Genital human papillomavirus screening by gene chip in Chinese women of Guangdong province

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Introduction

Human papillomavirus (HPV) infections are associated with benign and malignant lesions of cutaneous and mucosal epithelia.1,2 Over 90% of cervical cancer tissues have been demonstrated DNA sequences from high-risk human papillomavirus (HR-HPV) types.3 Specific types of HPV cause the initiating infection that could lead to cervical cancer. Therefore, HPV typing has an important prognostic or therapeutic value, as it can distinguish between HPV types of high and low oncogenic risks. Identification of high-risk HPV genotypes may permit selection of those patients who are at increased risk for disease and may therefore provide additional clinical value. An important requirement for diagnostic approach is that HPV testing and identification of high-risk HPV types should be highly sensitive and specific.4

In China, nearly 200 000 cases of cervical cancer are diagnosed annually, with approximately 20 000 deaths in 2001 alone, and cervical cancer was becoming a major health hazard for women in the countryside.5 There were only a few reports and detailed data about epidemiological research of HPV infection of general population in Guangdong areas. For development of prevention measures against cervical cancer and trials of HPV vaccines aimed specifically at Guangdong population, it was very important to carry out HPV screening in Guangdong women. The commercially available hybrid capture (HCII) assay is used widely in routine analysis of cervical scrapings but does not allow typing of this virus.6,7 This test permits the detection of only the limited number of genotypes. In our study, a flow-through hybridisation and gene chip was used to screen Chinese women for HPV infection.

Here, we reported a population screening of 1705 Chinese women in eastern area (Chaozhou)
of Guangdong province. It allowed an insight into the complex distribution of HPV types in the population.

Methods

Patients

This screening was organised by Chaozhou municipal government, with the cooperation of gynaecological practitioners in three hospitals (Chaozhou Central Hospital, Chaozhou City Hospital and Chaozhou Gynecological and Pediatric Hospital). All studies were approved by Chaozhou Central Hospital Ethics Committee. The purpose was aimed at estimating overall HPV prevalence, type-specific prevalence, age-specific prevalence, and extent of multiple infections. The population eligible to this study included 1705 women (age range from 20 to 68 years). These women received HPV screening from gynaecological practitioners in three abovementioned hospitals between October 2006 and December 2006. The population consisted of hospital staff, policewomen, teachers, workers, civil servants and retirees. The participants were required to fulfil the following criteria: (i) sexually active women of any age, (ii) having no previous histological diagnosis and treatment for gynaecological diseases, (iii) had given birth at least once, and (iv) are willing to undergo HPV testing.

HPV DNA extraction

HPV DNA specimens were collected using plastic cervical swabs. Each plastic swab was mixed well with 1 mL of specimen transport medium (physiological saline) for HPV DNA testing, and stored immediately at 4°C. All swabs and store bottle with specimen transport medium were from Chaozhou Hybribio Biotechnology Limited Corporation, Guangdong, China. All specimens were finally sent to our clinical laboratory for HPV analysis.

The brush and supernatant were removed after the cells were centrifugated for five minutes with relative centrifugal force 9660 g. The sediments obtained were extracted by Alkali lysis using DNA extraction Kits (Chaozhou Hybribio Biotechnology Corp.).

PCR and flow-through hybridisation and gene chip

HPV DNA was amplified with the L1 consensus HPV PGMY09/PGMY11 primer set, as previously described. One microlitre supernatant of HPV DNA extraction was mixed with 24 μL reaction system (Hybribio Biotechnology PCR Kit). The amplification mixture contained 4 mmol/L MgCl₂, 50 mmol/L KCl, 7.5 U of AmpliTaq Gold DNA polymerase, a 200 μmol/L concentration (each) of dATP, dCTP, and dGTP, 600 μmol/L of dUTP, 1 U of uracil-N'-glycosylase, 100 pmol of each biotinylated PGMY primer, and 2.5 pmol each of the 5'-biotinylated β-globin primers GH20 and CP04. HPV was amplified in a KP-TC48 (Chaozhou Hybribio Biotechnology) thermal cycler at 95°C for three minutes, followed by denaturation for 40 s at 93°C, annealing for 40 s at 55°C, and extension at 72°C for 40 s for a total of 40 cycles. Amplification was followed by a seven-minute terminal extension step at 72°C. In some experiments, the polymerase chain reaction (PCR) products were separated by electrophoresis in 2.0% agarose gel.

Genotyping for HPV was done by flow-through hybridisation and gene chip by HybriMax (Chaozhou Hybribio Limited Corporation, Chaozhou, China). The gene chip contained type-specific oligonucleotides immobilised on a nylon membrane. The final results were detected by colourimetric change on the chip under direct visualisation. The chip could identify 13 high-risk HPVs (HR-HPVs) (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), five low-risk HPVs (LR-HPVs) (6, 11, 41, 42, 44) and popular HPV type 53, 66, CP8304 in Chinese population.

