

RESEARCH COMMUNICATION

Human Papilloma Virus Prevalence, Genotype Distribution, and Pattern of Infection in Thai Women

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Abstract

Background: The pattern of infection in cervical lesions with respect to HPV subtype has not been systematically studied in Thai women. The aim here was to determine HPV prevalence, genotype, and infection pattern in cervical lesions and to estimate the potential efficacy of an HPV prophylactic vaccine. **Design:** Formalin-fixed paraffin-embedded cervical tissue blocks of 410 Thai patients from 8 institutes in 4 regions of Thailand (northern, southern, north-eastern, and central) were studied. The samples included 169 low grade squamous intraepithelial lesions (LSILs), 121 high grade squamous intraepithelial lesions (HSILs), and 120 squamous cell carcinomas (SCCs). HPV-DNA was amplified by PCR using consensus primers GP5+ and GP6+. The HPV genotype was then determined by reverse linear blot assay that included 37 HPV-specific 5'-amino-linked oligonucleotide probes. Patterns of infection were classified as single infection (one HPV type), double infection (two HPV types), and multiple infection (three or more HPV types). **Results:** The mean age of the subjects was 42 years. The prevalence of HPV infection was 88.8%. The highest HPV prevalence was found in the southern region (97.1%) and the lowest in the central region (78.6%). HPV-DNA was detected in 84.6% of LSILs, 90.1% of HSILs, and 93.3% of SCCs. A total of 20 HPV genotypes were identified. The five most common high risk HPV were HPV16 (83.2%), HPV18 (59.3%), HPV58 (9.3%), HPV52 (4.1%), and HPV45 (3.8%). In double and multiple infection patterns, the most common genotypes were HPV16/18 (27.8%) and HPV11/16/18 (54.9%). HPV6 was found only in LSIL and never in combination with other subtypes. HPV11 was most common in LSIL. **Conclusion:** There is no difference of HPV type distribution in women from 4 regions of Thailand with prominent HPV16 and HPV18 in all cases. The bivalent and quadrivalent vaccines have the potential to prevent 48.6% and 74.5% of cervical cancers in Thai women. The potential of cancer prevention would rise to 87.6% if other frequent HR-HPV types (HPV58, 52, and 45) were also targeted by an HPV vaccine.

Keywords: Human papilloma virus - HPV genotype - HPV prevalence

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Introduction

Cervical cancer is the most common cancer and the third common cause of death in women in Thailand (Parkin, 2005). The overall cervical cancer incidence reported as an age-standardized rate (ASR) is 19.8 per 100,000. However, the ASR varies between the four geographical regions of the country. The highest incidence is in the north (Chiang Mai, ASR=25.6), followed by the central region (Bangkok, ASR=20.7), the south (Songkhla, ASR=16.1), and lastly the northeast (Khonkaen, ASR=15.0) (Parkin, 2005).

A key etiologic factor for the development of

cervical cancer is infection with certain types of human papillomavirus (HPV) (Walboomers, 1999). More than 100 HPV types have been identified and characterized at the molecular level, of which approximately 40 are able to infect the epithelial lining of the anogenital tract and other mucosal areas of the body (Tiersma, 2005). Based on their associations with cervical cancer and precancerous lesions, these HPV types can be divided into low risk types such as HPV 6, 11, 42, 43 and 44, which are associated with benign genital warts, and high risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70 which are associated with the development of anogenital cancer. These high risk types can be detected

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more than 90% of the cervical tissues of patients with cervical carcinoma or squamous intraepithelial lesion (SIL) (Lowy, 1994; Burd, 2003; Hwang, 2004).

The overall prevalence of HPV infection and distribution of HPV genotype varies with patient age, cytology stage, geographic region including regions within one country. Previous studies have reported that two most prevalent types worldwide are HPV16 and HPV18, accounting for more than 70% of cervical cancer (Walboomers, 1999; Bosch, 2002; Usubutun, 2009). However, in Asia, HPV18 is the fourth most frequent type after types 16, 52 and 58 (Bosch, 2008; Bosch, 2008).

