RESEARCH ARTICLE

Detection of Human Papillomavirus in Male and Female Urine by Electrochemical DNA Chip and PCR Sequencing

Pornjarim Nilyanimit[1], Nasamon Wanlapakorn[1], Somchai Niruthisard[2], Natkrita Pohthipornthawat[2], Anant Karalak[3], Piyawat Laowahutanont[3], Nittaya Phanuphak[4], Nobuhiro Gemma[5], Yong Poovorawan[1]*

Abstract

Background: Cervical cancer is the second most common cancer in Thai women after breast cancer. Currently, the Papanicolaou (Pap) smear is the recommended procedure for cervical cancer screening in Thailand, but only a relatively small percentage of women follow this screening program. An alternative method to detect HPV genotypes associated with cervical cancer is self-sampling of urine, which is a more widely accepted method. Our study aimed to evaluate the prevalence of HPV in Thai women using urine and cervical swabs and prevalence of HPV in Thai men using urine samples. Materials and Methods: Tumorigenic HPV detection was accomplished by electrochemical DNA chip and PCR/direct sequencing. In addition to HPV prevalence, we report the concordance between different methods and sample types. One-hundred and sixteen women and 100 men were recruited. Histological examination revealed normal cytology in 52 women, atypical squamous cells of undetermined significance (ASCUS) in 9, low-grade squamous intraepithelial lesions (LSIL) in 24, and high-grade squamous intraepithelial lesions (HSIL) in 31. One-hundred men were classified as heterosexuals (n=45) and homosexuals (n=55). Results: The most prevalent HPV genotype in our study was HPV16. The HPV detection rate was generally lower in urine samples compared with cervical samples. Overall, there was good agreement for the detection of carcinogenic HPV from female cervical samples between the DNA chip and PCR/sequencing, with 88.8% total agreement and a kappa value of 0.76. In male urine samples, the level of agreement was higher in heterosexuals compared with homosexuals. Conclusions: Further improvement is required to increase an overall yield of HPV DNA detection in urine samples before clinical application of a urine-based HPV screening program. The electrochemical DNA chip test is a promising technique for carcinogenic HPV detection.

Keywords: HPV - HPV genotyping - cervical cancer - urine - electrochemical DNA chip

Asian Pac J Cancer Prev, **14 (9)**, 5519-5525

Introduction

Human papillomavirus (HPV) is a non – enveloped, DNA virus that is transmitted through skin-to-skin contact with the penis, scrotum, vagina, vulva or anus of an infected person. More than 100 types of HPV have been identified, of which more than 40 can infect genital areas in human (National Center for Immunization and Respiratory Diseases, 2012). HPV infection is quite common among sexually active people. It can cause both benign diseases and malignant neoplasms of the genital tract (Koutsky, 1997). An individual can be infected with multiple types of HPV, and HPV infection in males can be found in many parts of genital organs such as penis, scrotum and anus (Goldstone et al., 2011). HPV prevalence in Thai women is 7.8%, with HPV16 most frequently detected (17.9%) (Chansaenroj et al., 2010), whereas prevalence of anal HPV infection in homosexuals or men who have sex with men (MSM) was 85% in HIV-positive and 58.5% in HIV-negative individuals (Phanuphak et al., 2013). Hadzisejdic et al. has suggested that HPV DNA can be detected in more than 90% of cervical lesions in women (Hadzisejdic et al., 2006).

Cervical cancer is the second most common cancer in Thai women (Globocan, 2008), and several studies have shown that certain types of HPV are the etiological cause of this disease (Gheit et al., 2011). HPV16 and HPV18 are the two most prevalent carcinogenic HPVs found in normal female cytological samples, and they are the genotypes correlated with the highest increased risk of developing precancerous lesions (Khan et al., 2005). The high anal HPV prevalence in MSM is also associated with anal cancer incidence, which is estimated to be 44 times higher in MSM than among the general population (Goldstone et al., 2011). Similar to women, HPV16 was found to be the most common high-risk HPV type in MSM

*[1]Center of Excellence in Clinical Virology, Department of Pediatrics, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, [3]Department of Pathology, National Cancer Institute, [4]The Thai Red Cross AIDS Research Centre, Bangkok, Thailand, [5]Research and Development Center, Toshiba, Japan *For correspondence: Yong.P@chula.ac.th*

(Phanuphak et al., 2013).

