RESEARCH COMMUNICATION

Prevalence and Genotypes of Human Papillomavirus among Thai Women

Jira Chansaenroj[1], Woradee Lurchachaiwong[1], Wichai Termrungruanglert[2], Damrong Tresukosol[2], Somchai Niruthisard[2], Prasert Trivijitsilp[2], Pichet Sampatanukul[3], Yong Poovorawan[1]*

Abstract

One of the most common cancers in women worldwide is cervical cancer, with death rates highest in less developed countries, including Thailand. This study was conducted to explore the prevalence of human papillomavirus (HPV) and its related cytological abnormalities among women attending cervical screening clinics in Thailand using the polymerase chain reaction (PCR). LBC specimens (ThinPrep®, Hologic, West Sussex, UK) were subjected to PCR of the E1 region to identify the most prevalent HPV types. Information on age and cytology grade was also collected. Among a total of 1,662 women, 29 different HPV types were found and the overall HPV prevalence was 8.7%. HPV prevalence among the general population amounted to 7.8%. The following HPV types were identified: HPV16 (17.9%), HPV90 (16.6%) and HPV71 (10.3%). The rates of other types were as follows; HPV66 (6.9%), HPV52 (6.2%), HPV34 (5.5%), HPV31 (5.3%), HPV42 (4.8%) and HPV39 (3.4%). HPV infection peaked in women aged around 20-39 years and thereafter gradually declined. As expected, HPV DNA can be found in normal cytology specimens. These results which elucidate HPV distribution in Thailand could be useful for vaccine development and the national cervical cancer prevention program.

Key Words: Human papillomavirus subtypes - LBC specimens - relative prevalence - Thailand

Asian Pacific J Cancer Prev, **11**, 117-122

Introduction

More than 400,000 women throughout the world are affected by cervical cancer every year, the second most malignant cancer after breast cancer (Gheit et al., 2006). Results obtained from epidemiological studies associate high-risk genotypes of human papillomavirus (HPV) with cervical carcinoma and malignant transformation of cervical epithelial cells. Recent research has suggested that HPV DNA can be detected in more than 90% of cervical cancers, while some studies have demonstrated HPV in all cases of cervical cancer (Hadzisejdic et al., 2006). HPV is classified as either cutaneous or mucosal depending on the type of epithelium host cells. The mucosal types are sub-divided depending on their oncogenic potential. Low-risk types such as HPV6 and HPV11 cause benign warts, whereas the high-risk types HPV16 and HPV18 are associated with female malignant disease of the lower genital tract (Crosbie and Kitchener, 2006).

The second most common cancer in women worldwide, cervical cancer contains DNA sequences from a high-risk oncogenic genital HPV. HPV16 and HPV18 are the most prevalent types found in 50–70% and 7–20% of cases, respectively (Parkin and Bray, 2006). Cervical cancers are differentiated based on the spectrum of intra-epithelial abnormalities. The spectrum in the cervix ranges from CIN 1 (low-grade cervical intra-epithelial neoplasia) over moderate CIN2 to high-grade CIN3. High-grade CIN3 lesions are the necessary precursor lesions for cervical cancer and approximately 90% of high-grade CIN harbor high-risk HPV, with HPV16 most frequently detected (Clifford et al., 2006). Normally, the risk of HPV infection is age related and increases with promiscuity. Thus, the highest incidence is observed in the 15- to 25-year age group while it declines in women above the age of 65. Over the last 50 years, both incidence and mortality due to invasive cervical cancer in the United States and in other developed countries have decreased to 70% as a consequence of more frequent cytological screening (Pap smear) which can facilitate detection of abnormal cervical cytology and intraepithelial neoplasia at an early stage and thus, result in efficient treatment. During previous years, various organizations have provided screening recommendations on which screening guidelines of most primary care practices have been based (Ogunmodede et al., 2007).

Recently, along with the advances in molecular biology techniques, highly sensitive and effective HPV detection tests have been developed. Among these are

[1]Center of Excellence in Clinical Virology, Department of Paediatrics, [2]Department of Obstetrics and Gynecology, [3]Department of Pathology and Clinical Epidemiology Unit, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
**For correspondence: Yong.P@chula.ac.th*

hybrid capture (HC), filter in situ hybridization (FISH), southern hybridization (SH), and polymerase chain reaction (PCR) (Khair et al., 2009). More recent data have demonstrated that the risk for acute infection to proceed to malignant transformation is substantially different for individual HR-HPV types. Several clinical and epidemiological studies have indicated that women without cervical abnormalities but detectable HPV16 and 18 infections tend to develop more severe preneoplastic lesions within a shorter period of time than women infected with other types (Vinokurova et al., 2008).