Infection with more than one HPV type is found in 20-50% of infected women (Franco, 1999; Liaw, 2001; Gargiulo, 2007). Infection with multiple HPV types has been observed frequently in patients with cytological abnormalities (Ho, 1998; Herrero, 2000). Some studies have reported multiple HPV types less frequently in cervical carcinoma than in normal cytology and in precancerous lesions (An, 2003), whereas other studies have found multiple HPV infection to be associated with a significantly increased risk of high grade SIL/invasive cancer compared to infection with a single HPV type (Spinillo, 2009).

Archival formalin-fixed, paraffin-embedded (FFPE) tissues are important resources for elucidating the clinical implications of HPV infection and the role of HPV in the pathogenesis of cervical carcinoma (Huang, 2004). HPV DNA can be detected in up to 100% of archival cervical carcinoma specimens using PCR-based methods (Baay, 1996; Kleter, 1998). The purpose of this study was to investigate the overall HPV prevalence, distribution of HPV genotypes and pattern of multiple type infections using archival FFPE cervical biopsies in women from four regions of Thailand. This information would then be useful to estimate the potential benefit of HPV vaccination in Thai women.

Materials and Methods

Specimens

410 FFPE cervical tissue blocks and the corresponding hematoxylin and eosin-stained slides were collected from the archives of eight institutes in four regions of Thailand (north, Chiang Mai; north east, Khonkaen; central, Bangkok; and south, Songkla). The cervical tissues were obtained between 2004 and 2007 and were composed of 3 diagnostic categories : low grade squamous intraepithelial lesion (LSIL), 169 cases; high grade squamous intraepithelial lesion (HSIL), 121 cases; and squamous cell carcinoma (SCC), 120 cases. The cases were almost equally divided between the northern region (104 cases), northeastern region (99 cases), central region (104 cases) and southern region (103 cases). Cases excluded from the study included: maximum size of the lesion <2 mm, invasive squamous cell carcinoma lower than Stage Ib; cervical lesions other than squamous, and the presence of more than one type of epithelial lesion. All slides were reviewed simultaneously by at least 6 pathologists using a multi-headed microscope to confirm the histologic diagnosis.

DNA extraction from FFPE tissue

Tissue samples underwent deparaffinization with xylene, and rehydration in graded ethanol. Subsequently DNA was extracted using QIAamp® DNA mini Kit (Qiagen). The DNA samples were stored at -20oC for further analysis. For sample quality control, PCR amplification was performed for the human β-globin using primers PC04: 5'-CAACTTCATCCACGTTCCACC-3' and GH20: 5'-GAAGAGCCAAGGACAGGTA-3', which yields a 286 bp product (Invitrogen Life Technologies, Carlsbad, CA, USA).

HPV DNA detection and genotyping

β-globin-positive samples were used for HPV DNA detection. PCR amplification was performed using the GP5+/GP6+ consensus primers for a conserved 142 bp segment in the L1 open reading frame of HPV, as described previously (Jacobs, 1995; Chopjitt, 2009). The primer GP6+ was biotinylated to yield a labelled PCR product. The PCR product was subjected to electrophoresis using a 1.5% agarose gel and visualized by UV transilluminator. HPV DNA-positive cases then underwent HPV genotyping by reverse line blot (RLB) hybridization assay (van den Brule, 2002). Briefly, 37 HPV type-specific 5'-amino-linked oligonucleotides probes were immobilized onto a Biotyne C membrane (Pall Gelman Laboratory, Ann Arbor, MI, USA) using a miniblotted. Subsequently, biotin-labeled PCR products were pipetted into the parallel channels of the miniblotted in such a way that the channels are perpendicular to the rows of oligoprobes blotted previously. Following hybridization, membranes were incubated with a streptavidin-peroxidase-conjugate and hybridization detected using chemiluminescence and exposure to x-ray film.

