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Prevalence and Genotype Distribution of Human Papillomavirus in Cheonan, Korea

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Human papillomavirus (HPV) infection is considered to play a critical role in the development of cervical carcinoma, which is the third most common cancer among Korean females. Here, we performed a baseline study of HPV infection and genotyping using an HPV DNA chip, which is a type of oligonucleotide microarray. A total of 6,855 cervical swab specimens from 5,494 women attending Dankook University Hospital Health Improvement Center in Cheonan, Korea between 2006 and 2012, originally collected for HPV infection screening, were genotyped for HPV. The extracted DNA from the cervical specimens was investigated by an HPV DNA chip designed to detect 41 different HPV types. HPV was identified as positive in 1,143 (16.7%) of the 6,855 samples. The most frequently detected HPV genotypes were HPV types 16, 53, 56, 58, 39, 52, 70, 84, 68, 62, 35, 54, 81, 18, and 30, in descending order of incidence. The proportions of single and multiple HPV infections in the HPV-positive specimens were 78.1% and 21.9%, respectively. The average age of HPV-positive patients was 39.9 years, with the positive rate of HPV being the highest in the 10–29 age group (20.6%). We report here on the prevalence and distribution of 41 different genotypes of HPV according to age among women in Cheonan, Korea. These data may be of use as baseline data for the assessment of public health-related issues and for the development of area-specific HPV vaccines.

Keywords: HPV DNA chip, HPV genotyping, prevalence, HPV type 58

Introduction

After breast cancer, cervical cancer is the second most common malignancy in women [11] and the leading cause of cancer-related deaths in females worldwide [24, 15]. Overall, in Korea, the incidence rate of cervical cancer is below only that of breast cancer, gastric cancer, and colorectal cancer, with over 5,000 new patients diagnosed every year [23].

Human papillomavirus (HPV) infection is the most common sexually transmitted infection around the world [2, 13], and epidemiological reports, as well as clinical studies, have clearly established HPV infection as the main cause of cervical cancer [8, 18]. At present, more than 100 HPV genotypes have been found and classified by molecular epidemiologic studies [17]. According to previous reports, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -69, -73, and -82 are classified as "high-risk types", while HPV-6, -11 -42, -43, and -44 are classified as 'low-risk types' [2, 5]. High-risk types of HPV are considered oncogenic, as they are found in more than 90% of cervical tissue samples of patients with cervical carcinoma or cervical intraepithelial neoplasia, whereas low-risk types generally only cause benign lesions [14]. Among the high-risk HPV types, HPV 16 and 18, which cause approximately 70% of all cervical cancers [15, 10, 26], are the most prevalent types in patients with cervical intraepithelial neoplasia and cervical carcinoma [1, 25], whereas the low-risk types HPV-6 and -11 are responsible for the majority of cases of genital warts and condylomas [15, 19]. Continuous and persistent exposure by the high-risk types of HPV genotypes is reported to increase the risk of severe dysplasia, cervical

cancer, and other anogenital malignancies [10, 19, 20], and these HPV genotypes, which are detected in more than 90% of all cervical cancer cases, are now classified as human carcinogens. Therefore, mortality due to cervical cancer can be lowered if it is diagnosed at the precancerous stage by a proper screening test for HPV and promptly treated [22]. Because the distribution and prevalence of HPV vary by geographic region and the immunity conferred by vaccines is type-specific, the need for population-specific HPV genotyping routine screenings is increasingly recognized [14]. Accordingly, identification of the precise HPV genotype in diagnostic practices is highly important to ensure accurate diagnosis and prognosis, as well as effective monitoring and therapeutic options of the disease [21, 6]. HPV genotyping helps in understanding the cause of HPV infection and progress of the disease; in addition, it can be used as a health-screening test to prevent cervical cancer in asymptomatic women [27].

In this study, we report on the distribution of HPV genotypes and the extent of multiple HPV infections in women from Cheonan, Korea, with no obstetric and gynecologic symptoms, in order to identify the frequency of the HPV genotypes in this population. To the best of our knowledge, this is the first description of epidemiologic data on HPV genotypes in a general female population from a local area in Korea using the HPV DNA chip. The results from our analysis will provide essential information for planning prevention by HPV vaccines and for public health programs based on HPV testing.