The American Cancer Society recommends that HPV testing should be used as an adjunct to cervical cytology for cancer screening in women more than 30 years of age (Wright et al., 2004). Pap smear is a method for cancer screening based on detecting abnormal cellular changes or premalignant lesions of cervical tissues. However, HPV testing is a better method for screening high-risk HPV infection in samples with normal cytology or low-grade squamous intraepithelial lesions (LSIL) (Wang et al., 2013). While Pap smear cytology screening has proved to be effective in reducing the incidence and mortality from cervical cancer (Fahey et al., 1995), this screening method requires a pelvic exam, which is invasive and uncomfortable for patients and time consuming for health-care workers (Mandelblatt et al., 2002). Cervical cancer screening in Thailand is still currently based on the Pap smear, but only a small percentage of women follow this screening program (Rugpao et al., 2009). HPV detection in urine sample is another alternative, which is more widely accepted because it is easily collected, non-invasive and readily available (Manhart et al., 2006). However, urine samples have some limitations as it doesn't directly come from the original sites of the disease. In addition, some reports (Song et al., 2007; Bissett et al., 2011) have demonstrated a lower sensitivity of HPV detection in urine compared with cervical swab samples, although other reports (Daponte et al., 2006; Gupta et al., 2006) have shown comparable sensitivity.

The detection of HPV DNA usually focuses on the L1 region of the major capsid-forming gene (Gravitt et al., 2000), and various techniques can be used to detect it such as polymerase chain reaction (PCR) with specific primers, Hybrid capture2 test (HCII) and linear array (De Antonio et al., 2008). Recently, a new technique for HPV DNA detection using an electrochemical DNA chip system combined with loop-mediated isothermal amplification (LAMP) was introduced. This technique can detect 13 high-risk (HR) genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) by using a single compact piece of equipment. The electrochemical DNA chip can detect single and multiple infections of high risk genotypes of HPV with higher sensitivity, specificity, rapidity and simplicity when compared with other methods such as direct sequencing (Hagiwara et al., 2007). Other studies have also demonstrated good agreement in electrochemical DNA chip testing when compared to direct sequencing (Chansaenroj et al., 2012; Satoh et al., 2013).

The aim of this study was to evaluate HPV detection rate in Thai women using urine and cervical samples. We also evaluate the prevalence of HPV in Thai men, both homosexuals and heterosexuals, using urine samples. In order to evaluate the performance of electrochemical DNA chip, the results of HPV genotypes detected were compared with those obtained from PCR/sequencing methods, which is the current gold standard.

Materials and Methods

The research protocol was approved by the Institutional Review Board (IRB number 389/2555 in female and IRB number 411/2555 in male) of the Faculty of Medicine, Chulalongkorn University. The objective of the study was explained to the patients and written consent was obtained for all participants. All specimens were sent and kept at -20°C until tested. The coding of all samples was anonymous.

Population study

Female subjects were recruited from King Chulalongkorn Memorial Hospital and the National Cancer Institute between June 2012 and February 2013. The collected samples were divided into 2 groups. The first group was from healthy women who came for a routine Pap smear checkup with normal cervical cytology confirmed by a cytotechnologist and pathologist. The second group consisted of female patients older than 15 who came for a follow-up visit at the colposcopy clinic. All of the patients in the second group had abnormal cytology at various stages and were classified into 3 groups; atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL).

Male urine samples were also collected from 2 groups of people. The first group was recruited from the Thai Red Cross AIDS Research Centre in Bangkok, Thailand. These subjects consisted of 18 and older Thai MSM. The second group was recruited from healthy Thai subjects who attended a routine health checkup at the Bangpakok 9 International Hospital, Bangkok.

Sample collection and preparation

Cervical swabs: cervical swabs were collected from the Department of Obstetrics and Gynecology, Chulalongkorn hospital, Bangkok and Department of Gynecology, National Cancer Institute, Bangkok, Thailand. The cervical swab was collected before the gynecologist performed Pap smear, and all specimens were kept in phosphate buffered saline (PBS) and stored at -20°C until tested. Cytological results were assessed by a specialized cytotechnologist and pathologist. The specimens were sent as anonymous with the coding number and patient's age to the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Urine: female urine samples were collected before the Pap smear test was performed. Male urine was collected before seeing the doctor during clinical visits. All of the urine specimens were collected in sterile 50ml tubes (Invitrogen co., Carlsbad, CA) and stored at 4°C for not more than 1 day. The urine was processed by centrifugation at 13,000rpm for 20min, the supernatant was discarded, and then the pellet was re-suspended with 1ml of PBS buffer and stored at -20°C until tested.

Pathological classification of cytological findings

Cytological determinations were characterized according to the 3 types of the Bethesda System, which is the international standard for reporting Pap smear results. The 3 types are; ASCUS, LSIL and HSIL (Solomon et al., 2002). Each type was diagnosed by a specialized cytotechnologist and confirmed by a pathologist.

Laboratory method

DNA extraction: DNA extraction and purification was performed using the Qiamp DNA mini kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. After extraction, all of specimens were tested for the β-globin gene to serve as an internal control. The DNA samples were stored at -20°C until tested.

HPV detection and genotyping.

