

Original Article

Prevalence of high-risk human papillomavirus in Hangzhou, Eastern China

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Abstract: Human papillomavirus (HPV) has been proved to play a crucial role in the development of cervical cancer and precancerous lesions. The prevalence and distribution of high-risk HPV (HR-HPV) genotypes varies in countries, even in China. The present study aimed to investigate the genotypes prevalence, age- and season-specific prevalence, and frequency of HR-HPV infection in gynecology examination (GE) patients and healthy volunteers (HV) in Hangzhou, Eastern China. A total of 8,918 study subjects were enrolled and assigned into the gynecology examination (GE) group ($n = 6,469$) and the healthy volunteer (HV) group ($n = 2,449$), and 15 HR-HPV genotypes were characterized in cervical specimens. HPV-52, -58, -16, and -51 were the four most common genotypes, and higher prevalence of HR-HPV genotypes was seen in the GE group than in the HV group except HPV-59 and -45. In the GE group, the age-specific prevalence of HPV genotypes appeared a "U-shaped" curve; however, no significant difference was seen in the HV group. The HR-HPV genotypes exhibited clear seasonal rhythms, and various genotypes presented season-specific characteristics. A single HR-HPV genotype infection was most frequently observed in both groups and the proportion of infection with a single or two HPV genotypes was comparable between the two groups. Our data indicate that HR-HPV has high prevalence and may have special characteristics in Hangzhou, Eastern China. These findings may provide supplementary data for the surveillance of HPV infection and for the cervical cancer control and prevention in China.

Keywords: Human papillomavirus (HPV), high-risk HPV, genotyping, prevalence, Eastern China

Introduction

Cervical cancer is the third most common cancer among women, with approximately 530,000 new cases annually worldwide [1]. Cervical cancer develops through a multistep process, with three cervical intraepithelial neoplasia grades, 1 to 3 (CIN1-3) [2]. However, it will take several years, even decades, from pre-cancer to invasive cervical cancer, which offers us many opportunities for interventions [1]. Therefore, early detection of cervical precancerous lesions and their causes likely contribute to reducing the incidence and mortality of the malignancy.

Human papillomavirus (HPV) has been identified as a cause of premalignant and malignant tumors in the lower genital and digestive tracts

[3]. Molecular and epidemiologic evidence has shown that persistent infection with high-risk (HR) HPV plays a crucial role in the development of both cervical cancer and cervical precancerous lesions [4, 5].

HPV is a small, non-enveloped virus that belongs to the Papillomaviridae family with approximately 8 kb circular double-stranded DNA [6]. To date, more than 200 HPV genotypes have been identified, of which approximately 40 genotypes may infect the genital tract [7]. According to oncogenic potential, HPV is classified into HR and low-risk (LR) types. HR-HPV infection accounts for more than 99% of cases with cervical cancer, whereas non-oncogenic HPV types, such as HPV-6 and HPV-11, are

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responsible for 90% of anogenital warts (papilloma) [8].

The prevalence and distribution of HR-HPV genotypes varies in countries and regions. In China, the prevalence of HPV genotypes also differs greatly in regions. Additionally, HPV vaccine has been commercially available in Mainland China since July 2016, using the extended schedule (0, 1, and 6 months) [9]. Therefore, it is essential to investigate the characteristics of female HR-HPV infection among different geographic regions.

The present study aimed to investigate the prevalence and genotypes of HR-HPV among women in Hangzhou, Eastern China, in order to provide supplementary data for screening cervical cancer and developing vaccination strategies in this region.

Material and methods

Ethical approval

This study was approved by the Ethical Review Committee of the Second Affiliated Hospital of Zhejiang University (No. 2016-111) and performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Signed informed consent was obtained from all participants following a detailed description of the purpose of the study.

