RESEARCH COMMUNICATION

Can Self Vaginal Douching for High Risk HPV Screening Replace or Assist Efficacy of Cervical Cancer Screening?

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Abstract

Objective: To study the accuracy of self vaginal douching and collection for HPV types 16, 18, 31 and 33 in women visiting Thammasat Hospital for the explicit purpose of cervical screening. Methods: A pelvic examination and Pap smear were performed for all women who came for cervical screening. Specimens were also collected by self vaginal douching before cervical screening and sent to the cell and molecular biology laboratory for analysis of human papillomavirus (HPV) types 16, 18, 31 and 33 using the polymerase chain reaction (PCR). Results: HPV prevalence was 3.6% overall from 250 women in this study. Twenty-four (9.6%) women had an abnormal cytology screening result. No cancer was found. Four women had a high grade squamous intraepithelial lesion (HSIL) and 14 had a low grade squamous intraepithelial lesion (LSIL) from colposcopic biopsy. Self vaginal douching for HPV 16, 18, 31 and 33 was used to predict abnormal Pap smear. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 12.5%, 97.5%, 33.3% and 91.3%, respectively. Conclusion: From our analysis of self vaginal douching for HPV detection using cases from Thammasat university hospital, it cannot replace the Pap smear.

Keywords: DNA - HPV - PCR - self collection

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Introduction

Cervical cancer is the second most common cancer worldwide. It is the highest incidence cancer among Thai women (Deerasamee and Srivatanakul, 1999). High-risk human papilloma virus (HPV) is the cause of cervical cancer. Pap smear is the standard method for cervical cancer screening. Frequent screening per individual is needed due to the disease's long progression from precancerous to cancerous status. Unfortunately most Thai women avoid gynecological checkup and cervical cancer screening because of modesty (Suwannarurk et al., 2004).

The clinical use of HPV-DNA testing in cervical cancer has been applied for cervical cancer screening. Atypical Squamous Cells of Undetermined Significance-Low-Grade Squamous Intraepithelial Lesion (ASCUS-LSIL) in a Triage Study (ALTS) was sponsored by the National Cancer Institute (Wright et al., 2007). When HPV-DNA and Pap smear both show negative result then the next screening appointment is scheduled every 3 years (Saslow et al., 2002).

Pap smear is an established cervical cancer screening that requires a trained cytopathologist to interpret the result. HPV testing, on the contrary, is a test utilizing a routine laboratory procedure. Thus HPV testing can process large numbers of samples readily. As a result, gynecological professional society proposed the use of HPV-DNA as the first cervical cancer screening before the use of Pap smear (Franceschi et al., 2009).

This investigation determined the accuracy of self vaginal douching and self sampling collection for HPV testing in detecting high grade squamous intraepithelial neoplasia (CIN2 or 3). Self collection was explored as a more private mean of sample collection. The possibility of using this method as the primary cervical cancer screening was also explored.

Materials and Methods

The study was conducted in a healthy population in an urban setting of Pathumthani, Thailand. The protocol was approved by the Faculty of Medicine, Thammasat University Ethical Committee on Clinical Research in 2007. Participants consisted of walk in patients for cervical cancer screening checkup at the outpatient gynecologic clinic at Thammasat University Hospital between April to May 2008. Scope of the study was explained to each subject. Those that could accept the additional self collecting sample for HPV with the normal Pap smear test agreed to sign the consent form. Health specific and sexual

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behavior data were also collected from all participants using a structured questionnaire. The demographics of these sample subjects are summarized in Table 1.

Self douching and self sample collection

Participants were given a gynecological checkup and cervical cancer screening. Self vaginal douching and collection was performed before pelvic examination with a modified vaginal douching method (Coutlée et al., 1997). Self douching and self sample collection were explained to participants orally by trained female personnel. The kit consisted of a 10 ml sterile disposable plastic syringe (Nipro, Thailand) containing 10 ml of normal saline and a clean disposable plastic cup (Aro, Thailand) with a case number on it. Participants were instructed to sit in a squatting position, to insert syringe tip into their vaginal opening, to inject normal saline content into the opening, then to collect the over spilled vaginal fluid by placing the cup under the vaginal opening. After checking that the participants understood the procedure, each participant was given the privacy of a lavatory stall to carry out the sample collection. The nurse then collected the plastic cup, transfer the content by another sterile syringe to a 10 ml centrifuge tube (Eppendorf, Hamburg, Germany). The sample were sent to Cellular and Molecular Microbiology Laboratory, Faculty of Medicine, Thammasat University for the HPV study. The sample was stored at -20°C until further processing.