ThinPrep liquid-based cytology test

Because of a variety of factors, we could not get ThinPrep liquid-based cytology test (TCT) data of the other two hospitals (Chaozhou City Hospital and Chaozhou Gynecological and Pediatric Hospital). In this paper, all 97 HPV-positive women from Chaozhou Central Hospital were analysed for TCT in 1053 population. The specimens of 97 women were collected, then were mixed with 3 mL specimen stored liquid (Guangzhou Da-An Gene Limited Corp., Guangzhou, China) and stored in room temperature. All the specimens were sent to clinical laboratory of Guangzhou Da-An Gene Limited Corporation for analysis. The results were evaluated using the Bethesda system. The evaluation system included: (i) negative, (ii) atypical squamous cells (ASC), (iii) low-grade squamous intraepithelial lesion, (iv) high-grade squamous intraepithelial lesion, and (v) squamous cell carcinoma.

Histology

Women diagnosed as HPV-positive and have abnormal TCT (with grade higher than ASC) were
referred for biopsy. Specimens of these women were collected by punch biopsy or loop electrosurgical excision procedure cone biopsy. Specimens were fixed in 10% formalin and routinely processed for paraffin embedding. Histological sections cut at 4-μm thickness were stained with haematoxylin and eosin by standard method. Then the cervical biopsy specimens were histologically examined and classified according to the cervical intraepithelial neoplasia) system.

Statistical analysis

Data were analysed using \( \chi^2 \) test, with a value of 0.05 considered the threshold level for significance. All analysis was completed using Microsoft Excel.

Results

PCR products identification

The PCR products for HPV were separated by electrophoresis in 2.0% agarose gel, the products of HPV gene were 450 bp, and \( \beta \)-globin products were 268 bp as internal control (Fig. 1).

Distribution of HPV genotypes

HPV genotypes of the products were identified by flow-through hybridisation and gene chips, positive results were observed in chips as follows (Fig. 2).

The population consisted of 1705 Chinese women in eastern area (Chaozhou) of Guangdong province. The age of participants ranges from 20 to 68 years. Participants were divided into four groups according to their age. Three hundred and twenty-three women were aged between 20 and 30 years, 644 women between 31 and 40 years, 559 women between 41 and 50 years, and 179 women were older than 51 years. In this study, the internal controls of all samples were positive and adequate for HPV analysis. Overall, 154 women (9.03%, 154 of 1705) were HPV DNA positive; 126 (7.39%) women were confirmed as having HR-HPV infection. Among the women with HPV infection, HR-HPV-52 was the most prevalent type (23.37%, 36 of 154), followed by HR-HPV-58 (16.2%, 25 of 154) and LR-HPV-CP8304 (16.3%, 25 of 154) (Fig. 3).

By age distribution, the prevalence of HR-HPV was lowest (3.4%) among women aged 20 to 30 years and progressively increased with age to a peak at 11.17% in group who are older than 51 years; these results present an increasing trend with age (Fig. 4). For women aged 31 to 40 and those aged 41–50, the overall HR-HPV prevalence was 8.07% and 8.23%, respectively, and there was no significance statistically between them, but there was significance statistically between them and other groups (3.4% and 11.7%, \( P < 0.005 \)). Twenty-four women in the study carried multiple genotypes (range two to five genotypes). The multiple-infection prevalence was 1.47% (24 of 1705).
The most popular genotypes were HPV-16 (25%, six of 24), HPV-18 (20.8%, five of 24) and HPV-52 (20.8%, five of 24) in those women. The prevalences of each group for multiple infection were as follows: aged 20–30 group – 0.6% (two of 323); aged 31–40 group – 1.4% (nine of 644); aged 41 to 50 group – 1.6% (nine of 559); and those older than 51 – 2.2% (four of 179). The multiple-infection prevalence of every age group also increased with age.

The results of TCT

Table 1 shows the 97 TCT results in 1053 women that we retrieved in Chaozhou Central Hospital. Furthermore, 98% (95 of 97) women had cervical inflammation.

<table>
<thead>
<tr>
<th>HPV positive</th>
<th>Case (n)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>82</td>
<td>84.5%</td>
</tr>
<tr>
<td>ASC</td>
<td>12</td>
<td>12.4%</td>
</tr>
<tr>
<td>LSIL</td>
<td>2</td>
<td>2.1%</td>
</tr>
<tr>
<td>HSIL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Amount</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

ASC, atypical squamous cells; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

1705); the most popular genotypes were HPV-16 (25%, six of 24), HPV-18 (20.8%, five of 24) and HPV-52 (20.8%, five of 24) in those women. The prevalences of each group for multiple infection were as follows: aged 20–30 group – 0.6% (two of 323); aged 31–40 group – 1.4% (nine of 644); aged 41 to 50 group – 1.6% (nine of 559); and those older than 51 – 2.2% (four of 179). The multiple-infection prevalence of every age group also increased with age.

