RESEARCH ARTICLE

Comparison of Detection Sensitivity for Human Papillomavirus between Self-collected Vaginal Swabs and Physician-collected Cervical Swabs by Electrochemical DNA Chip

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Abstract

Background: Human papillomavirus (HPV) DNA testing is an effective method to screen for precancerous changes in the cervix. Samples from self-collection rather than Pap smear can potentially be used to test for HPV as they are more acceptable and preferred for use in certain settings. The objective of this study was to compare HPV DNA testing from self-collected vaginal swabs and physician-collected cervical swabs. Materials and Methods: A total of 101 self-collected vaginal and physician-collected cervical swabs of known cytology from Thai women were tested by electrochemical DNA chip assay. The specimens were divided into 4 groups: 29 with normal cytology, 14 with atypical squamous cells of undetermined significance (ASCUS), 48 with low-grade squamous intraepithelial lesion (LSIL), and 10 with high-grade squamous intraepithelial lesion (HSIL). Results: Positive detection rates of HPV from self-collected swabs were similar to those from physician-collected swabs. Among specimens with abnormal cytology, HPV was found in 50% of self-collected swabs and 47.2% of physician-collected swabs. In specimens with normal cytology, 17.2% of self-collected swabs and 24.1% of physician-collected swabs were positive for HPV. Concordance was relatively high between results from self-collected and physician-collected samples. The most common HPV genotype detected was HPV 51. Conclusions: HPV DNA testing using self-collected swabs is a feasible alternative to encourage and increase screening for cervical cancer in a population who might otherwise avoid this important preventive examination due to embarrassment, discomfort, and anxiety.

Keywords: HPV - HPV genotyping - self-collected - electrochemical DNA chip

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Introduction

Human papillomavirus (HPV) causes benign and malignant neoplasms of the genital tract including cervical cancer (Koutsky, 1997). HPV genotypes 16 and 18 correlate with an increased risk of developing precancerous lesions and are the most common causes of cervical cancer (Khan et al., 2005). HPV DNA testing is an effective screening method for precancerous changes in the cervix, especially for specimens with normal cytology or low-grade squamous intraepithelial lesions (LSIL) (Wang et al., 2013). Papanicolaou (Pap) test is a simple technique used for screening cervical cancer since the 1940's and has been shown to reduce the incidence of mortality from cervical cancer in countries with an active screening program (Safaeian et al., 2007). Test results help physicians detect precancerous lesions and determine the course of treatment prior to the development of malignancy.

In developing countries with few or no screening for cervical cancer by Pap test, the incidence of cervical cancer is high (Parkin et al., 2001). Women generally do not visit their doctor for gynecologic examination when the disease is asymptomatic, which creates a barrier to HPV screening. Other barriers include the embarrassment, discomfort and fear of the results. Moreover, the cost of testing may discourage patients from choosing to screen for HPV (Oranratanaphan et al., 2014). To improve screening coverage, self-administered sample collection could be an alternative to Pap smear test (Nilyanimit et al., 2013; Scarinci et al., 2013). Previous studies examined the sensitivity and predictive value of HPV detection by comparing self-collected and physician-collected samples for HPV screening. They found that HPV self-collection was an acceptable and feasible method to confirm cytology results in cervical cancer screening (Garcia et al., 2003, Safaeian et al., 2007). In Thailand, most screening of cervical cancer is done by Pap smear, but few women follow this screening program (Rugpao et al., 2009). Patients who avoid HPV testing may do so because of

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cultural and behavioral factors specific to certain countries.

The universal detection of HPV DNA usually focuses on the L1 region of the major capsid gene (Gravitt et al., 2000). Detection of the HPV DNA can be done by various techniques such as polymerase chain reaction (PCR) with specific primers, hybrid capture test, and linear array (De Antonio et al., 2008). Recently, a new technique to detect HPV DNA uses electrochemical DNA chip system combined with loop-mediated isothermal amplification (LAMP). This technique can detect 13 high-risk (HR) HPV genotypes. It can also detect single and multiple infections of high risk genotypes of HPV with higher sensitivity, specificity, simplicity, and speed when compared with other methods (Hagiwara et al., 2007).

Due to the many advantages of using self-collected samples for HPV testing, this study aimed to compare the HPV DNA test results between self-collected and physician-collected cervical swabs in Thai women.

Materials and Methods

All self-collected and physician-collected specimens were obtained from the King Chulalongkorn Memorial Hospital and Bangpakok 9 International Hospital in Bangkok, Thailand, between October 2013 and March 2014. The Pap smears were evaluated by a specialized cytotechnologist and confirmed by a pathologist. The research protocol was approved by the Institutional Review Board (IRB number 519/56) of the Faculty of Medicine, Chulalongkorn University. The objective of the study was informed to participants and written consents were obtained. The specimens were sent as anonymous.

