Human Papillomavirus Prevalence and Genotypes Distribution Among Female Outpatients in Qingdao, East China

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Persistent infection with human papillomavirus, especially high risk ones, is a necessary cause of cervical cancer. This study aimed to investigate the distribution of HPV genotypes in female outpatients from Qingdao, East China. A total of 4,534 cervical swabs from women visiting this medical institution for gynecologic care were included. HPV genotypes were examined by a PCR-based hybridization gene chip assay and liquid-based cytology analysis was used to evaluate cervical cytology. The overall HPV prevalence in this study was 32.2% (1,459/4,534). A total of 23 HPV genotypes were identified and the five most prevalent ones were HPV16 (16.1%), HPV52 (8.9%), HPV58 (7.9%), HPV6 (7.0%), and HPV53 (6.5%). Age-specific prevalence of HPV exhibited one peak at the youngest age group and the HPV positive rate decreased gradually with age growth. But high risk HPV infections were more prevalent among aged women. Besides, association between cervical cytology and HPV infection was also determined, 27.2% (1124/4,126) of women with normal cytology were HPV positive while 82.1% (335/408) of women with abnormal cytology were HPV positive. These findings give new epidemiological data and may provide guidance for the vaccination program in this area.

INTRODUCTION

Cervical cancer is a main cause of mortality among women. There were an estimated 5,27,600 new cervical cancer cases and 2,65,700 deaths worldwide in 2012. It is the second most commonly diagnosed cancer and third leading cause of cancer death among females in less developed countries [Torre et al., 2015]. The burden of cervical cancer is disproportionately high in less developed countries with substantially lower survival rates due to presentation at relatively advanced stages [Forman et al., 2012]. Human papillomavirus (HPV) infection, the commonest sexually transmitted infection, has been established as the main cause of cervical squamous intraepithelial lesions and invasive cervical cancer. More than 200 HPV genotypes have been identified, and approximately 40 distinct HPV types infect the mucosal epithelium of the genital tract [Munoz et al., 2003]. On the basis of their ability to cause cervical cancer, they are generally grouped into high-risk HPV types (such as HPV16, 18, 52, and 58) and low-risk HPV types (such as HPV6, 11, 42, and 43). Besides, geographic differences in the prevalence of HPV genotypes have been reported [Munoz et al., 2004] and the geographic variation is also obvious in China [Shi et al., 2012]. Moreover, the attribution of different HPV genotypes to cervical neoplasia, especially intraepithelial lesions, varies ethnogeographically [Chan et al., 2012]. Therefore, accurate evaluation of the regional distribution of HPV genotypes is essential for preventative strategies for cervical cancer.
The present study assessed the HPV genotypes distribution, age-specific prevalence of HPV and association between cervical cytology and HPV infections among women in Qingdao, East China. These epidemiological findings may provide guidance for vaccination program and prevention of cervical cancer in this region.

MATERIALS AND METHODS

Study Population and Sample Collection

Qingdao is a main city on the east coast of China. This study was carried out between November 2011 and October 2014 included a total of 4,534 samples collected from 4,005 women (age 17–76 years, median 46.5 years) who visited Department of gynecology and Department of gynecological oncology of Qingdao Central Hospital, a tertiary institution with 1,600 beds and an affiliated hospital of Qingdao University. A woman was eligible if she (a) had a sexual life history, (b) was a gynecological outpatient and with symptoms of genital tract diseases such as cervicitis and vulvar discomfort, (c) was neither previously miscarriage nor presently pregnant, (d) had not undergone a total uterus or cervix resection, and (e) agreed to undergo HPV test and cervical cytology evaluation. Procedures for this study were approved by Research Ethics Boards at Qingdao University Medical College.

Cervical exfoliated cell samples were obtained by a gynecologist according to the routine procedures in the hospital. For each patient, two separate cervical exfoliated cell specimens were collected independently for HPV genotyping assay and cytological diagnosis, respectively.

DNA Extraction and HPV Genotyping

For HPV DNA extraction, cervical exfoliated cell samples were stored at a specimen transport medium (Yaneng Biotechnology Limited Corp., Shenzhen, China). High-quality DNA was obtained from disruption of the cells, DNA isolation and purification according to the manufacturer’s instructions for DNA extraction. (Yaneng Biotech).

HPV detection and genotyping were performed using the HPV Genotyping Kit (Yaneng Biotech), a PCR-based flow-through hybridization and gene chip system. The gene chip contains 23 type-specific probes that recognize 18 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) and five low-risk HPV genotypes (6, 11, 42, 43, and 81). The PCR reaction to amplify the extracted DNA was according to the manufacturer’s protocols. The final results were obtained by colorimetric change on the chip under direct visualization. HPV negative and positive controls, provided in the Kit, were simultaneously detected in every test.