Identification of individual HPV types is essential to investigate epidemiology and clinical characteristics of particular types. At present, several consensus PCR systems have been modified for use in large-scale epidemiological studies (Jiang et al., 2006). Recently, clinical trials have been performed to test the efficacy of prophylactic vaccines which target the two oncogenic types, HPV-16 and HPV-18 (Gravitt et al., 2007). Detection of high-risk HPV types in genital specimens has been approved in several countries for the triage of women with a cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) and also for primary cervical cancer screening in women aged 30 years and above as an adjunct to cytology (Coutlée et al., 2007). In the management of women with ASCUS, HPV characterization helps improve the understanding of prevalence, individual risk stratification, persistence, re-infection, co-infection and development of the most effective vaccine (Gillio-Tos et al., 2006). At present, the molecular detection and typing techniques have been proposed to be applied instead of the current cytological screening. The conventional methods for DNA or RNA extraction from clinical samples present the most labor-intensive and critical part in current diagnostic assays (Broccolo and Cocuzza, 2008). Type-specific HR-HPV prevalence in the population and in cervical cancer is essential to predict the burden (De Vuyst et al., 2009).

The 8-kilo base pair genome comprises the genes E1, E2, E4, E5, E6 and E7 which are essential for viral DNA replication, viral gene transcription and cellular transformation. In addition, two late (L) genes encode the viral capsid protein. DNA replication is one of the viral maintenance processes and thus, details of the mechanism involved would facilitate antiviral therapy design. The most interesting gene is E1 which is involved in all steps of replication initiation such as origin recognition, ATP-dependent DNA melting, and unwinding of DNA (Auster and Joshua-Tor, 2004).

In general, there is strong evidence of variation in HPV prevalence among the female population. Our study has aimed at elucidating prevalence and genome distribution of HPV DNA in hospital-based specimens in Bangkok, Thailand by using PCR for E1 gene amplification. The amplification assay detects target HPV-DNA from different carcinogenic HPV types (high risk genotypes: 16, 18, 30, 31, 33, 35, 39, 42, 45, 51, 52, 56, 58, 59, 68, 73 and 82, probably high-risk genotypes: 66, low risk genotypes: 6 and 11) These studies have improved our knowledge and can be used for preventive and control measures of cervical cancer in Thailand.

Subjects and Methods

Study population

This study protocol has been approved by the Ethics Committee of the hospital and faculty of Medicine, Chulalongkorn University. The cervical cells were randomly obtained in the course of the patients' routine check up or investigation and treatment between January 2008 and December 2009. These specimens were sent as anonymous with a coding number. The available data were based on cytology and age at sampling. All specimens were exclusively used for academic research.

Sample collection and preparation

All cervical cells were collected for cytology by LBC (ThinPrep®, Hologic, West Sussex, UK). These 848 samples were obtained during hospital-based routine check up or investigation and treatment at King Chulalongkorn Memorial hospital, Bangkok and 852 from Bangkok 9 International hospital, Bangkok. After centrifugation at 4,000 rpm for 10 min the cell pellets were separated from the supernatant and stored at -70°C until further tested.

Cytological diagnoses

All cervical smears were analyzed by a qualified pathologist using The Bathesda System 2001 (TBS 2001). To prevent bias the participating patients' personal details were not disclosed.