Materials and Methods

Specimen Collections

A total of 5,494 Korean women aged 19–78 years who attended the Health Improvement Center at Dankook University Hospital at Cheonan, Korea for an HPV genotyping test between October 10, 2006 and December 15, 2012 were included in this study. A total of 6,855 cervical swab specimens were obtained from the patients using a Digene cervical brush, and transferred into vials containing 1 ml of specimen transport medium (Qiagen, Hilden, Germany).

Nucleic Acid Extraction

The vials containing the cervical specimens and specimen transport medium were vortexed to dissociate the cells, and centrifuged at 10,000 rpm for 5 min. After removing the supernatant, DNA was extracted from 200 μl of the cervical cell specimens using the Chemagic Viral DNA/RNA Kit (PerkinElmer Chemagen, Rodgau, Germany) and Chemagic Magnetic Separation Module I automated nucleic acid isolation system (PerkinElmer Chemagen),

according to the manufacturer's protocol for DNA extraction.

HPV Genotyping

For each sample, HPV detection and genotyping were performed using the HPV DNA Chip (Goodgene, Seoul, Korea), a polymerase chain reaction (PCR)-based oligonucleotide microarray system. The HPV DNA Chip contains 41 type-specific probes that recognize 22 high-risk (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68a, 68b, 69, 70, 73, and 82) and 19 low-risk HPV types (6, 11, 30, 32, 34, 40, 42, 43, 44, 54, 55, 61, 62, 72, 81, 83, 84, 90, and 91). Genomic DNA amplification, labeling, hybridization, and analysis were performed according to the manufacturer's instructions. Briefly, the PCR cycle to amplify the extracted DNA was as follows: after a 5 min pre-denaturation at 94°C, a cycle of 30 sec at 94°C, 30 sec at 50°C, and 30 sec at 72°C was repeated 40 times. Finally, the final extension was performed at 72°C for 5 min.

The PCR product (10 μl) was mixed with 50 μl of distilled water in a new tube and denatured at 95°C. Subsequently, the tube was immediately chilled on ice and spun down at 10,000 rpm for 30 sec before being mixed with 65 μl of the HPV DNA hybridization solution. The HPV DNA hybridization solution was dispensed to each chip plate and the hybridization reaction was performed at 48°C for 30 min. After the reaction was complete, the HPV DNA chip plate was washed thoroughly, and the hybridization signals were visualized and analyzed with a GenePix Personal 4100A scanner (Axon Instruments, USA). The risk attribution of the HPV genotypes was performed as previously described [10].

Results

This retrospective study was carried out on 5,494 patients referred for a HPV test through a health screening to the Dankook University Hospital between October 10, 2006 and December 15, 2012. During the study period, 6,855 specimens were collected and tested for HPV genotyping. The total number of HPV-positive specimens was 1,441. A total of 789 patients (14.4%) and 1,143 specimens (16.7%) were found to be positive for HPV.

We next explored the prevalence and distribution of individual HPV genotypes among these 789 women (Fig. 1). Among the 1,143 detected viruses, HPV-16 was the most prevalent type (106/1,141 specimens; 7.36%), followed by HPV-53 (101/1,141 specimens; 7.01%), HPV-56 (96/1,141 specimens; 6.66%), HPV-58 (76/1,141 specimens; 5.27%), and HPV-39 (75/1,141 specimens; 5.20%).