Subjects

A total of 8,947 female volunteers, who visited the Yuhang Branch of the Second Affiliated Hospital of Zhejiang University (Hangzhou, China) during the period between January and November, 2016 for screening of HPV infection were recruited in this study. All cases were assigned into the gynecology examination (GE) group ($n = 6,469$) and the healthy volunteer (HV) group ($n = 2,449$). The inclusion criteria included (1) age of 18 years or greater; (2) a history of sexual life for more than one year; (3) no sexual intercourse during the past 3 days; (4) having an intact uterus; (5) non-pregnant; and (6) without a history of cervical therapy. Finally, a total of 8,918 women that met the inclusion criteria were enrolled in the study. There were 6,469 subjects in the GE group,

with a median age of 37 years (range, 18 to 84 years), and 2,449 subjects in the HV group, with a median age of 41 years (range, 20 to 85 years).

Collection of cervical specimens

All cervical specimens were collected by well-trained physicians. Cervical secretions were firstly removed with cotton swabs and then exfoliated cervical cells were collected with a cervical brush (Shanghai ZJ Bio-Tech Co., Ltd.; Shanghai, China). The brush was inserted 1 to 1.5 cm into the endocervical canal and rotated for 3 to 5 full turns. Then, each specimen with brush containing cellular materials and the mixture with 1.5 mL of specimen transport medium, was stored at 4°C immediately prior to detection for less than 72 hours. All specimens were labeled with subjects' information.

DNA extraction and HPV genotyping

DNA extraction and HPV genotyping were carried out using the High-Risk HPV genotype Real-Time PCR kit exactly according to the manufacturer's instructions (Shanghai ZJ Bio-Tech Co., Ltd.; Shanghai, China), and 15 HR-HPV genotypes were characterized on an ABI 7500 Real-time PCR System (Applied Biosystem; Foster City, CA, USA), including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68 and -82. Positive and negative controls, provided by the detection kit, were simultaneously detected in each batch of test.

Statistical analysis

HPV infection was defined as positive for one or more HPV genotypes. All data were stratified by genotype, age (18-24, 25-29, 30-34, 35-39, 40-49, 50-59, and 60 years and greater), and season (Spring: March to May, Summer: Jun to August, Autumn: September to November, Winter: December to the following February). Differences of proportions were tested for statistical significance with a chi-square test. All statistical analyses were performed using the statistical software SPSS version 17.0 (SPSS, Inc.; Chicago, IL, USA), and a P value < 0.05 was considered statistically significant. The software Graphpad Prism version 5.0 (GraphPad Software, Inc.; San Diego, CA, USA) was used for diagram drawing.

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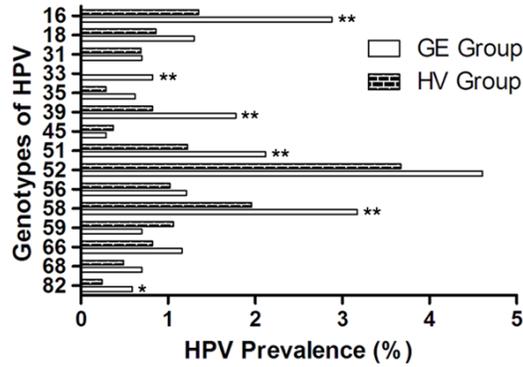


Figure 1. Prevalence of HR-HPV genotypes in the study subjects. There were significant differences in the prevalence of HPV-16, -33, -39, -51, -58 and -82 genotypes between the GE group and the HV group. * $P < 0.05$; ** $P < 0.01$.

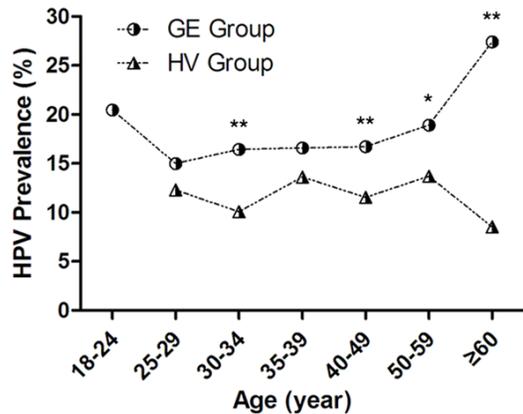


Figure 2. Age-specific prevalence of HR-HPV genotypes in the study subjects. There were significant differences in the prevalence of HR-HPV genotypes between the GE group and the HV group at age of 30-34, 40-49, 50-59 and 60 years and greater old group. * $P < 0.05$; ** $P < 0.01$.