Polymerase Chain Reaction (PCR)/Sequencing: the DNA was amplified by polymerase chain reaction (PCR) using a nested MY/GP primer set to amplify the L1 gene. The MY/GP primer set can detect 39 different HPV genotypes which overlap the 13 carcinogenic HPV genotypes detected by the electrochemical DNA chip (Qu et al., 1997). The PCR reaction mixture contained 2μL DNA template, 0.5μL of 10mmol of each primer, 15μL of 2.5× PerfectTaq Plus MasterMix (5 PRIME Inc., Hamburg, Germany) and sterile distilled water to a final volume of 25μL. The nested PCR reactions were performed by using Eppendorf Thermocycler (Eppendorf, Hamburg, Germany). The PCR conditions were previously reported (Lurchachaiwong et al., 2009) and are the followings: the first PCR amplification was initially denatured at 94°C for 5 min, followed by 40 cycles at 94°C for 30s (denaturation), at 55°C for 45s (annealing), at 72°C for 1.30s (extension) and a final extension step at 72°C for 7 min. The second PCR amplification used 50°C for 45s (annealing). All amplified products were separated by electrophoresis in a 2% agarose gel (FMC Bioproducts Rockland, ME) stained with ethidium bromide and visualized with UV transillumination (Gel Doc1000, BIO-RAD, Hercules, CA). The PCR products were purified from agarose gel with the PCRExtract&GelExtract Mini Kit (5 PRIME, Hamburg, Germany). The product of GP primer was subjected to direct sequencing by First BASE Laboratories Sdn Bhd (Selangor Darul Ehsan, Malaysia). Nucleotide sequences were edited and assembled using SEQMAN (LASERGENE program package, DNASTAR) and consequently subjected to BLAST program on the GenBank Database (www.ncbi.nlm.gov/BLAST) for genotype diagnosis.

Electrochemical DNA chip: the electrochemical DNA chip package consists of six Loop mediated isothermal amplification (LAMP) reagents, an intercalation reagent and an electrochemical DNA chip, which has L1 specific DNA probes for 13 carcinogenic high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The conditions of the LAMP procedure are the following; denaturation at 95°C for 5 min, followed by 65°C for 90 min and 80°C for 5 min. The automated hybridization of probe and primer and the subsequent quantification of the resulting electrochemical signals was done on the GLH-2C601 Genelyzer™ (Toshiba, Tokyo, Japan). A previous study done in our lab using the same electrochemical DNA chip reported no cross hybridization (Chansaenroj et al., 2012).

Statistical analysis

The PCR/sequencing technique was compared with the electrochemical DNA chip by using analyses of agreement (Kappa value and percent total agreement). The Kappa values ranging from 0.0-0.20 were considered as poor agreement, from 0.21-0.40 as fair agreement, from 0.41-0.60 as moderate agreement, from 0.61-0.80 as good agreement and from 0.81-1.00 as excellent agreement. We also compared HPV detection rate in urine and cervical samples. Sensitivity, which is the probability of an HPV-positive urine sample given an HPV-positive cervical sample, and specificity, which is the probability of an HPV-negative urine sample given an HPV-negative cervical sample were reported. Concordance was measured as the percentage of paired urine and cervical samples that yielded the same results. Type-specific concordance was calculated as the percentage of paired urine and cervical samples that were positive for the same HPV genotypes. Two-sided p value was calculated with values less than 0.05 being considered statistically significant. All of these analyses were carried out using SPSS Software version 17.0 (IBM Corporation, Somers, NY).

Results

Patient's characteristics

There were a total of 116 women recruited for this study. Histological examination revealed normal cytology in 52, ASCUS in 9, LSIL in 24, and HSIL in 31 women. The average age of women with normal cytology was 43.1 years (range, 27-61 years), while in women with abnormal cytology the average age was 40.0 years (range, 15-63 years). In male patients, there were a total of 100 men recruited, of which 45 were heterosexual males who visited hospitals for health check-up and 55 were documented as homosexuals or MSM. Among 55 MSM, 21 (38.1%) were HIV-positive. The average age of MSM was 28.1 years (range, 18-46 years).

HPV concordance in paired urine and cervical sample by PCR/sequencing technique

Table 1 showed that the highest prevalence of any-type HPV detected by PCR was found in women with HSIL; 61.3% in urine samples and 83.9% in cervical samples.The most prevalent HR-HPV types detected by PCR in cervical samples with precancerous lesions were HPV16 (26.56%), HPV31 (6.25%) and HPV58 (6.25%). Among 116 women, there were 14 samples (12.07%) that were high-risk HPV (HR-HPV) positive in the cervical samples but negative in urine samples. In contrast, there were 7 samples (6.0%) that were HPV positive in urine but negative in cervical samples. The sensitivity of HPV detection in urine samples by PCR ranged between 44.4-66.7%, while specificity ranged between 60-100% and concordance ranged between 64.5-82.7%.