DNA extraction and house keeping gene detection

DNA was extracted by organic extraction (phenol–chloroform) of the samples. Briefly, cellular pellets were re-suspended in 400 µl of lysis buffer. Samples were incubated at 95˚C for 30 min, mixed for 2 min, and digested with 50 µl of proteinase K (20µg/l). After overnight incubation at 50∞C, samples were heated to 95˚C for 10 min to inactivate the proteinase K. Phenol–chloroform extraction followed by high-salt isopropanol precipitation was performed as described previously (Broccolo et al., 2005) and purified material was re-suspended in a final volume of 30 µl deionized water, respectively. The γ-globin gene was selected to serve as an internal control for DNA extraction, using conventional PCR as a detection method. Primer sequences for the _-globin gene have been previously described (Shadrina et al., 2007). The reaction mixture consisted of 2 µl DNA, 0.5 µM γ-globin forward primer and γ-globin reverse primer, 10 µl 2.5X Eppendorf masterMix (Eppendorf, Hamburg, Germany), and nuclease-free water to a final volume of 25 µl. The amplification reaction was performed in a thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: Denaturation at 94°C for 3 min, followed by 40 amplification cycles consisting of denaturation at 94°C for 30 sec, primer annealing at 60°C for 30 sec, and extension at 72°C for 30 sec, and concluded by a final extension at 72°C for 7 min.

HPV detection by PCR and direct sequencing

To detect HPV DNA the primers chosen were part of the E1 gene sequence. Based on a previous study

(Lurchachaiwong et al., 2009), the E1 gene can be used for HPV genotype detection. The amplification reaction was performed based on this previous study. To identify HPV DNA positive samples, the PCR products were subjected to electrophoresis in 2% agarose gel (FMC Bioproducts, Rockland, ME). The DNA bands were stained with ethidium bromide and visualized by UV transillumination (Gel Doc 1000, BIO-RAD, CA). These were further purified using the HiYield Gel/PCR DNA Fragments Extraction kit (Bioscience) according to the manufacturer's specifications. The purified DNA served as templates for DNA sequencing performed by FirstBASE Laboratories SDNBHD (Selangor Darul Ehsan, Malaysia).The nucleotide sequences were analyzed in both directions using forward and reverse primers to confirm the consistency of the sequencing result and ensure that variations of nucleotide sequences were not due to sequencing errors.

Sequence analysis

The resulting sequences were analyzed by BLAST and genotyping was analyzed by comparing with the sequences stored at GENBANK database (www.ncbi.nlm.nih.gov).

Statistical analysis

HPV prevalence was determined as individual genotypes. The proportion of positive samples was analyzed in relation to age-groups.

Results

Of a total of 1698 ThinPrep specimens collected from hospital-based routine check-up or investigation and treatment in Thailand, 36 had inadequate cytology results and age. In total, 1662 specimens were included. The average age of the participants was 43.4 years. In this study, we used E1 primers designed for semi-nested PCR. The PCR primers were type-specific for high-risk HPV genotypes. Data were classified according to cytological type, with 1,622 (97.6%) showing normal, and 40 (2.4%) abnormal cytology.

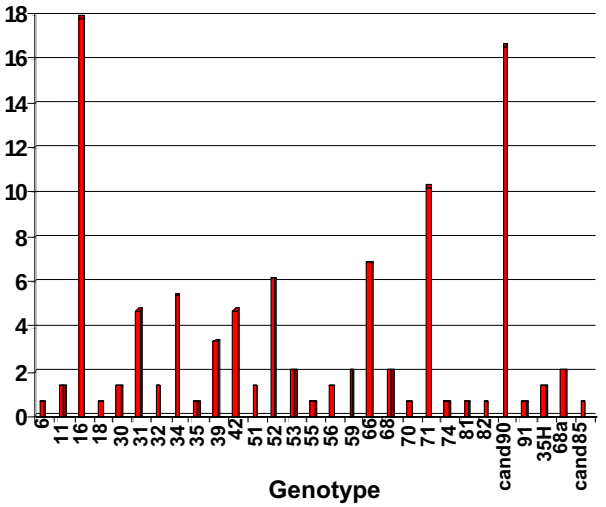

Figure 1. Distribution of Positive HPV Specific Types by PCR-sequencing Assay

In the single infection group, the prevalence analysis was based on 1,662 specimens. According to the results obtained, of those 1662, there were 145 specimens (8.7%) were positive for HPV DNA, with HPV16 most frequently detected (26;17.9%), followed by candHPV90 (24;16.6%), HPV71 (15;10.3%), HPV66 (10;6.9%), HPV52 (9;6.2%), HPV34 (8;5.5%), HPV31 (7;4.8%), HPV42 (7;4.8%) and HPV39 (5;3.4%) whereas each of the remaining HPV types was found in less than 3.0% (Figure 1). Of all HPV DNA positive samples 65 (44.8%) were high-risk HPV. Twelve different high-risk HPV types except for genotypes 33, 45, 73 were identified among the women. Age specific prevalence increased from 22.8% at the age of 20-29 years to 37.2% at 30-39 and decreased from 17.2% to 15.9 and 4.8% at the age of 40-49, 50-59 and 60-69, respectively. As shown in Table 1, HPV infection is common among the female population below the age of 30, with HR-HPV particularly prevalent in women between 30 and 39 and gradually decreasing with advancing age. All the sequences were submitted to the Genbank database under the accession number GQ161664