Results

Prevalence of HR-HPV genotypes

There were 14 HR-HPV genotypes characterized in the HV group, including HPV-16, -18, -31, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68 and -82. Of the 2,449 subjects, 12.3% (290/2,449) were infected with one or more HR-HPV genotypes, and HPV-52 was the most common genotype (3.67%), followed by HPV-58 (1.96%), HPV-16 (1.35%), HPV-51 (1.22%), HPV-59 (1.06%), and HPV-56 (1.02%), while less than 1% prevalence of HPV-18, -31, -35, -39, -45, -66, -68 and -82 genotypes was detected (Figure 1).

In the GE group, all 15 HPV genotypes were detected, and 17.08% (1,105/6,469) of the subjects were infected with one or more genotypes. HPV-52 was the most common genotype (4.61%), followed by HPV-58 (3.17%), HPV-16 (2.88%), HPV-51 (2.12%), HPV-39 (1.78%), HPV-18 (1.30%), HPV-56 (1.21%), and HPV-66 (1.16%), while less than 1% prevalence of HPV-33, -68, -59, -31, -35, -82, and -45 genotypes was found (Figure 1).

HPV-52, -58, -16, and -51 were the four most common genotypes characterized in both groups, and higher prevalence of HR-HPV genotypes was seen in the GE group than in the HV group except HPV-59 and -45. There were significant differences in the prevalence of HPV-16, -33, -39, -51, -58 and -82 genotypes between the GE group and the HV group ($P < 0.05$ or 0.01) (Figure 1).

Age-specific prevalence of HR-HPV infection

Age-specific prevalence of HR-HPV genotypes is shown in Figure 2. The prevalence of HR-HPV genotypes varied in ages in the HV group, ranging from 8.52% to 13.69%; however, there was no significant difference in the HV group (Chi-square test, $\chi^2 = 7.869$, $P = 0.248$). In the GE group, the age-specific prevalence of HR-HPV genotypes appeared a “U-shaped” curve, with the first peak seen at ages of 18 to 24 years (20.47%, 78/381) and the second peak at ages of 60 years and greater (27.42%, 51/186) (Chi-square test, $\chi^2 = 23.3$, $P = 0.001$). In addition, higher HR-HPV genotype prevalence was detected in the GE group than in the HV group at all age groups. There were significant differences in the age-specific prevalence of HR-HPV infection between the GE group and HV group at ages of 30-34, 40-49, 50-59 and 60 years and greater ($P < 0.05$ or 0.01).

Season-specific prevalence of HR-HPV infection

The season-specific prevalence of HR-HPV genotypes is presented in Figure 3. The prevalence of HR-HPV genotypes fluctuated with season in the HV group (Chi-square test, $\chi^2 = 7.837$, $P = 0.05$), with the lowest prevalence detected in spring (9.79%, 47/480), and the highest seen in autumn (14.42%, 111/770). In the GE group, the lowest HR-HPV genotype prevalence was also found in spring (13.84%, 242/1749), and

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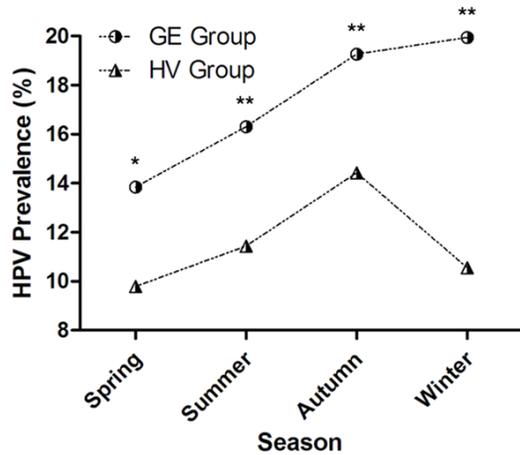


Figure 3. Season-specific prevalence of HR-HPV genotypes. There were significant differences in the prevalence of HR-HPV genotypes between the GE group and the HV group in spring, summer, autumn and winter. * $P < 0.05$; ** $P < 0.01$.