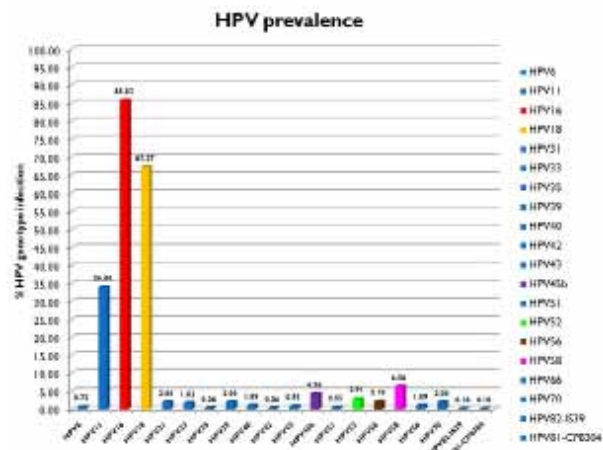


Figure 1. HPV Genotype Distriburion among HPV-positive Samples from Women Diagnosed with LSIL, HSIL and SCC

Table 1. Number of Cases Positive for HPV DNA by PCR Amplification for Each Histological Diagnosis

Histological Diagnosis	HPV DNA Detection		Total
	Positive (%)	Negative (%)	
LSIL	143 (84.6)	26 (15.4)	169
HSIL	109 (90.1)	12 (9.9)	121
SCC	112 (93.3)	8 (6.7)	120
Total (%)	364 (88.8)	46 (11.2)	410

Table 2. HPV Prevalence in Thai Women from the Four Regions of Thailand

Region	North(n=104)		Northeast(n=99)		South(n=104)		Central(n=103)		Total	
	P(%)	N(%)	P(%)	N(%)	P(%)	N(%)	P(%)	N(%)	P(%)	N(%)
LSIL (169)	38 (86.4)	6 (13.6)	30 (75)	10 (25)	41 (93.2)	3 (6.8)	34 (82.9)	7 (17.1)	143 (84.6)	26 (15.4)
HSIL (121)	29 (96.7)	1 (3.3)	27 (93.1)	2 (6.9)	30 (100)	0	23 (71.9)	9 (28.1)	109 (90.1)	12 (9.9)
SCC (120)	28 (93.3)	2 (6.7)	30 (100)	0	30 (100)	0	24 (80)	6 (20)	112 (93.3)	8 (6.7)
Total (410)	95 (91.3)	9 (8.7)	87 (87.9)	12 (12.1)	101 (97.1)	3 (2.9)	81 (78.6)	22 (21.4)	364 (88.8)	46 (11.2)

P, positive; N, negative

Table 3. Pattern of HPV Infection in Precancerous and Cancerous Lesions

Diagnosics (n=HPV positive)	HPV Single Infection (%)	HPV Double Infection (%)	HPV Triple Infection (%)	Unknown (%)	Not Done (%)
LSIL (143)	44 (30.8)	43 (30.1)	54 (37.8)	2 (1.4)	0
HSIL (109)	38 (34.9)	34 (31.2)	35 (32.1)	0	2 (1.8)
SCC (112)	42 (37.5)	33 (29.5)	36 (32.1)	1 (0.9)	0

Statistical analysis

Statistical analysis was performed by the chi-square test. Statistical significance was set at $P < 0.05$.

Results

The mean age of the studied subjects was 42 years (range 17-84 years). The extracted DNA samples from all cases were positive for the β -globin gene indicating acceptable quality for HPV analysis. The prevalence of HPV DNA was 84.6% in LSIL, 90.1% in HSIL and 93.3% in SCC (Table 1). A total of 20 HPV genotypes were identified in Thai women. The most common genotype was 16 (83.2%). The others in descending order included 18 (59.3%), 11 (34%), 58 (9.3%), 52 (4.1%), 45 (3.8%), 56 (2.2%), 31, 39, and 70 (all 2%), 33 (1.8%), 40 and 66 (both 1.1%), 43 (0.9%), 6 (0.7%), 51 (0.6%), 35 and 42 (both 0.4%), and 81 and 82 (both 0.2%) (Figure 1). The 5 most common high risk HPV genotypes in Thai women were 16, 18, 58, 52 and 45.