HPV DNA testing

Vaginal douche liquid was thawed at 25°C. Exfoliated cervical cells were processed by centrifugation at 10,000 rpm (25°C) for 5 minutes. The supernatants were then removed, leaving the precipitates for further processing and DNA extraction by use of the Viral Nucleic Acid Extraction Kit (Real Genomics, Taipei County, Taiwan) following its protocol. DNA content from the extract was determined by calculation based on the UV absorbance at 260nm. DNA extracts so obtained were further processed by PCR for DNA amplification using the Taq DNA Polymerase (Bio-Rad Laboratories, Tokyo, Japan) and Oligonucleotide primers (synthesized by Operon, Huntsville, AL) specific to HPV type 16, 18, 31 and 33 accordingly. The amplification products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining.

Liquid-based Pap smear

A liquid based preparation Papanicolaou smear was collected from all subjects. All the cervical cytological specimens were collected from the cervix with cervical brush by 5 rounds in scraping pattern. The brush was immersed and manually stirred vigorously in collecting vial containing preserved cell solution (Cytyc, Boxborough, MA). Collecting vials were then submitted to the Department of Pathology, Thammasat University Hospital for daily processing. The results were read per normal routine by the resident cytopathologist. The lab result was later rechecked and confirmed by the senior pathologist.

Patients who had abnormal results of equal to or over "abnormal squamous/glandular cells of undetermined significance" (ASC-US or AGUS) were later assigned for colposcopy and colposcopic biopsy including endocervical curettage to further confirm the result. The resulting data were analyzed by Fisher's Exact Test using the implementation in SPSS version 11.

Table 1. Socio-demographic Characteristics and Sexual Behaviors for Cervical Human Papillomavirus (HPV) Infection among 250 Women at Thammasat University Hospital, Pathumthani, Thailand

	Current Study HPV infection status		
	Infected	Uninfected	p-value *
Age, years			N/A
<20	0	3 (100.0)	
21-30	1 (2.1)	47 (97.9)	
31-40	3 (3.6)	80 (96.4)	
41-50	3 (3.9)	73 (96.1)	
>51	2 (5.0)	38 (95.0)	
Age group, years			0.4204
<30	1 (2.0)	50 (98.0)	
>30	8 (4.0)	191 (96.0)	
Religion			0.6625
Buddhist	9 (3.7)	236 (96.3)	
Muslim	0	5 (100.0)	
Education			0.05
Primary and lower[1]	2(5.9)	32 (94.1)	
Secondary and higher[2]	7(3.3)	205 (96.7)	
Marital status			1.00
Married[3]	8(4)	193 (96)	
Single	1(2.3)	42 (97.7)	

Results

Demographic data

Demographic data are shown in Table 1. The mean age of the subjects was 40.3 ± SD 10.5 years, the mean age at first intercourse 23.9 ± SD 4.4 years. Nearly half (46.8%) had completed high school. Approximately two-third (70.5%) was living with a partner and 13.6% had more than 1 sexual partner.

Prevalence of HPV from self-collecting sample

The prevalence of type-specific human papillomavirus (HPV) infection of the cervix from this study was 3.6% (9/250). The genotype groups 16, 18, 31 and 33 were 4, 2, 2 and 1 cases respectively as reported in Table 2. Percentage of sensitivity and specificity of HPV test and abnormal Pap smear is shown in Table 3.

Age-specific prevalence of HPV infection

As shown in Table 1, rate of HPV DNA positive cases showed no difference between all age groups. Overall high-risk HPV infections were not significantly different among women <30 years of age (2%) and >30 years of age (4%) in this study. When patients were grouped according to other attributes for their socio-demographic characteristics, like religion, level of education and marital status, there was no significant difference for any attribute.

Prevalence of abnormal Pap smear

Liquid-based cytology for cervical cancer screening

Table 2. Prevalence of Type-Specific Human Papillomavirus (HPV) Infection of the Cervix Among 250 Women at Thammasat University Hospital, Pathumthani, Thailand

		Women with abnormal cytology no (%)*			Women with normal cytology no (%)
		CIN negative	LSIL	HSIL	
HPV DNA negative	241 (96.4)	6 (100)	12 (85.7)	3 (75.0)	220 (97.3)
HPV DNA positive*	9 (3.6)	0 (0)	2 (14.3)	1 (25.0)	6 (2.7)
Total	250	6	14	4	226
* Detail HPV subtype					
16	4 (1.6)	0	1	0	3
18	2 (0.8)	0	1	0	1
31	2 (0.8)	0	0	0	2
33	1 (0.4)	0	0	1	0

* Six subjects had CIN negative result (false positive screening).

