Human Papillomavirus in Thai Women and Men with Anogenital Warts

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Abstract

Objective: Anogenital warts are caused by human papillomavirus (HPV). Globally, HPV genotypes 6 and 11 are most often associated with anogenital warts. However, the diversity of HPV genotypes found in patients with genital warts in Thailand is unknown. The aim of this study was to investigate HPV-associated anogenital warts in the Thai population and to assess whether genotypes found are represented in the bivalent and quadrivalent HPV vaccine. Methods: This study included 206 anogenital swab samples from patients who were diagnosed with anogenital warts. Detection of HPV DNA was performed using polymerase chain reaction to amplify the L1 gene and sequencing. Results: HPV was identified in 88.3% (182/206) of the samples. The majority of HPV genotypes were low-risk genotypes HPV6 (36.9%) and HPV11 (36.4%), which represented the most common infection found in genital warts in this study. Conclusion: Immunization with the quadrivalent vaccine (HPV6, HPV11, HPV16, and HPV18) could potentially prevent genital warts caused by HPV infection.

Introduction

Human papillomavirus (HPV) is most commonly transmitted through sexual contact. There are more than 100 HPV genotypes, and at least 40 genotypes are associated with infection in the anogenital area [1], the most common benign tumor in the anogenital region [2]. Typical anogenital warts appear as flesh-colored, exophytic lesions on the external genitalia, including the penis, scrotum, vulva, perineum, and perianal skin [3]. The highest occurrence of genital warts has been reported in individuals between 20 and 24 years of age [4]. Globally, genital warts are estimated to affect approximately 1% of the human population [5], although the incidence in sexually transmitted disease clinics is approximately 13% [6]. Although genital warts are benign, they may cause discomfort and affect psychological well-being because they are often untreated [7–9].

Risk factors for genital warts include multiple sexual partners, anal intercourse, immunodeficiency, tobacco use, ultraviolet radiation exposure, and pregnancy [1, 10]. As many as 50% of men who have had sex with men (MSM) with squamous cell carcinoma of the anus have a history of anorectal warts, while only 20% of women and heterosexual men have this reported history [10]. The
treatment of genital warts is often painful, expensive, and unsuccessful [11].

HPV genotypes 6 and 11 often infect the mucosa and skin of the anorectum and genitalia. They are considered low-risk genotypes due to their low oncogenic potential, and infections often result in genital warts or low-grade precancerous lesions [12]. HPV genotypes 31, 33, 35, 39, 40, 43, 45, 51–56, and 58 all have oncogenic potential, while HPV genotypes 16 and 18 are high-risk HPV genotypes associated with the development of high-grade intraepithelial neoplasia, carcinoma of the anus, vulva, vagina, cervix, and penis, and head and neck cancer [13].

HPV-related anogenital warts are assessed clinically by anoscopy, skin biopsy, and by HPV DNA detection [1, 14, 15]. Dacron swab is a simple method of collecting anogenital wart samples [16]. Immunization with a bivalent vaccine (containing HPV16 and 18) (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or the quadrivalent vaccine (Gardasil, Merck Sharp and Dohme Corp., NJ, USA) (containing HPV16, 18, 6, and 11) can provide excellent protection against the HPV types comprised in the vaccine. The quadrivalent vaccine has also demonstrated significant cross-protection to other oncogenic types in both naive and previously HPV-infected individuals [17]. Thailand has recently implemented nationwide two-dose HPV vaccination among grade 5 schoolgirls, whose average age is approximately 11 years. Both the bivalent and the quadrivalent vaccines are used depending on availability of the vaccine. A recently approved nonavalent vaccine (Gardasil9, Merck Sharp and Dohme Corp., NJ, USA) containing HPV16, 18, 6, 11, 31, 33, 45, 52, and 58 in the USA has been recommended for previously unvaccinated MSM and immunocompromised patients through the age of 26 years [18]. Here, we characterized the genotypes of HPV detected in Thai patients with anogenital warts to determine whether these genotypes are represented in the vaccine.