Table 1. Distribution of Type-specific Human Papillomavirus among 1662 Women in 10 Year Age Groups

Age group	<20	20-29	30-39	40-49	50-59	60-69	70-79	≥80
Total No.	6	208	455	506	325	131	25	6
HPV DNA-ve No (%)	6 (100)	175 (84.1)	401 (88.1)	481 (95.1)	302 (92.9)	124 (94.7)	23 (92.0)	5 (83.3)
HPV DNA +ve No (%)	0 (0.0)	33 (15.9)	54 (11.9)	25 (4.9)	23 (7.1)	7 (5.3)	2 (8.0)	1 (16.7)
LR-HPV No.		4	6	2				
PHR-HPV No.		5	3	1	3		1	
HR-HPV No.		14	24	10	11	4	1	1
Any type No.		10	21	12	9	3		

Table 2. Prevalence of Human Papillomavirus Infection in Cervical Lesions among 1,662 women in Thailand

Cytology	Normal	ASC-US	ASC-H	AGC	LSIL	HSIL	CA	All	Total No (%)
Total No.	1,622 (97.6)	13	2	3	12	8	2	40 (2.4)	1,662 (100)
HPV DNA negative	1,495 (90.0)	9 (69.2)	2 (100)	2 (66.7)	6 (50)	3 (37.5)		22 (1.3)	1517 (91.3)
HPV DNA positive	127 (7.6)	4 (30.8)		1 (33.3)	6 (50)	5 (62.5)	2 (100)	18 (1.1)	145 (8.7)
LR-HPV No.	8	2			1	1		4	12
PHR-HPV No.	10				2	1		3	13
HR-HPV No.	55	2		1	2	3	2	10	65
Any types No.	54				1			1	55

- GQ161751 and GU447239 - GU447289.

We compared the results of cytology with HPV genotype. Abnormal Pap smear indicated ASCUS 32.5% (13/40), ASC-H 5.0% (2/40), AGC 7.5% (3/40), LSIL 30.0% (12/40), HSIL 20.0% (8/40) and carcinoma 5.0% (2/40). HPV DNA positive samples with normal cytology amounted to 7.8% (127/1622).

The number of specific HPV types in relation to cytology is shown in table 2. A high percentage of HR-HPV is associated with abnormal cytology. As for subtypes, the carcinogenic HPV type 9 species (e.g. HPV16, HPV31) were highly prevalent in samples of both normal and abnormal cytology. Second in prevalence are non-carcinogenic HPV type 15 species (e.g. HPV71, HPV90) which are only found in samples with normal cytology. Third are 6 and 7 species.

Discussion

Information on HPV cervical infection in Thailand is scarce. Our aim was to determine the prevalence of HR-HPV types causing cervical infections by amplifying the E1 gene of HR-HPV by semi-nested PCR performed on 1662 hospital-based specimens. Altogether, 8.7% were HPV DNA positive. HPV DNA positive samples with normal cytology amounted to 7.8% (127/1622). HR-HPV prevalence was 44.8%

There are various HPV types involved in cervical cancer depending on geographic distribution. HPV types rare in cervical cancer are commonly found in the general population. In future, HPV testing will focus on primary screening and cervical cancer prevention. HPV genotype distribution will provide basic knowledge for HPV-based cervical cancer screening, cost-effective prophylactic HPV vaccine and assessment of vaccination against specific HPV infection in each geographic area (Lai et al., 2007). In routine set-up or high-throughput, HPV typing is feasible.