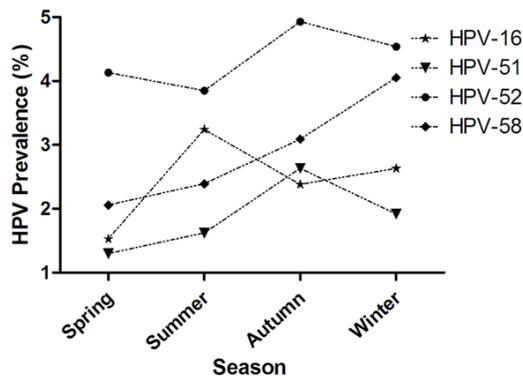


Figure 4. Season-specific prevalence of HR-HPV genotype infection.

the prevalence increased remarkably in summer and remained at a high level in autumn (19.26%, 313/1,625) and winter (19.94%, 249/1,249) (Chi-square test, $\chi^2 = 26.426$, $P = 0$). In addition, higher HR-HPV genotype prevalence was observed in the GE group than in the HV group at all four seasons. There were significant differences in the season-specific prevalence of HR-HPV genotype infection between the GE group and HV group in spring, summer, autumn and winter ($P < 0.05$ or 0.01).

In addition, HPV-52, -58, -16, and -51 were detected as the most common four HPV genotypes in both the GE and HV groups, and various HPV genotypes exhibited their specific characteristics with seasons. HPV-52 was the

primary genotype of infections, and its prevalence fluctuated with season, with the highest infection (4.93%) seen in autumn. The HPV-58 prevalence increased remarkably from spring to winter, while the prevalence of HPV-16 and -51 showed a peak. The highest prevalence of HPV-16 (3.24%) and -51 infection (2.63%) was detected in summer and autumn, respectively (**Figure 4**). Also, the four-year data pertaining to the season-specific prevalence of HR-HPV genotype infection in this area was very similar with **Figure 4** (data not shown).

Frequency of HR-HPV genotype infection

Among the 8,918 subjects enrolled in this study, there were 1,395 subjects with infection of one or more HR-HPV genotypes. In the HV group, 80% of the 290 HPV-positive samples were infected with a single HR-HPV genotype, 16.55% with two genotypes, 2.07% with three genotypes, and 1.38% with more than three genotypes (**Table 1**). In the GE group, 76.11% of the 1,105 HPV-positive specimens were infected with a single HR-HPV genotype, 17.83% with two genotypes, 4.16% with three genotypes, and 1.9% with more than three genotypes (**Table 1**). Our data indicated that a single HR-HPV genotype infection was most frequently observed in both groups and the proportion of infection with a single or two HR-HPV genotypes was comparable between the two groups.

Discussion

As the most common sexually transmitted infection, HPV has been identified as a cause of cervical cancer [1, 3, 5]. The prevalence and genotypes of HPV has been extensively investigated worldwide [10, 11]. Since the prevalence and distribution of HR-HPV genotypes varies in different regions of China [12, 13], there is still a great need of more knowledge on HR-HPV genotype prevalence in Eastern China. The current study was therefore designed with aims to investigate the prevalence and genotypes of HR-HPV in Hangzhou, Eastern China. Our data showed 15.64% prevalence of HR-HPV genotypes in Hangzhou, which is similar to the findings reported in other regions located in Eastern China, but different from the results detected in Southern and Northern China [14-17].