Cytological Diagnosis Criteria

Cervical slides were prepared using a liquid-based cytology method and the cytological classifications of disease grade were made in conformity to the Bethesda 2001 criteria. The evaluation system included negative for intraepithelial lesion or malignancy (NILM) and epithelial cell abnormality such as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and invasive cervical cancer (ICC) [Solomon et al., 2002]. The slides were evaluated for cervical cytology by three academic cytopathologists from Qingdao Central Hospital and Qingdao Cancer Hospital. A conclusion was made only when all the three cytopathologists reached an agreement. Then, the association between cervical cytology and HPV infection was investigated.

Statistical Analysis

Frequencies of clinical data were calculated. All statistical analyses were performed using SPSS 17.0 for Windows (Chicago, USA). Differences between groups were estimated by Pearson’s $\chi^2$ test and $P < 0.05$ was considered significant.

RESULTS

HPV Prevalence and Genotypes Distribution

During the 3 years surveillance, a total of 4,534 specimens were collected and detected for HPV genotypes. The genotyping tests in this study showed that 1,459 specimens were HPV positive and the overall prevalence of HPV was 32.2% (1,459/4,534). As shown in Figure 1A, among the 1,459 positive samples, 62.6% (914/1,459) were HPV single infection samples and 37.4% (545/1,459) were HPV multiple infections samples. Further, according to Figure 1B, among the 1,459 positive samples, the frequencies of high-risk HPV (HR-HPV) genotypes and low-risk HPV (LR-HPV) genotypes were 1,768 and 585, respectively.

The details of HPV genotypes distribution were also presented. The total frequency of HR- & LR-HPV infections was 2,353. This number exceeded the number of positive samples (1,459) because each single genotype was counted separately in multiple infections. As shown in Figure 2A, among HR-HPV genotypes, the top ten types were as follows: HPV16 (379/16.1%), HPV52 (210/8.9%), HPV58 (185/7.9%), HPV53 (154/6.5%), HPV66 (110/4.7%), HPV56 (98/4.2%), HPV68 (95/4.0%), HPV51 (92/3.9%), HPV59 (75/3.2%), and HPV18 (72/3.1%). Among LR-HPV genotypes (Fig. 2B), HPV6 was the most prevalent one (164/7.0%), followed by HPV81 (145/6.2%), HPV11 (99/4.2%), HPV43 (97/4.1%), and HPV42 (80/3.4%). Besides, as shown in Figure 3A, for single HPV infection samples, prevalence of HPV types was as follows (top ten): HPV16 (208/22.8%), HPV58 (86/9.4%), HPV52 (84/9.2%), HPV53 (63/6.9%), HPV81 (58/6.1%), HPV66 (46/5.0%), HPV6 (43/4.7%), HPV56 (42/4.6%), HPV51 (34/3.7%), and HPV68 (32/3.5%).
For multiple HPV infections samples (Fig. 3B), prevalence of each type was as follows (top ten): HPV16 (171/11.9%), HPV52 (126/8.8%), HPV6 (121/8.4%), HPV58 (99/6.9%), HPV53 (91/6.3%), HPV81 (89/6.2%), HPV11 (81/5.6%), HPV43 (67/4.7%), HPV66 (64/4.4%), and HPV68 (63/4.4%).

Age-Specific Prevalence of HPV

Patients were stratified into six groups according to age (<20 years, 21–30 years, 31–40 years, 41–50 years, 51–60 years, >60 years) and presence of HPV infections of each age group was counted. As shown in Table I, age-specific prevalence of HPV exhibited one peak at the youngest age group (19/45, 42.2%). The positive rates of HPV infections decreased gradually with age growth (Table I). As shown in Figure 4, age-specific prevalence of pure high-risk HPV infections kept on a relatively high level at the 31–40, 41–50, 51–60, and over 60 years group (78.0%, 73.3%, 76.4%, and 65.9%, respectively). Besides, age-specific prevalence of high and low-risk mixed HPV infections exhibited a distinctly high level at the youngest age group (68.4%). Further, pure low-risk HPV infections were not that prevalent and did not exceed 20% in any age group.