Materials and Methods

This cross-sectional analysis used data from enrollment visits of a cohort study at King Chulalongkorn Memorial Hospital in Bangkok, Bang Rak Sexually Transmitted Infections Center in Bangkok, and Chiang Mai University Hospital in Chiang Mai. This study was approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University (IRB No. 509/57). Written informed consent was obtained from all participants. The specimens were anonymized by number codes.

Study Population

Individuals older than 18 years of age who came for medical check-up visits at the hospitals were recruited between December 2014 and October 2015. A total of 206 specimens were categorized into three groups: females (n = 69), males (n = 91), and MSM (men who self-reported to have sex with other men or who were bisexual) (n = 46). All of the patients had anogenital warts (condylomatosa acuminata, genital warts, anal warts). Patient demographics, clinical, and behavioral data were extracted from medical records and questionnaires.

Sample Collection and Preparation

All anogenital wart swab samples were collected by using Flexible minitip flocked swab (Copan Diagnostics, Murrieta, CA, USA). Swabbed specimens were added to 1 mL of phosphate-buffered saline, vortexed, and stored at −20 °C until tested.

HPV Detection and Genotyping

The total viral nucleic acid was extracted from 100 µL of clinical specimens using the Qiap DNA mini kit (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions. HPV DNA was amplified using polymerase chain reaction (PCR) and MY/GP universal primer sets specific to the L1 gene [19]. The PCR reaction mixture contained 2 µL DNA template, 0.5 µL of 10 mmol of each primer, 15 µL of 2.5× PerfectTaq Plus MasterMix (5 PRIME Inc., Hamburg, Germany), and sterile distilled water to a final volume of 25 µL [20]. For the first PCR amplification, cycling parameters were initial denaturation at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 s (denaturation), 55 °C for 45 s (annealing), 72 °C for 1.30 s (extension), and a final extension at 72 °C for 7 min. The second PCR amplification was identical except that annealing was at 50 °C for 45 s. The β-globin gene served as an internal control and was successfully amplified from all samples. Primer sequences of the β-globin gene have been previously described [21]. The reaction mixture contained 2 µL DNA template, 0.5 µL of 10 mmol of forward and reverse primer, 15 µL of 2.5× PerfectTaq Plus MasterMix (5 PRIME Inc., Hamburg, Germany), and sterile distilled water to a final volume of 25 µL. The amplification reactions were performed in thermal cycler (Eppendorf, Hamburg, Germany) under the following condition: denaturation at 94 °C for 3 min, followed by 40 amplification cycles consisting of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, and final extension at 72 °C for 7 min. All amplified HPV PCR products were separated using 2% agarose gel electrophoresis and purified using an Expin Gel SV kit (GeneAll, Seoul, South Korea). HPV was genotyped by Sanger sequencing using GP5+ primer. Sequences were analyzed using the BLAST program on the GenBank Database (www.ncbi.nlm.gov/BLAST).

Statistical Analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). χ² and odds ratio tests were used to assess the statistical significance of any factor, which may be related with anogenital warts in each group. Statistical significance was defined as p < 0.05.

Results

The average age of patients in this study was 28.9 years (Table 1). The average age of female patients in this study was 28.7 years (range, 15–49 years). The average age of
Male and MSM patients was 30.9 (range, 16–58) and 27.2 years (range, 18–54), respectively. HPV was detected in 88.3% (182/206) of all samples. HPV positivity was highest in MSM (95.7%, 44/46). In this study, the relationship between alcohol consumption, smoking status, and number of sexual partners and HPV positivity was not statistically significant ($p > 0.05$). HPV6 and HPV11 were the most frequent genotypes in all groups (Fig. 1). The most frequently detected HPV genotypes among 206 samples were HPV6 (76/206, 36.9%), HPV11 (75/206, 36.4%), HPV16 (13/206, 6.3%), HPV18 (5/206, 2.4%), and HPV66 (2/206, 1.0%). Regardless of sexual orientation, men were more likely to be infected with HPV11. In contrast, HPV6 was more frequently detected in women than men. Self-identified straight men and women were more likely to have HPV16 than MSM.