The overall prevalence by age is reported in Table 1. The average age of all patients referred for diagnosis was 39.9 years (range, 19.6–78.6 years), and the average age of HPV-positive patients was comparable. The age-dependent positive rate relative to the referral rate was the highest for the 10–29 age group (20.43%), followed by the 60–79 age
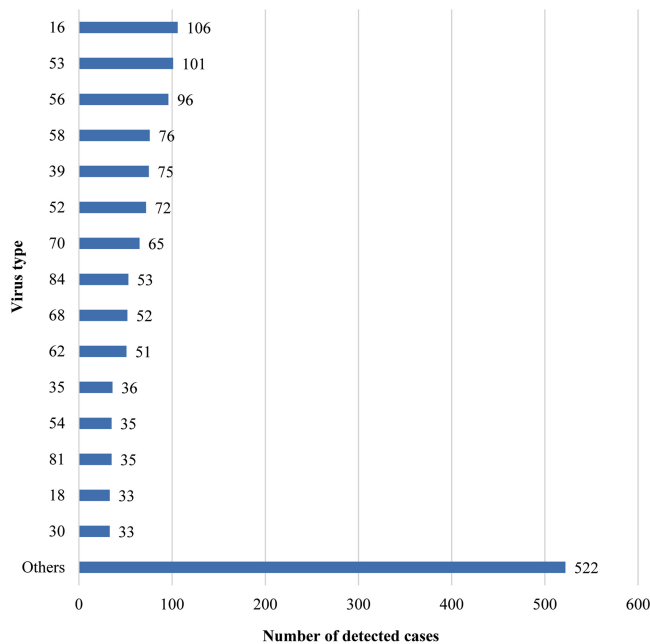
Fig. 1. Number of specimens infected with various human papillomavirus (HPV) types.
Each reported HPV type includes both single and multiple infections. HPV-16 was the most prevalent HPV type in HPV-infected women, followed by HPV-53, HPV-56, HPV-58, HPV-39, and HPV-52.

Table 1. Distribution of human papillomavirus-positive specimens by age.

Age (years)	No. of specimens	No. of positive specimens (%)
10–29	323	66 (20.43)
30–39	3702	607 (16.40)
40–49	1932	316 (16.36)
50–59	644	104 (16.15)
60–79	254	50 (19.69)

group (19.69%) (Table 1).

The prevalence of the different types of HPV according to the number of concomitant infections of HPV is summarized in Table 2. Two hundred-fifty multiple infections (21.87%) were detected in the 1,143 positive specimens, as compared with 893 cases (78.13%) of single HPV infection (Table 2). Fig. 2 shows the age-specific incidence of single and multiple infections of HPVs in the HPV-positive groups. Among the positive cases, the single infection rate was the highest in the 30–39 year group (78.70%), whereas the multiple infection rate was highest for patients in the 10−29 year group (27.30%). Overall, the proportions of single and multiple infections of HPV were

Table 2. Single and multiple infections of human papillomavirus.

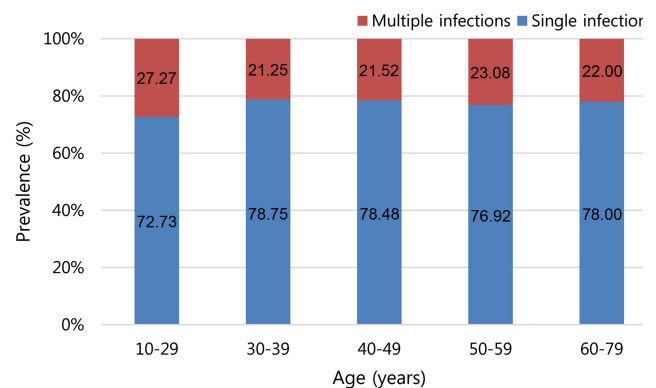| Infection type	No. of specimens	Proportion of positive specimens (%)
Single infection	893	78.13
Multiple infection	250	21.87
Double infection	210	18.37
Triple infection	32	2.80
Quadruple infection	8	0.70
Total	1143	100.00

Fig. 2. Proportion of single and multiple infections of human papillomavirus (HPV) in the HPV-positive groups according to age.
There were no significant differences in the overall prevalence of single and multiple infections between the age groups.

Table 3. Mean age of patients according to human papillomavirus (HPV) types.

HPV type	No. of patients	Mean age of patients (years)
HPV-35	36	38.0 (28.8–65.9)
HPV-39	75	38.7 (21.6–70.6)
HPV-70	65	39.0 (28.2–74.1)
HPV-18	33	39.1 (29.4–65.3)
HPV-53	101	39.1 (26.9–67.9)
HPV-58	76	39.4 (25.6–71.0)
HPV-62	51	39.6 (29.7–64.7)
HPV-16	106	40.0 (29.7–68.2)
HPV-52	72	40.0 (29.3–74.1)
HPV-54	35	40.9 (29.6–63.1)
HPV-56	96	40.9 (28.9–64.1)
HPV-84	53	40.9 (28.9–71.8)
HPV-30	33	41.4 (29.7–66.0)
HPV-68	52	42.4 (29.7–71.2)
HPV-81	35	43.8 (29.5–73.5)
Other	249	42.5 (28.1–69.3)

similar in all age groups (approximately 72–79% and 21–27%, respectively; Fig. 2).

