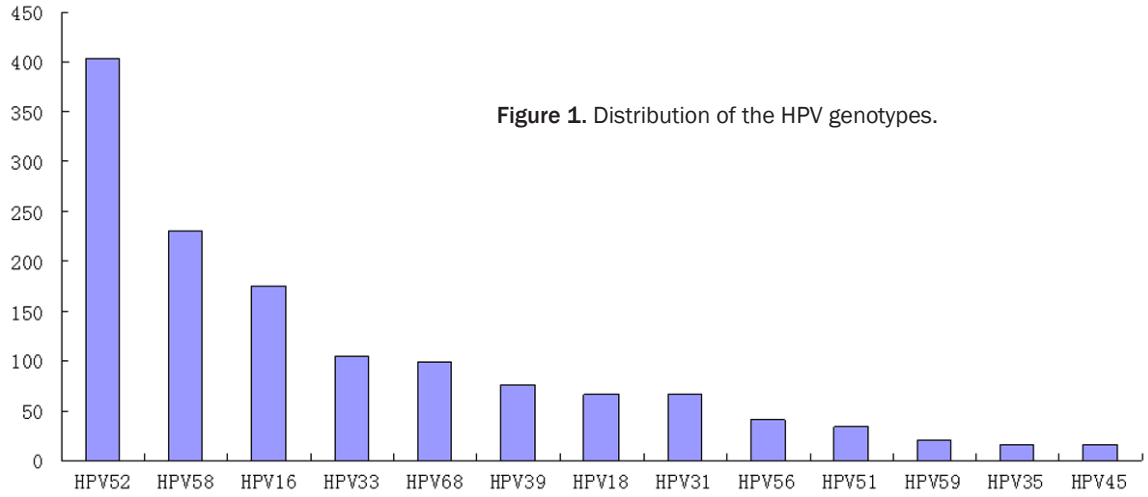

Figure 1. Distribution of the HPV genotypes.

grounds who received health check-up in the Health Management Center of the Third Xiangya Hospital of Central South University from January 1, 2013 to December 31, 2013 were enrolled. Inclusion criteria: a) aged 18-70 years; b) had lived in Changsha for at least six months (including locals and migrants); and c) sexually active. Exclusion criteria: a) with malignant tumors; b) with a history of cervical surgery; c) pregnant; d) with a history of pelvic radiotherapy; and e) having received gynecological screening and treatment within the past 12 months. All the subjects signed informed consents. During the screening, 211 (2.11%) did not meet the inclusion criteria, and 302 (3.02%) refused to participate for reasons including "too busy", "in the menstrual period", and "useless". Finally, 9,487 qualified questionnaire forms (94.9%) entered the statistical analysis.

Sample collection

A specially designed HPV sampler (Guangdong Kaipu Biotech Co., Ltd, Guangdong, China) was inserted into the external orifice of the cervix and then rotated 5 times clockwise or counterclockwise. Then, the sample was slowly withdrawn and put into a sampling tube containing special cell preservation solution. Tighten the cap and label the tube with patient's information. Store the sample in a 4°C refrigerator before it was determined by trained personnel within 24 hours. The HPV-DNA level in the sample was determined using a HPV genotyping kit (PCR+membrane hybridization) (Guangdong Kaipu Biotech Co., Ltd, Guangdong, China), which allowed the detection of 21 HPV types including 13 high-risk types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 5 low-risk types (HPV-6, 11, 42, 43, and 44), and 3 common types among Chinese populations (HPV-53, 66, and CP8304) within a single attempt. The experimental steps were as follows: a) Nucleic acid extraction: The nucleic acid was extracted in strict accordance with the kit instructions. If no immediate amplification was required, the extracted DNA templates could be temporarily stored in a refrigerator at 4°C, or at -20°C if longer storage was required. b) Amplification: Amplification was performed in strict accordance with the kit instructions in a TC-48 amplification instrument. The amplification products were denaturated into single DNA strands at 95°C before hybridization. c) Hybridization: The base sequence probes for the known 21 HPV types were coated in biotin films and hybridized with the amplified and denatured DNA products in the 45°C hybrid solution. The DNA was then detected and typed based on the ELISA principles.

Questionnaire-based survey

A questionnaire concerning the risk factors of HPV infection, including demographic data (age, marriage status, and education background), menstruation, fertility status, sexual activity, and family history) and the awareness of cervical lesions (e.g. the relationship between cervical lesion and HPV) was designed. After the subjects signed the informed consent forms, a trained interviewer carries out face-to-face survey. Uniformly trained gynecologists

Table 1. HPV infections among women with different occupations, education backgrounds, living habits, and obstetric histories