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Table 1. HR-HPV genotypes characterized in the cervical specimens captured from the study subjects

No. of HPV genotypes detected in cervical specimens positive for HPV	Prevalence in the GE group (<i>n</i> = 1,105, %)	Prevalence in the HV group (<i>n</i> = 290, %)
1	76.11 (841/1,105)	80.00 (232/290)
2	17.83 (197/1,105)	16.55 (48/290)
3	4.16 (46/1,105)	2.07 (6/290)
4	1.45 (16/1,105)	1.03 (3/290)
5	0.36 (4/1,105)	0 (0/290)
6	0.09 (1/1,105)	0.34 (1/290)
Total	100 (1,105/1,105)	100 (290/290)

In the current study, the age-specific HR-HPV genotype prevalence varied from 8.52% to 13.69% in the HV group; however, no significant difference was seen in the prevalence of HR-HPV genotypes in the HV group (Chi-square test, $\chi^2 = 7.869$, $P = 0.248$), while the HR-HPV genotype prevalence exhibited a “U-shaped” change with age in the GE group, with two peaks seen at ages of 18 to 24 years, and 60 years and greater, which is similar to other previous reports [18-19]. In addition, significantly higher HR-HPV genotype prevalence was detected in the GE group than in the HV group at all age groups, which may be explained by the following contributors. Young women may have more active sexual intercourse, and their genital systems are not mature enough; as a result, the prevalence of HR-HPV infection exhibited its first peak in women at ages of 18 to 24 years [20]. If elder women, which have alterations of hormone levels and weaker immune function, still have active sex activities, the second peak of HPV prevalence may occur since their viral clearance abilities decrease [21].

It has been shown that HPV genotypes are linked to cervical cancer [1, 3], and the severity of cervical lesions is associated with some specific HPV genotype infection [2]. If the capability of viral clearance reduces, persistent HPV infection may occur, thereby inducing cervical lesions. In this study, HPV-52, -58, -16, and -51 were characterized as the four most common HPV genotypes in both the GE and HV groups. Our findings showed HPV-52, rather than HPV-16 or -18, as the most common genotype in Hangzhou, Eastern China, which may indirectly infer the incidence of cervical cancer and the severity of cervical lesions in this region. The

prevalence of HPV-33 genotype was low in both the GE group (0.82%) and the HV group (0). HPV-33 is a HR-HPV type within the same phylogenetic species (alpha-9) as HPV-16 [22] and accounts for approximately 5% of cervical cancer cases worldwide [4]. The prevalence and distribution of HPV-33 genotype varies in different countries and regions, and it may have formed 5 phylogenetic groups [23].

In this study, HPV-52, -58, -16, and -51 were detected as the four most common HPV genotypes. It has been shown that the HPV genotypes exhibit clear seasonal rhythms, and various genotypes present season-specific characteristics [24]. Previous studies have demonstrated a relationship between HPV genotypes and sunlight exposure [25]. It is therefore assumed that the well-known phenomenon of UV-mediated suppression of immune surveillance may be causally related to this [26], which may explain the variation of HPV genotypes in different geographic regions.

Our findings showed that one HR-HPV genotype infection was predominant in both the HV and GE groups, followed by infection with two genotypes. Both HPV virus and host are found to affect HPV infection, such as genetic susceptibility or host genome, HPV variants, co-infection of HPV, first sexual intercourse, numbers of sexual partners during the past last year, sex partners having sex with others, female educational level, healthy habits, and other life-style factors [27].

This study is the most current (from January to December 2016) and relatively large-scale epidemiological survey pertaining to HR-HPV genotype prevalence in Eastern China. However, there are still some limitations that should be noted. Since the commercial HPV genotyping kit used in this study can only detect 15 types of known HR-HPV genotypes, underestimation of HPV genotype prevalence cannot be excluded. In addition, occupation-specific HPV prevalence was not analyzed in the present study,

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since subjects' occupation was not fully recorded.

The results of the present study demonstrate high prevalence of HR-HPV genotypes in Eastern China. Since July 2016, HPV vaccine has been made commercial in Mainland China, using the extended schedule (0, 1, and 6 months). Considering the harm and public health significance of HPV infection, effective interventions are required to reduce HPV infection, such as health education, HPV screening and genotyping in women aged more than 18 years, and monitoring and treatment of HPV infections. Our findings may provide supplementary data for the surveillance of HPV infection and for the cervical cancer control and prevention in China.

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Disclosure of conflict of interest

None.

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