### Table 1: Association of risk factors with HPV infection

<table>
<thead>
<tr>
<th></th>
<th>Mean age, years</th>
<th>HPV positive</th>
<th>Alcohol consumption</th>
<th>Odds ratio</th>
<th>$p$ value</th>
<th>Smoking</th>
<th>Odds ratio</th>
<th>$p$ value</th>
<th>Sex partner &gt;1</th>
<th>Odds ratio</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases (n = 206)</td>
<td>28.9</td>
<td>182 (88.3%)</td>
<td>89 (43.4%)</td>
<td>1.128</td>
<td>0.782</td>
<td>32 (14.1%)</td>
<td>1.511</td>
<td>0.446</td>
<td>64 (32.1%)</td>
<td>0.903</td>
<td>0.830</td>
</tr>
<tr>
<td>Female (n = 69)</td>
<td>28.7</td>
<td>61 (88.4%)</td>
<td>31 (44.9%)</td>
<td>0.890</td>
<td>0.951</td>
<td>8 (11.6%)</td>
<td>0.859</td>
<td>0.723</td>
<td>20 (22.0%)</td>
<td>0.405</td>
<td>0.730</td>
</tr>
<tr>
<td>Male (n = 91)</td>
<td>30.9</td>
<td>77 (84.6%)</td>
<td>38 (41.8%)</td>
<td>0.806</td>
<td>0.663</td>
<td>4 (8.7%)</td>
<td>0.815</td>
<td>0.830</td>
<td>9 (9.9%)</td>
<td>0.405</td>
<td>0.560</td>
</tr>
<tr>
<td>MSM (n = 46)</td>
<td>27.2</td>
<td>44 (95.7%)</td>
<td>20 (43.5%)</td>
<td>1.136</td>
<td>0.813</td>
<td>20 (43.5%)</td>
<td>1.272</td>
<td>0.323</td>
<td>9 (9.9%)</td>
<td>0.405</td>
<td>0.820</td>
</tr>
</tbody>
</table>