Association Between Cervical Cytology and HPV Infections

Normal cytology (NILM) was observed in 4,126 cases (91.0%). Abnormal cytology was observed in 408 cases (9.0%) including 127 cases of ASCUS, 104 cases of LSIL, 71 cases of HSIL, and 11 cases of ICC. The association between cervical cytology and HPV infection was analyzed as presented in Table II. 1,124 cases (27.2%) with normal cytology were HPV positive and most infections (62.5%, 702/
1,124) were pure high-risk HPV infections. Besides, overall HPV prevalence was 75.5% (142/188), 84.7% (111/131), 91.0% (71/78), and 100% (11/11) in ASCUS, LSIL, HSIL, and ICC, respectively. Both high-risk HPV and low-risk HPV infections were involved in abnormal cytology. While the prevalence of pure HR-HPV was 76.7% (313/408) in abnormal cytology compared with 17.0% (702/4,126) in NILM ($\chi^2 = 761.7$, $P < 0.01$). Besides, pure HR-HPV infections accounted for a larger proportion than LR-HPV infections both in ASCUS (67.5% vs. 4.8%) and in LSIL (79.4% vs. 3.0%). Only HR-HPV infection was involved in HSIL and ICC. Furthermore, HPV16 was the most prevalent genotype in both normal cytology and abnormal cytology, followed by HPV52 and 58. Table III listed the distribution and attribution of the three major genotypes in women with normal, ASCUS, LSIL, HSIL, and ICC cytology.

**DISCUSSION**

Cervical cancer is one of the most frequent cancers in Chinese women. Annually, 85,000 new cases are estimated to develop HPV-related cancers and 75,000 are diagnosed to be cervical cancer in China [Forman et al., 2012]. Undoubtedly, HPV infection is the main causal agent associated with cervical cancer development [zur Hausen, 2002].

In this study, the overall HPV prevalence, including high-risk and low-risk HPV, was found to be 32.2% among female outpatients who visited Qingdao Central Hospital. This rate was in conformity with the 36.5% positive rate observed from a similar study in Harbin, China [Sun et al., 2014]. But the women involved in this study were all outpatients. They could not represent an open population, thus, prevalence rate may vary if healthy women were included. A multi-center, population-based cross-sectional study in China reported a HPV
positive rate of 14.3% in the entire population [Wu et al., 2013]. In this study, HPV16 was the most prevalent type as in most other regions of the world. HPV52 and 58 were respectively the second and third common types, which is comparable with several previous studies in different areas of China [Ye et al., 2010; Shen et al., 2013; Sun et al., 2014]. Instead of HPV16, HPV52 was reported to be the most prevalent type in Hong Kong [Chan et al., 2009], Macao [Yip et al., 2010], and Taiwan [Wang et al., 2010]. Besides, HPV18 was not that common in this area other than in western countries. Knowledge of the distribution of specific HPV types in a

TABLE I. Age-Specific Prevalence of HPV

<table>
<thead>
<tr>
<th>Age group (years old)</th>
<th>Single HPV+ no. (%)</th>
<th>Multiple HPV+ no. (%)</th>
<th>HPV+ no. (%)</th>
<th>Total cases no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>5 (11.1%)</td>
<td>14 (31.1%)</td>
<td>19 (42.2%)</td>
<td>45</td>
</tr>
<tr>
<td>21–30</td>
<td>139 (19.2%)</td>
<td>118 (16.3%)</td>
<td>257 (35.5%)</td>
<td>725</td>
</tr>
<tr>
<td>31–40</td>
<td>238 (21.9%)</td>
<td>130 (12.0%)</td>
<td>368 (33.9%)</td>
<td>1086</td>
</tr>
<tr>
<td>41–50</td>
<td>308 (21.8%)</td>
<td>145 (10.2%)</td>
<td>453 (32.0%)</td>
<td>1415</td>
</tr>
<tr>
<td>51–60</td>
<td>170 (19.3%)</td>
<td>101 (11.5%)</td>
<td>271 (30.8%)</td>
<td>881</td>
</tr>
<tr>
<td>&gt;60</td>
<td>54 (14.1%)</td>
<td>37 (9.7%)</td>
<td>91 (23.8%)</td>
<td>382</td>
</tr>
<tr>
<td>Total</td>
<td>914 (20.2%)</td>
<td>545 (12%)</td>
<td>1,459 (32.2%)</td>
<td>4,534</td>
</tr>
</tbody>
</table>

given region will enable the development of optimal protective strategies. Until now, two HPV vaccines have been developed and proved to be effective in the prevention of HPV-related cervical neoplasia development. These two vaccines covered HPV6, 11, 16, and 18, thus it could prevent cervical intraepithelial neoplasia, cervical cancer, and genital warts. But in eastern Asia where HPV52 was more prevalent, the current vaccines were not enough. The present data provided some more regional instructions for the development and application of new HPV prophylactic vaccines.