Discussion

This study reports a large-scale evaluation of HPV DNA testing for Chinese population in the primary cancer screen program in Chaozhou, which is located at eastern area of Guangdong province of China. In order to increase the reliability, TCT and histology were added to screen the cervical cancer.

Seminested PCR and reverse hybridisation for the detection and genotyping of HR-HPV have been well described previously. In our study, a method that combined flow-through hybridisation with gene chip technology was used to screen 21 kinds of HPV genotypes. There were three kinds of most popular HPV genotypes for Chinese in the chip, so it was more suitable for HPV screening in Chinese women. The overall prevalence of HPV in the population was 9.03% of our study, and is higher than that detected in Hong Kong (7.13%). But the result was much lower than that in other areas such as Taiwan (19.3%), Colombia (14.9%) and Paraguay (20%).

Most of the previous reports showed a declining prevalence of HPV infection with increasing age in population screening. The subjects in the countries or areas such as Colombian, Japan and Brazil, were greatly affected by west sexual culture. However, an opposite tendency was found in our study. It was an interesting finding. He et al. had reported the similar results from Shenzhen of Guangdong Province. Cervical HPV infection generally occurred soon after sexual debut in young women, and it decreased with age. This reason had been demonstrated in many previous studies worldwide. However, in our study, it was found that the prevalence of HR-HPV s progressively increased with age. The reasons for the increasing prevalence of HPV infections with age were presumably the following. (i) In eastern Guangdong Province, Chaozhou, a traditional Confucian culture influential place, most women had only one sexual companion in their life, so the probability of HPV infection was lower than that in other areas. (ii) Because of power immunity, HPV infection in young women was spontaneously cleared within two years after the infection. With age, the infection opportunity increased, another explanation for infection peak in older women was the cohort effect or reactivation of a latent HPV infection in the setting of declining immunity among older population.

In three patients who are HPV-positive and have abnormal TCT (Table 1), we found two cervical cancers. The positive ratio was 0.19% (two of 1053). The genotype of the two women was HR-HPV 52.
Clinical trials of preliminary vaccines in humans look very positive, demonstrating that an HPV vaccine can prevent HPV infection and precancerous lesions in women.13 These data provide hope that an HPV vaccine may be a reality within five to ten years. Public health officials will then need to understand the type-specific HPV prevalence in a region as a vaccine would offer little cross-protection between various HPV types.22 We know that HPV-16 is now considered the most predominant subtype in countries around the world, followed by HPV-18 and 45.23 Therefore, most HPV vaccines nowadays are being designed against both HPV-16 and HPV-18.24 However, HR-HPV 52 and HR-HPV 58 were the highest in Chinese population in our research. The similar results were reported in Hong Kong and Taiwan.13,14 The data indicated that the ascendant types in eastern Asia were not the same as ascendant types of other areas in world. Those studies also demonstrated these two kinds of HPV genotype detection, and vaccines development was very important in Chinese population. It was necessary to carry out prophylactic work to protect women from getting infected with the two kinds of HR-HPV.

Twenty-four women in our study carried multiple genotypes (range, two to five genotypes). The multiple-infection prevalence was 1.47% (24 of 1705). The most popular genotypes were HPV-16 (25%, six of 24), HPV-18 (20.8%, five of 24) and HPV-52 (20.8%, five of 24) in those women. Multiple prevalence of every group increased with age. Prevalence in group who were older than 51 (2.2%, four of 179) was higher than in other group (0.6%, 1.4%, 1.6%). The result was lower than that in other Asian countries such as Taiwan.13 According to our knowledge, with age, the degree of people’s immunocompetence decreased, so the frequency of multiple HPV genotypes increased.

TCT was used to detect HPV-positive women in our research. According to previous studies, the two-method combination could increase the detection rate of cervical cancer and was the optimal approach for diagnosis of early cervical cancer and precancer lesions.25,26 Our data that could be taken in 1053 women were analysed, in 3 TCT and HPV positive women, two progressive cervical cancers were detected.

In conclusion, this study provides information about the prevalence of HPV and distribution of HPV genotypes in Chinese population in eastern area of Guangdong. Furthermore, it provided information of different age distribution from other areas in world. It could be useful for future trials of HPV vaccines aimed at Chinese population.

References


14 Lin H, Ma YY, Moh JS et al. High prevalence of genital human papillomavirus type 52 and 58 infection in women attending gynecologic practitioners in South Taiwan. *Gynecol Oncol* 2006; 101: 40–45.