### Discussion

HPV genotype distribution studies of genital warts in the Thai population and within the Southeast Asian region are limited. A previous study on genital warts in Thailand found that around 40% of women presenting with external anogenital warts who were not immunocompromised had recurrent warts within 6 months after completing the treatment [22]. Approximately 90% of genital warts are caused by low-risk genotypes HPV6 and HPV11 [23]. A screening of HPV in patients with genital warts showed that 88.7% in China, 75.0% in Spanish men, and 93.3% in Argentinian women were positive [16, 24, 25]. Although there are no reliable prevalence data of HPV infection in men with genital warts, a low-risk genotype such as HPV6 in men has been reported [26]. In this study, HPV6 and HPV11 genotypes were most commonly detected (155/206, 75.2%) [27]. This finding was simi-
<table>
<thead>
<tr>
<th>Country</th>
<th>Sample type</th>
<th>Total sample size (M/F)</th>
<th>Mean age/ range, years</th>
<th>HPV positive</th>
<th>Most frequent genotype, %</th>
<th>Other genotypes (HR/LR)</th>
<th>HPV detection assay</th>
<th>Collection method</th>
<th>Collection method</th>
<th>Study [Ref.], year</th>
</tr>
</thead>
<tbody>
<tr>
<td>France CA</td>
<td>CA</td>
<td>516 (260/256)</td>
<td>30.0/18–72</td>
<td>99.0%</td>
<td>69.0</td>
<td>16.0</td>
<td>9.0</td>
<td>INNO-LiPA assay</td>
<td>cytobrush</td>
<td>Aubin et al. [26], 2008</td>
</tr>
<tr>
<td>Sweden CA</td>
<td>CA</td>
<td>621 (576/45)</td>
<td>28.4/15–69</td>
<td>96.3%</td>
<td>61.7</td>
<td>10.3</td>
<td>12.9</td>
<td>Luminex assay</td>
<td>cytobrush</td>
<td>Sturegård et al. [49], 2013</td>
</tr>
<tr>
<td>UK GW</td>
<td>ND</td>
<td>31 (ND)</td>
<td>ND</td>
<td>100.0%</td>
<td>90.0</td>
<td>32.0</td>
<td>ND</td>
<td>linear array</td>
<td>biopsy</td>
<td>Ball et al. [50], 2011</td>
</tr>
<tr>
<td>Spain GW</td>
<td>GW</td>
<td>184 (184/0)</td>
<td>12.6/17–72</td>
<td>75.0%</td>
<td>51.6</td>
<td>17.7</td>
<td>42.3</td>
<td>linear array</td>
<td>Dacron swab</td>
<td>Arroyo et al. [16], 2016</td>
</tr>
<tr>
<td>South Korea GW</td>
<td>GW</td>
<td>40 (80/0)</td>
<td>35.4/ND</td>
<td>57.3%</td>
<td>51.6</td>
<td>17.7</td>
<td>42.3</td>
<td>linear array</td>
<td>Dacron swab</td>
<td>Kwon et al. [51], 2016</td>
</tr>
<tr>
<td>Czech Republic CA</td>
<td>CA</td>
<td>64 (ND)</td>
<td>ND/15–69</td>
<td>90.6%</td>
<td>71.9</td>
<td>20.3</td>
<td>1.6</td>
<td>PCR and reverse line blot hybridization</td>
<td>cervical scrape, biopsy</td>
<td>Tacherty et al. [52], 2011</td>
</tr>
<tr>
<td>Denmark GW, AW</td>
<td>201 (124/58)</td>
<td>270/18–85</td>
<td>74.0%</td>
<td>67.6 GW</td>
<td>7.1 GW</td>
<td>16.5 GW</td>
<td>2.0</td>
<td>PCR (modified MGP primer) and Luminex-based HPV genotyping</td>
<td>cytobrush</td>
<td>Kofod et al. [53], 2014</td>
</tr>
<tr>
<td>Italy CA</td>
<td>CA</td>
<td>109 (99/10)</td>
<td>26.1</td>
<td>73.0%</td>
<td>49.0</td>
<td>67.0</td>
<td>ND</td>
<td>PCR/GP5/GP6 and type-specific PCR, HPV6, 11</td>
<td>biopsy</td>
<td>Jatlineski et al. [15], 2012</td>
</tr>
<tr>
<td>USA CA</td>
<td>CA</td>
<td>39 (27/12)</td>
<td>28.1 M/ND</td>
<td>100.0%</td>
<td>94.0</td>
<td>8.0</td>
<td>ND</td>
<td>PCR MY09/11</td>
<td>Dacron swab</td>
<td>Greer et al. [54], 1995</td>
</tr>
<tr>
<td>China GW</td>
<td>GW</td>
<td>1.005 (440/56)</td>
<td>32.0/17–86</td>
<td>88.7%</td>
<td>41.3</td>
<td>37.6</td>
<td>10.4</td>
<td>PCR MY09/11, GP5+/6+</td>
<td>biopsy</td>
<td>Chang et al. [24], 2013</td>
</tr>
<tr>
<td>Argentina GW</td>
<td>GW</td>
<td>140 (90/10)</td>
<td>ND/15–45</td>
<td>93.3%</td>
<td>80.0</td>
<td>12.7</td>
<td>2.0</td>
<td>PCR MY09/11, GP5+/6+</td>
<td>biopsy</td>
<td>Fleider et al. [25], 2016</td>
</tr>
<tr>
<td>Thailand GW, AW</td>
<td>206 (137/69)</td>
<td>29.2/15–58</td>
<td>88.30%</td>
<td>37.9</td>
<td>37.4</td>
<td>6.8</td>
<td>8.8</td>
<td>PCR MY09/11, GP5+/6+</td>
<td>flocked swab</td>
<td>Present study</td>
</tr>
</tbody>
</table>