HPV concordance in paired urine and cervical samples by the electrochemical DNA chip

Prevalence of HR-HPV infection detected by the electrochemical DNA chip was also reported highest in HSIL group; 51.6% in urine samples and 87.1% in cervical samples. These are comparable numbers to the PCR technique. The most prevalent HR-HPV types in women with precancerous lesions detected in cervical samples by the electrochemical DNA chip were HPV16 (31.25%),

Table 1. HPV Concordance in Paired Urine and Cervical Samples by PCR/Sequencing and the Electrochemical DNA Chip

Population	Technique	HPV type	HPV positivity		Sensitivity	Specificity	Concordance	Type-specific Concordance (%)
			Urine sample n (%)	Cervical sample n (%)				
Female with normal cytology (n=52)								
	PCR	Any type HPV	8 (15.4)	9 (17.3)	44.4	90.7	82.7	23.1
	DNA chip	HR-HPV	4 (7.7)	7 (13.4)	57.1	100	94.2	42.8
Female with abnormal cytology								
Total (n=64)	PCR	Any type HPV	33 (51.6)	46 (71.9)	65.2	83.3	70.3	57.1
	DNA chip	HR-HPV	27 (42.2)	42 (65.6)	64.3	100	75	62.8
HSIL (n=31)	PCR	Any type HPV	19 (61.3)	26 (83.9)	65.4	60	64.5	57.1
	DNA chip	HR-HPV	16 (51.6)	27 (87.1)	59.3	100	61.3	57.1
LSIL (n=24)	PCR	Any type HPV	9 (37.5)	14 (58.3)	64.3	100	79.2	50
	DNA chip	HR-HPV	9 (37.5)	11 (45.8)	81.8	100	91.7	72.7
ASCUS (n=9)	PCR	Any type HPV	5 (55.5)	6 (66.6)	66.7	66.7	66.7	42.8
	DNA chip	HR-HPV	2 (22.2)	4 (44.4)	50	100	77.8	50

Table 2. Comparison of HPV Detection in Cervical and Urine Samples Detected by the Electrochemical DNA Chip

	Cervical swab –		Cervical swab +		%agreement	Kappa	95%CI	p value
	Urine-	Urine+	Urine-	Urine+				
All(n=116)	67	0	18	31	84.48	0.67	0.52-0.81	<0.001
Normal cytology group (n=52)	45	0	3	4	94.23	0.7	0.37-1	<0.001
Abnormal cytology group (n=64)	22	0	15	27	76.56	0.55	0.36-0.75	<0.001
HSIL(n=31)	4	0	11	16	64.52	0.27	0.00-0.62	0.01
LSIL(n=24)	13	0	2	9	91.67	0.83	0.60-1	<0.001
ASCUS(n=9)	5	0	2	2	77.78	0.53	0.00-1	0.04

followed by HPV58 (10.94%) and HPV52 (9.38%). Among 116 women, dual or multiple HPV infections were detected in 5 of 116 (4.3%) samples extracted from urine and in 9 of 116 (7.8%) cervical samples. There were 18 samples (15.5%) that were HR-HPV positive in the cervix but negative in urine, however, none of the samples were positive in urine but negative in cervical samples. Sensitivity of HPV detection in urine samples by the electrochemical DNA chip ranged between 50-81.8%, while specificity was 100% and concordance ranged between 61.3-94.2%.

Agreement in HR-HPV detection by the electrochemical DNA chip in cervical and urine samples

Among 116 women, the overall agreement for the detection of HR-HPV genotypes by electrochemical DNA chip in urine and cervical samples was 84.48% with a kappa value of 0.67. There was good agreement in women with normal cytology (k=0.70) and moderate agreement in women with abnormal cytology (k=0.55). The highest level of agreement was found in women with LSIL, which reported 91.67% total agreement with a kappa value of 0.83 as shown in Table 2.

HPV positivity in male urine sample

Among heterosexual males (n=45), HPV DNA was detected in 3 urine samples (6.7%) by PCR and 2 urine samples (4.4%) by electrochemical DNA chip. Among HIV-positive MSM (n=21), HPV DNA was detected in 2 urine samples (9.52%) by PCR and 4 urine samples (19.05%) by electrochemical DNA chip. When considering only HIV negative MSM (n=34), HPV DNA was detected in 5 urine samples (14.7%) by PCR and 2 (5.88%) by electrochemical DNA chip. The genotypic distribution of HPV is shown in Table 3.