HPV-DNA detection in the Cervicovaginal tract has a higher sensitivity than sampling from urine specimens, although obtaining samples from the population is more labor intensive. Despite differences in collection methods and techniques, it has become apparent that prevalence differs notably between geographical regions but still, HPV16 is typically most prevalent (Manhart et al., 2006). This corresponds with the literature which suggests that the prevalence of HPV in women with normal cytology does not exceed 10% in the North-Western part of Europe (Baay et al., 2005). For example, of a population of 3,305 women between 15 and 69 years of age with normal cytology examined in the Netherlands, 4.6% were HPV positive and subsequently subjected to GP5+/bioGP6+ polymerase chain reaction-enzyme immunoassay (PCR-EIA) (Jacobs et al., 2000). Research conducted on samples obtained from a cervical screening program of 6,123 women between 32 and 38 years old from 5 different regions in Sweden detected HR-HPV in 6.8% by using HPV primer (GP5+/GP6+) PCR-EIA (Forslund et al., 2002). According to a previous report, prevalence in Asia and Australia amounts to between 5% and 11%. For example, based on GP5+/GP6+ PCR-EIA performed on randomly, selected married women aged between 15 and 69 years, 2.0% were HPV positive in Hanoi and 10.9% in Ho Chi Minh City (Pham et al., 2003). In Indonesia, of 2,686 women aged 15-70 years, 11.4% were HPV positive (Vet et al., 2008).

According to a study, HPV16 and/or HPV18 can be detected in more than 50% high-grade squamous intraepithelial lesions, 70% invasive cervical cancer and 81.5% adenocarcinomas. We implied that HPV16 and HPV18 are more prone to persist and progress to cervical lesion than other high-risk types (De Sanjosé et al., 2007). Among the HR-HPV types in many regions, type 16 is dominant. HPV prevalence varies depending on many factors such as age and region. As a result, most cervical carcinomas the worldwide contain HR HPV DNA. A decrease in HR-HPV infection with advanced age is the main cause for the decline in HPV prevalence observed upon screening this population group (Jacobs et al., 2000). Therefore, prevalence tendencies observed in this study may be used as baseline profiles to monitor the efficacy of HPV vaccination strategies in Thailand. Previous study of infection by multiple HPV genotypes showed that single infection of HPV genotype have more significant effect on increasing risk of high-grade cervical lesions than multiple infection of HPV genotypes. Moreover, the multiple HPV infections are less frequent in high-grade than low-grade cervical neoplasia and less common in cervical adenocarcinoma (ADC) (Zielinski et al., 2003; Cuschieri et al., 2004; Hadzisejdi et al., 2006).

This study has been in agreement with previous reports in that screening of a population aged between 30 and 60 years revealed a significant decline in HR-HPV prevalence. Also, de novo HR-HPV infections will decrease with advancing age. HPV prevalence peaks at the age of 40 and thereafter declines for all HR-HPV types.

Pap smear is not replaced by HPV test but they can complement each other to reduce negative results in samples with normal cytology or LSIL. A recommendation by the American Cancer Society suggests that HPV testing should be used in women after the age of 30, because infection is mainly asymptomatic in populations under 30 years of age and often transient (Wright et al., 2004). In Southeast Asia, the age-standardized incidence rate (ASR) is 18.3. In Thailand, 6,300 new cases of cervical cancer are diagnosed per year with ASR=19.5 per 100,000 with variations according to geographical region. In the north ASR=25.6, in the center ASR=20.7, in the south ASR=16.1 and in the north-east ASR=15.0 (Sriplung et al., 2005). Epidemiological studies on HPV genotype distribution are limited. As reported by the World Health Organization, a large percentage of women living in cities had their first sexual experience once they had reached the age of 24. Hence, most studies reported a high incidence of HPV infection in healthy populations below 30 years of age (Suwannarurk et al., 2009). For example, the study conducted in Lampang and Songkla provinces in 1997-2000 reported only 11% HPV infection rate in the age group of 25 or less. HPV DNA was found in 82-91% of cervical carcinomas and in 9-20% of normal cervical smears (Sukvirach et al., 1994).

HPV9 (e.g. HPV16, HPV31) species are high-risk-

malignant mucosal lesions and 5 (e.g. HPV51, HPV82), 6 (e.g. HPV56, HPV66) and 7 (e.g. HPV18, HPV68) are species causing high-risk mucosal lesions, but 5, 6 can also be present in benign lesions. In contrast, non-carcinogenic HPV type 15 species (e.g. HPV71, HPV90) are found in low-risk mucosal lesions (Narechania et al., 2005). In this study, we implied that 5, 6, 7 and 9 are the main species responsible for cervical lesions and that 15 may not elicit lesions in Thai women.