In LSIL, HSIL and SCC cases, this study found that more than 50% of HPV infection was due to types 16 and 18. HPV16 was the most common HPV genotype and its prevalence increased with severity of the lesion, namely 73.4% of LSIL, 87.2% of HSIL and 92.0% of SCC. On the other hand, the prevalence of low risk HPV types (6, 11, 40, 43, 70 and 82) decreased or was absent as the stage of the disease increased.

The prevalence of HPV in Thai women from 4 regions of Thailand is shown in Table 2. The highest prevalence of HPV infection was found in southern region (97.1%), followed by northern region (91.3%), north-eastern region (87.9%), and central region (78.6%). HPV was detected in 100% of cases of SCC from the north-eastern and southern region as well as HSIL from the southern region; however, the prevalence of HPV infection in each region was significantly different ($p = 0.002$).

HPV16 was the most prevalent genotype in women from all four regions of Thailand. Others high risk HPV genotypes showed a similar prevalence in the northern, north-eastern and central regions, with the five most common high risk HPV types (in descending order) as 16, 18, 58, 52 and 45. In contrast, in the southern region, there was a higher prevalence of 58 and 52.

Table 3 shows the pattern and frequency of single,

double, and multiple HPV infections in Thai women according to histological diagnosis. The overall prevalence was 27.6% for a single infection, 34.4% for double infection, and 38.0% for a multiple infection. In double and multiple infections, the most common genotypes were 16/18 (27.8%) and 11/16/18 (20.4%), respectively. There was no case of HPV 6 in combination with other genotypes; HPV6 was found only in LSIL whereas HPV11 was common in LSIL. The prevalence of HPV11/16/18 in each region was statistically different ($p = 0.0004$).

Discussion

The prevalence of HPV DNA in SCC, HSIL, and LSIL in this study was 93.3%, 90.1%, and 84.6 respectively. All groups showed a higher prevalence than other studies in Asian countries which have shown a prevalence for SCC ranging from 82.5%-91.1% (China, Korea, and Taiwan 82.5%, Japan 86.6%, Central Asia 90.5%, and South East Asia 91.1%). In the HSIL group, the prevalence ranged from 75.1% (China, Hong Kong, Taiwan) to 85.2% (Korea and Japan). For the LSIL group, the prevalence ranged from 33.3% (India) to 74.6% (Korea and Japan). (Bao, 2008)

For the high risk HPV group, HPV16 was the most common genotype in all neoplastic cervical lesions. This was followed by HPV18, 58, 52, 45, 56, 31, 39, 70, and 33. The findings were similar to other studies in Asian countries (Bao, 2008). In the low risk HPV group, HPV11 was the most common genotype with a prevalence rate of 34%.

However, this study was different from other studies that showed single HPV infections ranging from 12.5% (China), 56% (Mongolia), 71.3% (Japan), and 85.8% (Taiwan). Multiple infections ranged from 8.6% (Taiwan), 15.4% (Mongolia), 28.7% (Japan), and 80.65% (China) (Chen, 2006; Dai, 2008; Futai, 2009; Wu, 2009). The 5 most common HPV genotypes in single infections were 16 (61.5%), 18 (11.5%), 11 (8.1%), 58 (7.4%) and 52 (2.7%). In double and multiple infections, the most common genotype were HPV16/18 (27.8%) and HPV11/16/18 (20.4%).

When considering a prophylactic vaccine, the data from this study show that the bivalent and quadrivalent vaccines have the potential to prevent 48.6% and 74.5%

of infections in Thai women, respectively. The potential for cancer prevention would rise to 87.6% if other frequent high risk HPV type, such as 45, 52 and 58 were included in future HPV vaccines.

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