Table 3. HPV Prevalence in Male Urine Samples by PCR/Sequencing and the Electrochemical DNA Chip

Population	Technique	HPV type	HPV positivity in urine sample n (%) and HPV genotypes
Heterosexuals (n=45)	PCR	LR-HPV	2 (4.4); HPV6, 70
	PCR	HR-HPV	1 (2.2); HPV18
	DNA chip	HR-HPV	2 (4.4); HPV35+52, 18
Non-HIV MSM (n=34)	PCR	LR-HPV	4 (11.8); HPV6, 11, 84, 87
	PCR	HR-HPV	1 (2.9); HPV82
	DNA chip	HR-HPV	2 (5.9); HPV31+68, 45
HIV-positive MSM (n=21)	PCR	LR-HPV	1 (4.8); HPV81
	PCR	HR-HPV	1 (4.8); HPV45
	DNA chip	HR-HPV	4 (19); HPV35, 35+68, 35+52+68, 45

Agreement in HR-HPV detection in PCR/sequencing and DNA chip in female cervical and urine samples

In the PCR/sequencing technique, HPV positive results were excluded if the specimens showed negative results from the internal control (β-globin gene). All positive PCR products were sequenced in order to identify the HPV genotypes. The DNA chip used primer sets for each HPV and amplified only one or multiple matching types among 13 carcinogenic types.When we compared HPV detection between the electrochemical DNA chip and PCR/sequencing technique in female urine and cervical samples, the PCR/sequencing technique was considered positive only when they were positive in one of the 13 carcinogenic HPV types detectable by the DNA chip. Table 4 shows that among 116 women, overall agreement for the detection of HR-HPV genotypes by electrochemical DNA chip and PCR/sequencing was 88.79% and 86.21% in cervical samples and urine samples, respectively. In women with normal cytology, carcinogenic HPV detection rate showed fair agreement with a kappa value (k-value)

Table 4. Comparison between PCR/Sequencing and the Electrochemical DNA Chip in Female and Male Samples*

			DNA chip- PCR/ Sequencing -	DNA chip- PCR/ Sequencing +	DNA chip+ PCR/ Sequencing -	DNA chip+ PCR/ Sequencing +	%agreement	Kappa	95%CI	p value
Female all (n=116)		Cervical swab	66	1	12	37	88.79	0.76	0.64-0.88	<0.001
		Urine	80	6	10	20	86.21	0.62	0.45-0.80	<0.001
Normal cytology (n=52)		Cervical swab	45	0	5	2	90.39	0.41	0.00-0.90	<0.001
		Urine	47	1	1	3	96.15	0.73	0.36-1.00	<0.001
Patients (n=64)	HSIL (n=31)	Cervical swab	4	0	3	24	90.32	0.67	0.34-1.00	<0.001
		Urine	12	3	2	14	83.87	0.67	0.42-0.93	<0.001
	LSIL (n=24)	Cervical swab	12	1	3	8	83.33	0.66	0.36-0.96	<0.001
		Urine	15	1	6	2	70.83	0.22	0.00-0.59	0.09
	ACUS (n=9)	Cervical swab	5	0	1	3	88.89	0.77	0.34-1.00	0.009
		Urine	6	1	1	1	77.78	0.36	0.00-1.00	0.14
Male	All (n=100)	Urine	92	0	6	2	94	0.38	0.00-0.76	<0.001
	Heterosexuals (n=45)	Urine	43	0	1	1	97.78	0.66	0.03-1.00	<0.001
	MSM (n=55)	Urine	49	0	5	1	90.91	0.26	0.00-0.68	0.002

*PCR/sequencing technique was considered positive only when they were positive in one of the 13 carcinogenic HPV types detectable by the DNA chip

of 0.41 in cervical samples and 0.73 in urine samples. In women with HSIL, there was good agreement in HPV detection rate from cervical samples; 90.32% agreement and a k-value of 0.67. However, in urine samples the agreement dropped down to 83.87% with a k-value of 0.67. In women with LSIL and ASCUS, the level of agreement was also higher in cervical samples than in urine samples as shown in Table 4. In summary, the overall percentage of agreement in HR-HPV detection by the two methods in female samples ranged from 70.83-96.15% with the kappa value ranging from 0.22-0.77.

Agreement in HR-HPV detection in PCR/sequencing and DNA chip in male urine samples

Among healthy males (n=100), there was moderate agreement of HR-HPV detection in urine samples by the 2 methods, with 94.00% agreement, and a k-value of 0.38. As shown in Table 4, the level of agreement and k-value was higher in heterosexual male urine samples than in MSM urine samples.

Discussion

The present study evaluated the detection rate of HPV genotypes by comparing PCR/sequencing with an electrochemical DNA chip, which is a new automated DNA test that detects 13 carcinogenic HPV genotypes (Satoh et al., 2013). Both assays used the same DNA sample obtained from the same patient specimen. Moreover, we also compared HPV detection in cervical and urine samples in women, as urine is considered to be a preferable biological specimen for HPV DNA detection (Manhart et al., 2006).