Information about the age-specific prevalence of HPV infection among women is also important for designing vaccination and protective strategies to prevent cervical cancer. In this study, the overall HPV prevalence exhibited a peak status at the youngest age group and the HPV positive rate decreased gradually with age growth. This pattern was in accordance with a typical pattern reported previously [Wheeler et al., 2013] and it was also very common in Asia according to a global statistics [Forman et al., 2012]. It was worth noting that high-risk HPV infections became the predominant infections among HPV positive women with the age growing. This strongly indicated the risk of advanced cervix pathological changes among middle-aged women. Regular HPV detection should be recommended for women with previous HPV infections. Besides, the prevalence of high and low-risk mixed HPV infections exhibited a bimodal U-shaped curve, which was consistent with another typical pattern reported before [Franceschi et al., 2006], with the first major peak at the ≤20 years group and a second minor peak at the >60 years group. Explanation of the first peak appeared in younger women could be insufficient sex education and high-risk sexual behavior. Thus, early sex education is essential for the HPV infection control. While the second minor peak appeared in older women may be reactivation of latent HPV infections or hormonal and immunity changes [Gonzalez et al., 2010].

**TABLE II. Association Between Cervical Cytological Results and HPV Infections**

<table>
<thead>
<tr>
<th>Cytological diagnosis</th>
<th>Pure HR-HPV infection</th>
<th>Pure LR-HPV infection</th>
<th>HR-&amp;#126LR-HPV infection</th>
<th>Total HPV positive samples (%)</th>
<th>No. of HPV negative samples (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NILM</td>
<td>702 (17.0%)</td>
<td>193 (4.7%)</td>
<td>229 (5.5%)</td>
<td>1,124 (27.2%)</td>
<td>3,002 (72.8%)</td>
<td>4,126</td>
</tr>
<tr>
<td>ASCUS</td>
<td>127 (67.5%)</td>
<td>9 (4.8%)</td>
<td>6 (3.2%)</td>
<td>142 (75.5%)</td>
<td>46 (24.5%)</td>
<td>188</td>
</tr>
<tr>
<td>LSIL</td>
<td>104 (79.4%)</td>
<td>4 (3.0%)</td>
<td>3 (2.3%)</td>
<td>111 (84.7%)</td>
<td>20 (15.3%)</td>
<td>131</td>
</tr>
<tr>
<td>HSIL</td>
<td>71 (91.0%)</td>
<td>0</td>
<td>0</td>
<td>71 (91%)</td>
<td>7 (9.0%)</td>
<td>78</td>
</tr>
<tr>
<td>ICC</td>
<td>11 (100%)</td>
<td>0</td>
<td>0</td>
<td>11 (100%)</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

NILM, negative for intraepithelial lesion or malignancy; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer.
High-risk HPV infection is the principal etiological factor for cervical cancer. The severity of cervical cytopathology was in direct relation to increased prevalence of HR-HPV [Schiffman et al., 2007a]. In this study, the HR-HPV prevalence also exhibited an increasing tendency with the aggravation of cervical cytopathology. Among the 4,534 cases in this study, 1,124 cases were HPV positive with normal cytology and 73 cases were HPV negative with abnormal cytology. The explanation of women with a normal cytology but HPV positive could be low viral copy numbers in the early stages of HPV infection. Most HPV infections were transient with clearance mediated by cell-mediated immune mechanisms usually within 6 months [Schiffman et al., 2007b]. Besides, a few women were HPV negative with an abnormal cytology. The abnormality might not be caused by HPV infection but inflammation, hyperkeratosis, or dried skin cells [Schiffman et al., 2007b]. Furthermore, a meta-analysis study indicated that HSIL infected with HPV16, 18, and 45 could progress to invasive cervical carcinoma [Clifford et al., 2003]. In this work, HPV16, 58, and 52 were the most prevalent types both in HSIL and invasive cervical carcinoma. Globally, HPV16, 18, 33, 45, 31, 58, and 52 were the most prevalent HPV types in cervical cancer [Clifford et al., 2003]. But in eastern Asia, HPV58, 52, and 16 were among the five most common types in HSIL and ICC [Bao et al., 2008]. A meta-analysis about prevalence and attribution of HPV52 and 58 in cervical neoplasia also indicated that the attribution of HPV52 and HPV58 to cervical intraepithelial neoplasia and invasive cancer in eastern Asia were respectively 2.5–2.8 and 3.7–4.9 folds higher than elsewhere [Chan et al., 2014]. Therefore, for a given geographical region, knowledge of distribution of HPV genotypes in abnormal cervical cytology, especially advanced stages, is meaningful for effective cervical cancer preventive strategies.

In conclusion, this 3 years surveillance obtained the type-specific HPV prevalence data, age-specific HPV prevalence data and cervical cytological profiles of women in this region. These findings not only enhanced the hypothesis that the next generation HPV prophylactic vaccines including HPV52 and 58 may offer better protection for women in China and other Asian countries, but also provided essential information for determining the specific and effective clinical management strategies for cervical cancer prevention in this region.

ACKNOWLEDGEMENT

The authors acknowledge all the staff who participated in this work at Qingdao Central Hospital and Qingdao Cancer Hospital.

REFERENCES


SUPPORTING INFORMATION
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