Factors	Number of cases		Positivity	*P*
	Negative	Positive	(%)	value
Occupations				0.240
Government	1655	359	17.8	
Public institutions	4413	852	16.2	
Companies	1843	365	16.5	
Education background				0.140
High school or lower	265	55	17.2	
Junior college	1548	347	18.3	
Undergraduate	5066	983	16.3	
Master or higher	1032	191	15.6	
Smoking				0.157
Yes	179	45	20.1	
No	7732	1531	16.5	
Alcohol consumption				0.029
Yes	2097	460	18.0	
No	5814	1116	16.1	
Annual salary (RMB yuan)				0.228
<20,000	350	88	20.1	
20,000-50,000	4884	950	16.3	
>50,000-100,000	2600	522	16.7	
>100,000	77	16	17.2	
Age for first sex (years)				0.018
≤20	426	113	21.0	
20-35	7442	1456	16.4	
≥35	43	7	14.0	
Number of sexual partner (s)				0.037
1	6991	1361	16.3	
2	739	165	18.3	
≥3	181	50	21.6	
Sexual frequency (times/week)				0.037
1	7260	1421	16.4	
2	536	120	18.3	
≥3	115	35	23.3	
Times of getting married				0.037
1	7821	1548	16.5	
≥2	90	28	23.7	
Age for first pregnancy (years)				0.924
≤20	114	22	16.2	
20-35	7740	1544	16.6	
≥35	57	10	14.9	
Times of pregnancies and labors				0.045
0	84	13	13.4	
1	7323	1437	16.4	
≥2	504	126	20.0	
Times of abortions				0.009
0	3050	547	15.4	
1	2901	596	17.0	
≥2	1960	433	17.8	
Family history (malignancy)				0.016
Yes	40	16	28.6	
No	7871	1560	16.5	

finalized the survey, during which 20% of the questionnaire forms were reviewed and validated, and the pass rate was 100%.

Statistical analysis

The survey data were input into the EpiData 3.0 software. After the data were doubling checked, statistical analysis was performed using the SPSS 17.0 software package. The overall HPV infection with different features and due to different types was described using prevalence. The differences in the relevant variables between HPV-infected and non-HPV-infected subjects were compared using Ridit analysis and chi square test, so as to identify the potential risk factors of HPV infection. With the HPV infection as the dependent variable and the potential risk factors as the independent variables, we analyzed the contributors of HPV infection using the multivariate Logistic forward stepwise regression analysis.

Results

Prevalence and type distribution of HPV infection

Of these 9,487 eligible subjects, 1,576 were infected with HPV, yielding an overall HPV prevalence of 16.61%, which included 1,282 high-risk types (13.51%), 63 low-risk types (0.67%), and 231 intermediate-risk types (2.43%). Various genotypes were detected, with HPV52 being the most prevalent type (20.43%). The distribution of the top ten high-risk genotypes was shown in **Figure 1**.

Risk factors of HPV infection

The results of the univariate analysis of HPV infection are shown in **Table 1**. With the presence/absence of HPV infection as the dependent variable, the

Table 2. Results of multivariate analysis of the potential risk factors of HPV infection

Factors	OR value (95% CI)	*P* value
Number of sexual partner (s)	1.6 (1.1-2.3)	0.003
Sexual frequency (times/week)	1.1 (1.0-1.3)	0.036
Family history (malignancy)	1.3 (1.1-1.6)	0.025

Table 3. Comparison of HPV awareness among different areas

Authors	Country or area	Awareness rate of HPV
Alberto [25]	Italy	54%
Marlow [26]	United Kingdom	24.4%
Lee [27]	Hong Kong, China	10%
Li [28]	Mainland China	15.5%

variables that had been found to be statistically significant (P≤0.05) in the univariate analysis were introduced into the multivariate Logistic regression formula, in which the forward method was applied to screen the risk factors of HPV infection. It was found that multiple sexual partners (n>3), frequent sexual activities (>3 times/week), and a family history of malignancy were the major risk factors of HPV infection. In contrast, other factors such as alcohol consumption, age for first sex, times of getting married, times of pregnancies and labors, and times of abortions showed no significant correlation with HPV infection (**Table 2**).

Awareness of cervical lesions

Half (50%) of the subjects were not aware that HPV infection is a high-risk factor for cervical cancer, 21% had no idea whether a less chaotic/complicated sexual life may help to prevent cervical cancer, 19% did not agree that cervical cancer should be early diagnosed and treated. Up to 46% of the respondents did not know whether cervical cancer can be preventable.