The HPV may spontaneously disappear about 50% of new infections within 12 months. In young women, the most LSILs will regress spontaneously but a strong predictor of regression is follow-up HPV status whereas older women have a lower rate of clearance (Moscicki et al., 2004; Chumworathayi et al., 2010). The sternness of cervical lesions is parallel with prevalence of HPV increases. A Food and Drug Administration have a suggestion in women aged 30 years or more should have a HPV DNA testing. Women with inflammation, fungi or genital warts have a frequency of HR-HPV greater than in negative smears without any other finding. Women whose results are cytology negative, but HR-HPV DNA positive, should be repeated HPV DNA testing at 6 to 12 months. This guidance assists clinicians in effective manner and reducing unnecessary treatments (Jacobs et al., 2000; Wright et al., 2004).

In summary, the present study conducted on hospital-based specimens demonstrates that HPV infection is most prevalent in women between 20 and 39 years of age. We presume that the detection of carcinogenic HPV types in may reveal the presence of asymptomatic women who might be at risk to developing of cervical cancer (Ekalaksananan et al., 2001). These results show the age-specific prevalence on HPV in Thailand and could be useful for vaccine development and the national cervical cancer prevention program.

Acknowledgements

We would like to express our gratitude to the Commission on Higher Education, Ministry of Education, the Center of Excellence in Clinical Virology, Chulalongkorn University, CU Centenary Academic Development Project and King Chulalongkorn Memorial Hospital for their generous support. Finally we would like to thank Ms. Petra Hirsch for reviewing the manuscript.

References

Auster AS, Joshua-Tor L (2004). The DNA-binding domain of human papillomavirus type 18 E1. Crystal structure, dimerization, and DNA binding. *J Biol Chem*, **279**, 3733-42.

Baay MF, Tjalma WA, Lambrechts HA, et al (2005). Combined Pap and HPV testing in primary screening for cervical abnormalities: should HPV detection be delayed until age 35?. *Eur J Cancer*, **17**, 2704-8.

Broccolo F, Cocuzza CE (2008). Automated extraction and quantitation of oncogenic HPV genotypes from cervical samples by a real-time PCR-based system. *J Virol Methods*, **148**, 48-57.

Broccolo F, Drago F, Careddu AM, et al (2005). Additional evidence that pityriasis rosea is associated with reactivation of human herpesvirus-6 and -7. *J Invest Dermatol*, **124**, 1234-40.

Chumworathayi B, Thinkhamrop J, Blumenthal PD, et al (2010). Cryotherapy for HPV clearance in women with biopsy-confirmed cervical low-grade squamous intraepithelial lesions. *Int J Gynaecol Obstet*, **108**, 119-22.

Clifford G, Franceschi S, Diaz M, Muñoz N, Villa LL (2006). Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine*, **31 (supp 3)**, 26-34.

Coutlée F, Rouleau D, Ghattas G, et al (2007). Confirmatory real-time PCR assay for human papillomavirus (HPV) type 52 infection in anogenital specimens screened for HPV infection with the linear array HPV genotyping test. *J Clin Microbiol*, **45**, 3821-3.

Crosbie EJ, Kitchener HC (2006). Human papillomavirus in cervical screening and vaccination. *Clin Sci*, **110**, 543-52.

Cuschieri KS, Cubie HA, Whitley MW, et al (2004). Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol*, **57**, 68-72.

De Sanjosé S, Diaz M, Castellsagué X, et al (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis*, **7**, 453-9.

De Vuyst H, Clifford G, Li N, Franceschi S (2009). HPV infection in Europe. *Eur J Cancer*, **45**, 2632-9.

Ekalaksananan T, Pientong C, Kotimanusvanij D, et al (2001). The relationship of human papillomavirus (HPV) detection to pap smear classification of cervical-scraped cells in asymptomatic women in northeast Thailand. *J Obstet Gynaecol Res*, **27**, 117-24.

Forslund O, Antonsson A, Edlund K, et al (2002). Population-based type-specific prevalence of high-risk human papillomavirus infection in middle-aged Swedish women. *J Med Virol*, **66**, 535-41.

Gheit T, Landi S, Gemignani F, et al (2006). Development of a sensitive and specific assay combining multiplex PCR and DNA microarray primer extension to detect high-risk mucosal human papillomavirus types. *J Clin Microbiol*, **44**, 2025-31.