In this study, HPV prevalence was determined to be 17.3% in cervical samples with normal cytology by PCR, which is higher than what a previous survey found in Thai women (7.6%) (Chansaenroj et al., 2010), but slightly lower than a study of Japanese women (22.5%) (Onuki et al., 2009). In women with abnormal cytology, we found that HPV prevalence detected by PCR was higher than in the normal group with a prevalence of 83.9%, 58.3% and 66.6% for HSIL, LSIL and ASCUS, respectively. These results are consistent with another report (Onuki et al., 2009), which demonstrated a higher prevalence of HPV in precancerous lesions. The 3 most common types of HPV detected by PCR in our study were HPV 16 (26.6%), HPV 31 (6.3%) and HPV 58 (6.3%). This was different from a previous survey in Thai women which reported HPV16 as the most common genotype identified (17.9%), followed by HPV 90 (16.6%) and HPV 71 (10.3%) (Chansaenroj et al., 2010). Regardless of differences in collection methods and PCR techniques, HPV 16 is still the most prevalent HPV genotype in Thailand as well as in many other countries (Onuki et al., 2009; Bissett et al., 2011; Munoz et al., 2013).

Several other studies have compared HPV sampling rates between urine and cervical samples in order to gauge the ability to detect HPV prevalence independently of cervical cytology (Sellors et al., 2000; Brinkman et al., 2002; Daponte et al., 2006; Bissett et al., 2011; Munoz et al., 2013). Daponte et al. (2006) suggested that sensitivity of urine testing for HPV 16 and 18 was higher in cervical cancer (88.8%) than in high grade and low grade lesions (76.5 and 45.5%, respectively) (Daponte et al., 2006). A US study reported that the prevalence of any HPV genotype in HIV-infected women was lower in urine samples (58% for the cervical swab specimens and 48% for the urine specimens) but not significantly different (Brinkman et al., 2002). Our study demonstrated that the HPV detection rate was generally lower in urine samples compared with cervical samples, with sensitivity ranging between 44.4-81.8% and specificity 60-100% by PCR and electrochemical DNA chip. The sensitivity was also higher in the abnormal cytology group compared to the normal cytology group. Variation of sensitivity in urine samples is likely due to the relatively small amount of HPV DNA in urine or due to some PCR inhibitors in urine such as nitrites, which can inhibit amplification (Brinkman et al., 2004). The urine HPV DNA yield could be improved by using first-voided, epithelial cell-rich morning urine or collecting larger urine volumes. There were 7 samples (6.0%) that were HPV positive in urine but negative in cervical samples. This was probably due to HPV contamination in urine or improper cervical swab technique, which may have resulted in a low yield of HPV DNA. Concordance of HPV detection in urine and cervical

samples in our study ranged from 64.5-94.2%. The use of urine samples for HPV testing has some advantages as it is easy to collect and considerably less invasive, however, testing urine samples yields lower sensitivities than self-collected vaginal or vulva samples (Sellors et al., 2000). Previous studies concluded that further improvement in the PCR process was required in order to increase an overall yield before clinical application of a urine-based HPV screening program for precancerous lesions and cervical cancer (Brinkman et al., 2004; Daponte et al., 2006). Our study showed that urine HPV testing was helpful for the detection of HPV DNA in high grade cervical lesions, which need immediate treatment. HR-HPV detection in urine samples of women with normal cytology could help identify those who are at risk of developing precancerous lesions and may need frequent follow up. However, urine HPV testing cannot currently substitute for cytologic evaluation for cervical cancer screening.

The sensitivity of the electrochemical DNA chip for HPV detection has been found to be 10^2 copies/μl (Satoh et al., 2013), while it has been shown that PCR L1 gene detection requires at least 10^3 copies/μl (Lurchachaiwong et al., 2009). When comparing the two techniques for HPV detection, our results showed good agreement for the detection of HR-HPV genotypes in cervical samples from 116 women (k=0.76). In women with normal cytology, carcinogenic HPV detection rate showed moderate agreement (k-value=0.41) compared to women with HSIL (k value=0.67), LSIL (0.66) and ASCUS (0.77). A previous study (Satoh et al., 2013) showed better agreement between these two techniques with kappa values of 0.77, 0.94, 1.00 and 1.00 in women with normal cytology, cervical intraepithelial neoplasia grade I (CIN I), CIN II and CIN III, respectively. The difference in kappa values between our study and the previous study (Satoh et al., 2013) were probably due to a small sample size and an unequal distribution of samples between groups. Moreover, the electrochemical DNA chip can detect multiple HR-HPV genotypes while PCR with a consensus primer can detect only one predominant type due to possible amplification bias (Mori et al., 2011). Thus, in women infected with multiple types of HPV, the electrochemical DNA chip seems to be a better choice for rapid diagnosis and identification. However, the electrochemical DNA chip also has some limitations as it can currently detect only 13 of the 15 HR-HPV genotypes so far identified (Muñoz et al., 2003). This was apparent in our study as we found HPV82 in 2 of our samples by PCR/sequencing technique, which the electrochemical DNA chip is not designed to find.