Discussion

The type distribution of HPV shows remarkable geographic diversities across the world [10, 29]. Research has shown that the most prevalent HPV types were HPV16 and 18 in Europe and North America and HPV45 in Africa. In Asian countries, however, the proportions of HPV52 and 58 are much higher than those of HPV45, 31, and 33. By conducting survey on 9,487 women who received routine health check-up, we found the overall prevalence of HPV infection was 16.61% among this population in Changsha area, which was remarkably lower than those in Taizhou (20.79%) [17], Tianjin (34.05%) [18] and the rural area of Wuhan (57.03%) [19], but was slightly higher than those in Beijing (15.38%) [20] and the national average level (14.20%) [21]. Notably, all the subjects in the former four cohorts were patients in the gynecology clinics. In contrast, the vast majority of our subjects were female white-collars who were working in government, public institutions, or companies. Naturally, the HPV prevalence was lower in our cohort. Similarly, Zheng et al reported that the positive rate of HPV was 8.03% among 2,663 female employees from the public institution in Shantou City, Guangdong Province, which was lower than that of the general public [22]. It has been reported that the detection rate of HPV-52 and 58 ranged from 20% to 25% in the cervical cancers in Chinese women; [30, 31]. Meanwhile, HPV52 and 58 are more prevalent in southern China than in northern China [23]. Similarly, in our current study, the most prevalent HPV type in Changsha area was HPV52, accounting for 20.43% of the positive specimens. Also, we found the top ten high-risk HPV types were HPV58, 53, 16, 33, 68, 39, 66, 18, and 31, which was consistent with the findings in Bao et al's Meta analysis conducted among Chinese populations [32]. The currently available HPV vaccine only covers some high-risk types of HPV; It is still unclear whether this vaccine has cross protective efficacy for other high-risk HPV types. The high prevalence of HPV52 and 58 is somehow different from the global type distribution. The development of new HPV vaccines in China must consider such unique epidemiology, so does the prevention and clinical treatment of cervical cancer.

HPV infection is the result of the interaction of multiple factors. However, the exact risk factors of HPV infection remain controversial. Many studies have shown that sexually active women have higher rate of HPV infection; the number of sexual partners and the sexual frequencies of the women (and their partners) as well as the disease histories of their sexual partners are associated with the prevalence of HPV [12-15, 29]. Similarly, our current study found that larger number of sexual partners and higher sexual

frequency could increase the risk of HPV infection. HPV prevalence is related with the sexual behaviors in a specific population. Promiscuous individuals with frequent sexual activities are often more prone to cervical injury/infection, which decreases human body's capability in clearing viruses and thus results in the rapid virus replication. In addition, as shown in our current study, subjects with a family history of malignancy had a significantly higher HPV positive rate than other subgroups, which may be explained by the different susceptibilities of different genotypes to the specific HPV proteins (e.g. HPV E6 protein), which was consistent with the findings of Qiu et al [19].

Also, although alcohol consumption and obstetric history were found to be clinically significant during the univariate analysis, they were not significantly correlated with HPV infection in the multivariate analysis. However, this does not mean that alcohol consumption and obstetric history is not related with HPV infection; rather, they were ruled out due to the relatively high collinearity with the preserved variables in the final model. Furthermore, these two factors can, to certain extent, impair the health or physical status of an individual, causing the decrease in human defense system. Since the majority of our subjects were female employees in government, public institutions, and companies, their lifestyles and behaviors were quite simple and healthy; thus, these two factors showed no statistical significance in our analysis. Similar conclusion has also drawn by Wang et al [24].

In a survey conducted among 807 mothers in Italy by Alberto et al [25], up to 54% of the respondents had never heard of HPV. Marlow et al [26] carried out a large randomized survey among 16 to 97-year-old females in the United Kingdom and found the awareness rate of HPV was 24.4%. Li et al [28] surveyed 6,024 women in mainland China and found that only 15.5% of the subjects had heard of HPV. In a survey using purposive sampling method in Hong Kong, Lee et al [27] found only 10% of the participants had heard of HPV (**Table 3**). Therefore, even in developed countries and areas with relatively sophisticated cervical cancer screening programs, the awareness of HPV infection remains unsatisfactory among women. As shown in our current study, half (50%) of the subjects were not aware that HPV infection is a high-risk factor for cervical cancer, and 44.6% did not know cervical cancer is preventable. Among various viral diseases, viral hepatitis has been highly recognized and most people know its transmission mode and prognosis. Also, thanks to the awareness raising campaigns conducted by the government, the hazard of human immunodeficiency virus (HIV) has been well recognized. In contrast, half of our respondents had not heard of the HPV, a key contributor to cervical cancer, indicating that the health education and awareness-raising efforts in Changsha area still have a long way to go. Research has shown testing positive for HPV was associated with adverse psychological consequences. Anxiety and depression about the infection were widespread. Most women (3/4) described feeling stressed and angry, and two thirds were stigmatized; meanwhile, their sexual pleasures and activities dramatically dropped [33]. Therefore, it is important to learn about women's awareness of cervical lesions, so as to provide targeted educational interventions. Doctors, friends, family members, health education lectures, and mass media are credible sources of health education information. Therefore, efforts should be made to carry out health education at various levels, encourage positive media coverage, motivate the medical staff to be actively involved in health education, expand the coverage of health insurance, and promote the construction of grass-roots health facilities, so as to make more women be aware of the risk factors of HPV infection and the importance of cervical cancer screening. By doing so, we may achieve the early detection of cervical cancer, improve the patients' quality of life, and pay the way for the development and application of new HPV vaccines.

In conclusion, the prevalence and type distribution of HPV among women who receive health check-up in Changsha area have certain uniqueness. Early identification of highly risky populations, active interventions and health education for the risk factors, timely profiling, and regular follow-up visits are useful strategies to reduce the HPV prevalence in Changsha area.

Disclosure of conflict of interest

None.

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