Population study

Self-collected vaginal swabs and physician-collected cervical swabs were obtained from 101 females between ages 20-70 years. The specimens were separate into five groups: normal (n=29), atypical squamous cells of undetermined significance (ASCUS) (n=14), low-grade squamous intraepithelial lesions (LSIL) (n=48) and high-grade squamous intraepithelial lesions (HSIL) (n=10). Participations were voluntary and were solicited during colposcopy clinic and routine clinic. Both methods to collect the specimen were performed during the same visit for all participants.

Specimen collection and preparation

Physician-collected cervical swabs: were collected before the gynecologist performed Pap smear. The doctor inserted the Flexible minitip flocked swab (Copan Diagnostics, Murrieta, CA) into the cervix and twirled it for 3 seconds, after which the swab was placed in a collection tube and sent to the Center of Excellence in Clinical Virology Laboratory within 6 hours. After transportation to the laboratory, 1 ml of phosphate buffered saline (PBS) was added to the samples and vortexed. Then, the specimens were transferred to 1.5ml tube and stored at -20°C until used.

Self-collected vaginal swabs: Self-collected specimens were collected before Pap smear was performed. For self-collected vaginal swabs, patients were instructed to insert the Flexible minitip flocked swab (Copan Diagnostics, Murrieta, CA) into the vagina and twirl it 2-3 times. Self-collection was conducted in private room. All specimens were sent to the laboratory and the collected samples were treated by the same method as physician-collected cervical swabs.

Pathological classification

All specimens in this study were subjected to cytological evaluation to characterize the pathology. Cervical smears for cytology analysis were reported in accordance with the Bethesda System, which is the international standard for reporting Pap smear results. This system classifies histological morphology into 3 types; ASCUS, LSIL and HSIL (Solomon et al., 2002).

Laboratory method

DNA isolation: DNA was extracted from gynecological specimens using the Qiamp DNA mini kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. After extraction, the DNA samples were stored at -20°C until tested.

Electrochemical DNA chip: The electrochemical DNA chip 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 reaction conditions and detection were performed using Electrochemical DNA chip (Toshiba, Tokyo, Japan) according to manufacturer's instructions. Genotyping for HPV was accomplished by automated hybridization of probe and primer and the subsequent quantification of the resulting electrochemical signals was done on the GLH-2C601 GenelyzerTM (Toshiba, Tokyo, Japan). The specific primers amplified only respective target. Cross-hybridization of the electrochemical DNA chip was not observed. Nilyanimit et al., 2013.

Statistical analyses

The self-collected vaginal swabs were compared with physician-collected cervical swabs by using analyses of agreement (Kappa value and percent total agreement). The Kappa values ranging from 0.0 to 0.20 were considered poor agreement, from 0.21 to 0.40 as fair agreement, from 0.41 to 0.60 as moderate agreement, from 0.61 to 0.80 as good agreement and from 0.81 to 1.00 as

Table 1. Analysis of the Cytology Results and HPV Detected in Paired Samples (Self-collected Versus Physician-collected) Using Electrochemical DNA Chip

	Number of HPV positive samples/total (%)		Concordance (%)
	Self collected	Physician collected	
Total	41/101 (40.6)	41/101 (40.6)	91
Abnormal cytology	36/72 (50.0)	34/72 (47.2)	94
HSIL	5/10 (50.0)	4/10 (40.0)	90
LSIL	24/48 (50.0)	22/48 (45.8)	96
ASCUS	7/14 (50.0)	8/14 (57.1)	93
Normal cytology	5/29 (17.2)	7/29 (24.1)	83

Table 2. Concordance Comparison of HPV DNA Detection between Self-collected and Physician-collected Swab

	Self-collected + Physician collected +	Self-collected + Physician collected-	Self-collected - Physician collected +	Self-collected - Physician collected-	% agreement	Sensitivity	Specificity	Kappa	95% CI	P-value
Abnormal cytology (n=72)										
HSIL (n=10)	4	1	0	5	90	80	100	0.8	0.044-0.052	0.048
LSIL (n=48)	22	2	0	24	95.8	91.7	100	0.92	0	0
ASCUS (n=14)	7	0	1	6	92.9	100	85.7	0.86	0.004-0.007	0.006
Normal cytology (n=29)	4	1	3	21	86.2	80	87.5	0.58	0.005-0.008	0.006

excellent agreement. Concordance was measured by the percentage of paired self-collected vaginal swabs and physician-collected cervical swabs that yielded the same results. Type-specific concordance was calculated as the percentage of paired self-collected vaginal swabs and physician-collected cervical swabs samples that were positive for the same HPV genotypes. All statistical analyses used SPSS Software version 17.0 (IBM Corporation, Somers, NY).