We also demonstrated that prevalence of any type of HPV detected in MSM urine samples by the PCR technique was higher than in heterosexual males; 12.7% and 6.7%, respectively. The overall prevalence of HR-HPV detected by electrochemical DNA chip in HIV MSM (19%) was higher than in non-HIV MSM (5.9%). A previous report showed higher prevalence of HPV infection from anal samples in HIV-positive MSM (85%) than in HIV-negative MSM (58.5%) (Phanuphak et al., 2013). These results indicate that anal samples are superior to urine samples for the purpose of HPV detection. The low prevalence of HPV DNA in male urine samples can be explained by failure to extract small amounts of DNA from urine or the presence of PCR inhibitors (Geddy et al., 1993). The 2 most prevalent types of HPV detected in male in our study were HPV 35 (4%) and HPV 45 (3%). These results were different from a previous survey in Thailand which reported HPV16 as the most prevalent (Phanuphak et al., 2013). A survey in Italy demonstrated that the two most prevalent HPV genotypes from male anogenital sites samples were HPV 6 (13%) and 16 (7%) (Barzon et al., 2010). Our study showed that the electrochemical DNA chip has a higher detection rate of HR-HPV in male urine samples compared to PCR techniques. However, the differences in genotypes detected by these two techniques were most likely due to different reagents for amplification or PCR conditions. Overall, there was fair agreement in HR-HPV detection by the 2 methods (kappa value of 0.38). The level of agreement and the kappa value were higher in heterosexual male urine samples compared with MSM; 97.78% overall agreement with k-value of 0.66 in heterosexual group and 90.91% overall agreement and a k-value of 0.26 in MSM.

A limitation of our study was a small study population. Moreover, urine samples have limitations such as low cellular load and they were not taken directly from the HPV infection site. PCR inhibitors can affect these results as well. Thus, the results obtained from urine samples might not reflect the real prevalence of HPV infection.

In conclusion, there was good overall agreement between the electrochemical DNA chip and PCR/sequencing methods for HR-HPV detection in female cervical samples, while there was fair agreement in male urine samples. The detection rates of HR-HPV were generally higher in samples from cervical lesions than in samples extracted from urine. Further improvement of PCR process and specimen collection is required to increase an overall yield of HPV detection in urine samples before the technique can be used in a clinical setting. The electrochemical DNA chip test is a promising technique for carcinogenic HPV detection.

Acknowledgements

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (HR 1155A-55), Thailand Research Fund (DPG5480002), Dutsadi Piphat Scholarship, the Center of Excellence in Clinical Virology, Chulalongkorn University, Integrated Innovation Academic Center IIAC Chulalongkorn University Centenary Academic Development Project (CU56-HR01), the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530093) and King Chulalongkorn Memorial Hospital for their generous support. We would like to express our thanks to staff of King Chulalongkorn Memorial Hospital for their generous support and the staff of the Thai Red Cross AIDS Research Centre, Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital and National Cancer Institute for providing the samples. Finally, special

thanks is given to Mr. Brian Muchmore for reviewing the manuscript.

References

Barzon L, Militello V, Pagni S, et al (2010). Distribution of human papillomavirus types in the anogenital tract of females and males. *J Med Virol*, **82**, 1424-30.

Bissett SL, Howell-Jones R, Swift C, et al (2011). Human papillomavirus genotype detection and viral load in paired genital and urine samples from both females and males. *J Med Virol*, **83**, 1744-51.

Brinkman JA, Jones WE, Gaffga AM, et al (2002). Detection of human papillomavirus DNA in urine specimens from human immunodeficiency virus-positive women. *J Clin Microbiol*, **40**, 3155-61.

Brinkman JA, Rahmani MZ, Jones WE, et al (2004). Optimization of PCR based detection of human papillomavirus DNA from urine specimens. *J Clin Virol*, **29**, 230-40.

Chansaenroj J, Lurchachaiwong W, Termrungruanglert W, et al (2010). Prevalence and genotypes of human papillomavirus among Thai women. *Asian Pac J Cancer Prev*, **11**, 117-22.

Chansaenroj J, Theamboonlers A, Chinchai T, et al (2012). High-risk human papillomavirus genotype detection by electrochemical DNA chip method. *Asian Pac J Cancer Prev*, **13**, 1151-8.