Gillio-Tos A, De Marco L, Ghisetti V, et al (2006). Human papillomavirus typing with GP5+/6+ polymerase chain reaction reverse line blotting and with commercial type-specific PCR kits. *J Clin Virol*, **36**, 126-32.

Gravitt PE, van Doorn LJ, Quint W, et al (2007). Human papillomavirus (HPV) genotyping using paired exfoliated cervicovaginal cells and paraffin-embedded tissues to highlight difficulties in attributing HPV types to specific lesions. *J Clin Microbiol*, **45**, 3245-50.

Hadzisejdic I, Simat M, Bosak A, Krasevic M, Grahovac B (2006). Prevalence of human papillomavirus genotypes in cervical cancer and precursor lesions. *Coll Anthropol*, **30**, 879-83.

Jacobs MV, Walboomers JM, Snijders PJ, et al (2000). Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer*, **87**, 221-7.

Jiang HL, Zhu HH, Zhou LF, Chen F, Chen Z (2006). Genotyping of human papillomavirus in cervical lesions by L1 consensus PCR and the Luminex xMAP system. *J Med Microbiol*, **55**, 715-20.

Khair MM, Mzibri ME, Mhand RA, et al (2009). Molecular detection and genotyping of human papillomavirus in cervical carcinoma biopsies in an area of high incidence of cancer from Moroccan women. *J Med Virol*, **81**, 678-84.

Lai CH, Huang HJ, Hsueh S, et al (2007). Human papillomavirus genotype in cervical cancer: a population-based study. *Int J Cancer*, **120**, 1999-2006.

Lurchachaiwong W, Junyangdikul P, Payungporn S, et al (2009). Relationship between Hybrid capture II ratios and DNA amplification of E1, E6 and L1 genes used for the detection of human papillomavirus in samples with different cytological findings. *Asian Pac J Allergy Immunol*, **27**, 217-24.

Manhart LE, Holmes KK, Koutsky LA, et al (2006). Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. *Sex Transm Dis*, **33**, 502-8.

Moscicki AB, Shiboski S, Hills NK, et al (2004). Regression of low-grade squamous intra-epithelial lesions in young women. *Lancet*, **364**, 1678-83.

Narechania A, Chen Z, DeSalle R, Burk RD (2005). Phylogenetic incongruence among oncogenic genital alpha human papillomaviruses. *J Virol*, **79**, 15503-10.

Ogunmodede F, Yale SH, Krawisz B, Tyler GC, Evans AC (2007). Human papillomavirus infections in primary care. *Clin Med Res*, **5**, 210-7.

Parkin DM, Bray F (2006). The burden of HPV-related cancers. *Vaccine*, **24 (supp 3)**, 11-25.

Pham TH, Nguyen TH, Herrero R, et al (2003). Human papillomavirus infection among women in South and North Vietnam. *Int J Cancer*, **104**, 213-20.

Shadrina MI, Semenova EV, Slominsky PA, et al (2007). Effective quantitative real-time polymerase chain reaction analysis of the parkin gene (PARK2) exon 1-12 dosage. *BMC Med Genet*, **26**, 6.

Sriplung H, Sontipong S, Martin N, et al (2005). Cancer incidence in Thailand, 1995-1997. *Asian Pac J Cancer Prev*, **6**, 276-81.

Sukvirach S, Pitakpraisan P, Kosiyatrakul T et al (1994). Detection of human papillomavirus DNA in normal and cervical carcinoma tissue of Thai females. *Thai Cancer J*, **20**, 85-91.

Suwannarurk K, Tapanadechopol P, Pattaraarchachai J, Bhamarapravati S (2009). Hospital-based prevalence and sensitivity of high-risk human papillomavirus in Thai urban population. *Cancer Epidemiol*, **33**, 56-60.

Vet JN, de Boer MA, van den Akker BE, et al (2008). Prevalence of human papillomavirus in Indonesia: a populationbased study in three regions. *Br J Cancer*, **99**, 214-8.

Vinokurova S, Wentzensen N, Kraus I, et al (2008). Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. *Cancer Res*, **68**, 307-13.

Wright Jr TC, Schiffman M, Solomon D, et al (2004). Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol*, **103**, 304-9.

Zielinski GD, Snijders PJ, Rozendaal L, et al (2003). The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. *J Pathol*, **201**, 535-43.