Results

Among the 101 women participants in this study, the age ranges were between 20 to 70 years and the majority of the women were less than 50 years old. Among those with normal cytology, the average age was 58.3, while in women with abnormal cytology the average age was 41.8 years. In both the physician-collected and self-collected specimens, all 13 genotypes in which the DNA chip can detect were identified in the samples. As an internal control, β-globin gene was detected in all samples, indicating adequate DNA sampling. Among the samples with abnormal cytology, the DNA chip identified HR-HPV in 50% of self-collected samples and 47.2% of physician-collected samples (Table 1). From samples with normal cytology, HPV was identified in 17.2% of self-collected samples and 24.1% of physician-collected samples. The most common HR-HPV genotype found in both types of samples was genotype 51. Overall, HPV was detected in 40.6% of the samples in the self-collected and physician-collected specimens. The overall concordance between the results for the two collection methods was 91%. There was a 94% concordance in the abnormal cytology group and 83% in the normal cytology group.

The level of agreement was high between self-collected and physician-collected samples (Table 2). Among specimens with abnormal cytology, there was an excellent agreement in the HPV detection rate as measured by the kappa value (k-value). The k-value of HSIL was 0.80, LSIL was 0.92 and ASCUS was 0.86. For specimens with abnormal cytology, there was a fair agreement in HPV detection rate (k-value of 0.58). The sensitivity and specificity of HPV detection in self-collected and physician-collected ranged between 80-100% (Table 2).

Discussion

In this study, we assessed the concordance of HPV DNA detection between specimens from self-collected swabs and physician-collected swabs in a cohort of Thai women. We found that the agreement rate between self-collected and physician-collected specimens for HPV DNA detection was high. These findings were similar to a previous study (Alder et al., 2013). Among specimens with normal cytology, 6.9% of these samples later tested positive for HPV. This is comparable to the rate of 7.6% in a previous study of Thai women (Chansaenroj et al., 2010). Among samples with abnormal cytology, 33.7% of the specimens were positive for HPV, which was higher than in the normal group. Previous report showed a higher prevalence of HPV in precancerous lesions (Onuki et al., 2009). The detection of HPV DNA also depends on the grade of anogenital disease and position of sampling (Harper et al., 2002). Although our study population was small, our results showed high levels of agreement in the detection of HR-HPV among samples with abnormal cytology (k-value= 0.80-0.92) while there was a fair agreement (k-value=0.58) among the specimens with normal cytology. High agreement of HPV DNA detection between self-collected and physician-collected (k-value=0.75) was also observed in a study of women in Uganda (Safaeian et al., 2007). Study size, the technique used to collect samples, and methods used to detect HPV DNA can contribute to the differences in HPV detection.

Previous study in Thailand found that 25-38% of Thai women have had Pap smear test. In this group, women ages 30-65 have had only one Pap smear test done (Sriamporn et al., 2006). Reasons women avoid Pap test include embarrassment associated with gynecologic exam and the fear of pain from speculum, therefore self-collected swabs could be an alternative way to facilitate increased screening for HPV (Scarinci et al., 2012). Self-collection was well-accepted by the women in this study, although some women expressed doubt in their confidence in performing the collection correctly. Even if a simplified collection method is standardized, another barrier to the increased screening of HPV DNA in Thailand is cost (Oranratanaphan et al., 2014). Although the acceptability of urine sampling for HPV detection has been reported, sensitivity of this sampling method was not well-established (Sellors et al., 2000).

The most common type of HPV detected in our study was HPV51. It was different from a previous survey in Thai women, which reported HPV16 as the most common genotype identified (17.9%) (Chansaenroj et al., 2010). HPV 16 remained 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).

Self-collected vaginal swab for HPV DNA testing is a viable alternative for screening the HPV genotyping. The self-collected testing may be the alternative approach to clinician-collected specimens because it is less costly, less-invasive, and relatively practical in low-resource setting and in remote population (Petignat et al., 2007). In

addition, self-collected is overwhelmingly preferred over Pap smear test because it can be done in relative privacy and less invasively. In addition to requiring less resource on the healthcare system, this sampling method will help increase the number of women who choose to pursue HPV screening in the future.

In conclusion, testing for HPV using self-collected sampling is a feasible alternative to encourage and increase screening for cervical cancer in the population who might otherwise avoid this crucial preventive examination due to embarrassment, discomfort and anxiety.

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