CA, condylomata acuminata; GW, genital wart; AW, anal wart; M, male; F, female; ND, no data; HR, high risk; LR, low risk; MGP, modified GP5+/6+ primer. Italics indicate high-risk genotype.
lar to studies from the USA, which found HPV6 or HPV11 in 74–100% of genital wart samples [28, 29] (Table 2). Different HPV prevalence in published studies is likely due to variances in sampling techniques, study population, collection sites sampled, and the sensitivity of HPV DNA detection methods used [29]. Moreover, a higher rate of HPV detection resulted when samples were collected from a greater number of anatomical sites [30].

The risk of anogenital warts becoming cancerous may be related to the oncogenic potential of HPV. Although smoking, alcohol consumption, and sexual behavior (e.g., male homosexuality, higher sexual activity, or multiple sexual partners) are also known to be involved in oncogenic risk factors, they may be confounders [31–33]. In this study, we showed that smoking, alcohol consumption, and the number of sexual partners were not significantly associated with HPV DNA positivity in the genital wart samples. The result contrasted with a previous study [34–37] because of the limitation in specimen collection. Smoking is associated with weakened immune response against viral infections, and more smokers are HPV positive than nonsmokers [38]. Additionally, high intake of alcohol was significantly associated with an increased risk for HPV infections among men [33]. Alcohol is a potent modulator of immune function, which can lead to immune deficiency and increased susceptibility to various chronic and infectious diseases [39, 40]. Furthermore, multiple lifetime sexual partners increase the likelihood of oral-genital HPV co-infection [32].

Laryngeal papillomas can be found in infants and children who may have contracted HPV from their mothers during childbirth, although possible mechanisms of vertical transmission are not well understood. The prenatal transmission of HPV is also supported by the presence of HPV lesions in some infants at the time of birth [41]. HPV transmission from women without clinical evidence of HPV to the neonate is estimated to be between 1 and 18% [42, 43]. In women who had detectable HPV during pregnancy, the transmission rate ranged from 5 to 72% [41, 43, 44]. The laryngeal papilloma is not immediately apparent and may be quite difficult to diagnose until they become symptomatic. The HPV genotypes most often found in laryngeal papilloma were HPV6 and 11 [45–47]. Thus, preventing HPV6 and HPV11 infection can reduce the incidence of genital warts.

The HPV16, 18, and 45 are among the most prevalent types of HPV associated with cervical cancer worldwide [48]. The quadrivalent vaccine, which includes HPV6 and 11, may prevent infection in 84.5% of our patient population. The bivalent vaccine also contains the high-risk HPV16 and 18, genotypes found in the genital wart specimens in this study, and could theoretically prevent infection in an additional 9.2% of our patient population. Thus, Gardasil9 or Gardasil vaccination may prevent infection in 84.5%.

This study has several limitations. Our samples were derived from patients from only 3 hospitals, and therefore our findings may not represent the HPV genotypes in the general population. We were only able to identify genital warts from adolescents and young adults, individuals most likely to experience infection and seek medical care. We do not have information regarding the anatomical locations of the genital warts from which the samples were derived due to privacy and different coding practices on the medical records from different hospitals. The description of the anatomical location of genital warts was often affected by bias because in most circumstances physicians only performed a limited clinical examination on patients. We were therefore unable to analyze different HPV genotypes with sampling location.

**Conclusion**

HPV6 and 11 were the most frequently detected HPV genotypes identified in anogenital warts in this study. Since they are components in the quadrivalent HPV vaccine, appropriate immunization could potentially prevent a significant number of infections in Thailand.

**Acknowledgments**

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**Statement of Ethics**

This study was approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University (IRB No. 509/57). Written informed consent was obtained from all participants.

**Disclosure Statement**

The authors declare that they have no conflicts of interest.
References


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