Lastly, the prevalence and distribution of the specific HPV types were analyzed according to age (Table 3). Among the 1,143 detected viruses, HPV-16 was detected the most frequently (106 patients), followed by HPV-53 (101 patients), HPV-56 (96 cases), HPV-58 (76 cases), HPV-39 (75 cases), and HPV-52 (72 cases). No differences in age were noted between patients infected with the different HPV types.

Discussion

In 2012, Chen *et al.* [5] reported that the HPV-positive rate among healthy Chinese women was approximately 7.89%, whereas a 40.3% positive rate was reported in Kenya in 2010 [9], a 35.6% positive rate was reported in Croatia in 2001 [12], and a 21.7% positive rate was reported in Japan in 2009 [15]. As for Korea, positive rates of 17.6% and 19.2% were reported in 2011 and 2012 [17], respectively, which are similar to the 16.7% positive rate observed in this study.

Gargiulo *et al.* [11] reported that the multi-infection frequency is high in women aged less than 35 years, and Grinsztejn *et al.* [13] reported that young age is an important risk factor for HPV infection and that the prevalence rate decreases with age. Similarly, in Korea, Chung *et al.* [7] reported that the positive rate was the highest in women below 39 years old (27.7%), and Kim *et al.* [17] reported that women in their twenties show twice the rate of HPV positivity (40.5%) compared with women aged over 30 years. In accordance with these previous reports [7, 17], in this study, the prevalence rate in women aged 10–29 years was found to be 20.43%, which was higher than that for the older age groups. The average age of HPV-positive patients reported by Kim *et al.* [17] and Vidal *et al.* [26] was 40.1 ± 6.8 years and 40.3 ± 9.9 years, respectively; and the average age in this study was 39.9 years, which is similar to these previous studies.

HPV genotypes are classified into high-risk and low-risk groups depending on their cancer-causing capability, with the high-risk group having been reported to be an essential factor for the development of cervical cancer and precancerous lesions [3, 4]. Worldwide, among the high-risk group, HPV-16 is generally reported to show the highest infection rate, followed by HPV-18 [24]. However, several studies on HPV distribution in Asia and Korea have shown different results from those in the USA or Europe [1, 6, 8, 14]. For example, more HPV-58 than HPV-

18 cases were reported in this study, with HPV-58 and HPV-52 being the most common high-risk types after HPV-16. Similarly, the order of the HPV detection frequency in China in 2012 [5] was HPV-52, -16, and -58; whereas the order reported in Korea was HPV-16, -18, and -58 [7]; and the order reported by Shin *et al.* [24] in 2012 was HPV-16, followed by HPV-58 and 61. The HPV detection frequency order in this study conducted in the Cheonan region was types 16, 53, 56, and 58, which is very different from the reported HPV genotype distributions in USA and Europe. Thus, ethnicity-dependent sensitivity seems to be an important factor in the HPV detection frequency and distribution, in addition to the study subjects and test methods [24].

As mentioned, in this study, the most frequently detected virus genotype after HPV-16 was HPV-58, which is classified as a potential high-risk type by the World Health Organization. This is because there are only limited reports on HPV-58 in Europe and USA where its detection frequency is low relative to that in Asia, and this means that diverse and continuous studies in Korea and other areas of South East Asia are needed.

After the development of vaccines targeting HPV-16 and HPV-18 among the high-risk HPV genotypes, the Korea Food and Drug Administration approved the use of Gardasil™ in June 2007 and Cervarix™ in 2008 [7], and continuous studies on these are being performed [16]. It is judged that there will be a change in the future HPV prevalence and genotype distributions, and, after analyzing the specific HPV genotypes that are prevalent in any given area, development of preventive vaccines appropriate for these specific areas will be necessary.

Acknowledgments

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