Daponte A, Pournaras S, Mademtzis I, et al (2006). Evaluation of high-risk human papillomavirus types PCR detection in paired urine and cervical samples of women with abnormal cytology. *J Clin Virol*, **36**, 189-93.

De Antonio JC, Fernández-Olmos A, Mercadillo M, et al (2008). Detection of high-risk human papillomavirus by two molecular techniques: hybridcapture and linear array. *J Virol Methods*, **149**, 163-6.

Fahey MI, Irwig L, Macaskill P (1995). Meta-analysis of Pap test accuracy. *Am J Epidemiol*, **141**, 680-9.

Geddy PM, Wells M, Lacey CJ (1993). Lack of detection of human papillomavirus DNA in male urine samples. *Genitourin Med*, **69**, 276-9.

Gheit T, Tommasino M (2011). Detection of high-risk mucosal human papillomavirus DNA in human specimens by a novel and sensitive multiplex PCR method combined with DNA microarray. *Methods Mol Biol*, **665**, 195-212.

Goldstone S, Palefsky JM, Giuliano AR, et al (2011). Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. *J Infect Dis*, **203**, 66-74.

Gravitt PE, Peyton CL, Alessi TQ, et al (2000). Improved amplification of genital human papillomaviruses. *J Clin Microbiol*, **38**, 357-61.

Gupta A, Arora R, Gupta S, et al (2006). Human papilloma virus DNA in urine samples of women with or without cervical cancer and their male partners compared with simultaneously collected cervical/penile smear or biopsy specimen. *J Clin Microbiol*, **37**, 190-4.

Hadzisejdić I, Simat M, Bosak A, et al (2006). Prevalence of human papillomavirus genotypes in cervical cancer and precursor lesions. *Coll Antropol*, **30**, 879-83.

Hagiwara M, Sasaki H, Matsuo K, et al (2007). Loop-mediated isothermal amplification method for detection of human papillomavirus type 6, 11, 16, and 18. *J Med Virol*, **79**, 605-15.

Khan MJ, Castle PE, Lorincz AT, et al (2005). The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst*, **97**, 1072-9.

Koutsky L (1997). Epidemiology of genital human papillomavirus infection. *Am J Med*, **102**, 3-8.

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.

Mandelblatt JS, Lawrence WF, Gaffikin L, et al (2002). Costs and benefits of different strategies to screen for cervical cancer in less-developed countries. *J Natl Cancer Inst*, **94**, 1469-83.

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

Mori S, Nakao S, Kukimoto I, et al (2011). Biased amplification of human papillomavirus DNA in specimens containing multiple human papillomavirus types by PCR with consensus primers. *Cancer Sci*, **102**, 1223-7.

Munoz M, Camargo M, Soto-De Leon SC, et al (2013). Human papillomavirus detection from human immunodeficiency virus-infected Colombian women's paired urine and cervical samples. *PLoS One*, **8**, 56509

Muñoz N, Bosch FX, de Sanjosé S, et al (2003). International agency for research on cancer multicenter cervical cancer study group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*, **348**, 518-27.

Onuki M, Matsumoto K, Satoh T, et al (2009). Human papillomavirus infections among Japanese women: age-related prevalence and type-specific risk for cervical cancer. *Cancer Sci*, **100**, 1312-6.

Phanuphak N, Teeratakulpisarn N, Pankam T, et al (2013). Anal human papillomavirus infection among Thai men who have sex with men with and without HIV infection: prevalence, incidence, and persistence. *J Acquir Immune Defic Syndr*, **63**, 472-9.

Qu W, Jiang G, Cruz Y, et al (1997). PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol*, **35**, 1304-10.

Rugpao S, Koonlertkit S, Ruengkrist T, et al (2009). ThinPrep Pap-smear and cervical intraepithelial neoplasia in reproductive-aged Thai women. *J ObstetGynaecol Res*, **35**, 551-4.

Satoh T, Matsumoto K, Fujii T, et al (2013). Rapid genotyping of carcinogenic human papillomavirus by loop-mediated isothermal amplification using a new automated DNA test (Clinichip HPV™). *J Virol Methods*, **188**, 83-93.

Sellors JW, Lorincz AT, Mahony JB, et al (2000). Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. *CMAJ*, **163**, 513-8.

Solomon D, Davey D, Kurman R, et al (2002). Forum group members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*, **287**, 2114-9.

Song ES, Lee HJ, Hwang TS (2007). Clinical efficacy of human papilloma viruses DNA detection in urine from patients with various cervical lesions. *J Korean Med Sci*, **22**, 99-104.

Wang JL, Yang YZ, Dong WW, et al (2013). Application of human papillomavirus in screening for cervical cancer and precancerous lesions. *Asian Pac J Cancer Prev*, **14**, 2979-82.

Wright TC Jr, 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.