Table 3. Percentage of Sensitivity and Specificity (95% Confidence Interval) of HPV Test and Abnormal Pap Smear

	Abnormal Pap smear		Pathological result ≥ CIN 2(HSIL)	
	Pap +	Pap -	HSIL +	HSIL -
HPV +	3	6	1	2
HPV -	21	220	3	8
Sensitivity(95%CI)	12.5 (0,25.7)		25.0 (0,67.4)	
Specificity(95%CI)	97.4(95.2,99.4)		80.0 (55.2,100)	
PPV(95%CI)	33.3 (2.5,64.1)		33.3 (20,86.7)	
NPV(95%CI)	91.3(87.7,94.8)		72.7(46.4,99.0)	

Pap +: positive for cervical cytology; Pap -: negative for cervical cytology; HPV+: positive for HPV test; HPV-: negative for HPV test; HSIL: high-grade Squamous Intraepithelial Lesion (CIN 2/3)

was used in this study. Prevalence of abnormal Pap smear in this study was 9.6% (24/250) as reported in Table 2. There is no cancer detected in this group. Cervical pathology from colposcopic directed biopsy of CIN 1, CIN 2 & 3, and CIN negative were 14, 4 and 6 cases respectively. The prevalence of high-grade disease (CIN2+) was 1.6%.

Discussion

In comparison with a previous study at Thammasat University Hospital in 2006, the participants of this current investigation had slightly different demographical characteristics. This group of subjects had higher education levels. Their primary education to higher education was 13.8 and 86.2 percent respectively compared to 31.89 and 68.1 percent from the previous publication. The subjects in this study presented a lower sexual health risk due to higher level of formal education. Most subjects were older than 30 years of age 79.6 compared to 60 percent in year 2006. These changes contributed to the lower prevalence of high-risk HPV DNA than 33.5 percent observed at the same location in 2006 study (Suwannarurk et al., 2009).

Self vaginal douching was chosen as a method of sample collection for this investigation to see its effectiveness as a HPV sample collection in a normal setup. Prevalence of infected women by high-risk HPV from current study was 3.6%. However the abnormal Pap smear showed 9.6% incidence (Table 3). This brought the sensitivity (12.5%) to the unacceptable level. Sensitivity was 25.0 percents when compared to CIN2+. All comparisons landed this result in an unacceptable range.

Self collection was explored as a mean of sample collection to evaluate a favorable sample collecting method in an attempt to increase cervical cancer screening among the susceptible population (Ogilvie et al., 2007). Some investigations have utilized self sample collection for Pap smear work (Reowchopisakul et al., 1993). However the current interest is now shifted to HPV-DNA testing from self collection (Nobbenhuis et al., 2002; Daponte et al., 2008).

Thai women who came to gynecological clinic preferred minimal activity at the genital area during gynecological examination (Suwannarurk et al., 2004). They considered any sample collection intrusive. Many chose not to have any examination done and only came to the doctor when major problem arose, i.e., first pregnancy or major bleeding. Self vaginal douching was chosen for the study by the investigators because there was no harsh instrument involved. All subjects welcomed this method after hearing about it at the subject recruiting presentation, considered it less frightening than the use of brush/swab. Most Thai women dislike the idea of using tampon for the reason that it harbored moisture. The only complaint about self vaginal douching method heard by nurses from participants was that the procedure created unwanted puddle.

Self cervicovaginal collection was explored by many groups of investigators in recent years (Lorenzato et al., 2002; Nobbenhuis et al., 2002; Dannecker et al., 2004). The use of swab, brush, tampon, and douching were all explored with acceptable sensitivity. Self collecting works performed after year 2002 were mostly employed swab from commercial HPV kits. However, since the Thais had inhibition about foreign objects entering genital area (Suwannarurk et al., 2004) self vaginal douching was the first method of choice to be explored by this study. There was a study looking at HPV prevalence among sex workers in the south of Thailand, which employed tampons as a collection medium with a good sensitivity (Chandeying et al., 2006). However, that collection method was rejected by patients at our hospital during the exploratory stage of the research.

Self vaginal douching was earlier explored in a few investigations (Morrison et al., 1992; Coutlée et al., 1997; Nobbenhuis et al., 2002; Chen et al., 2008). Their results

Table 4. Accuracy of HPV Test to Predict Abnormal Cervical Cytology

Author (year)	Study origin	Number of patients	Method	Sensitivity %	Specificity%	PPV%	NPV%
Chen FC (2008)	Taiwan	132	Lavage	49.6*	29.6*	75*	12.1*
Nobbenhuis MA (2002)	Netherland	54	Lavage	71.4	100	100	50
Coutlee F (1997)	Canada	270	Lavage	93.9	80.5	85.2	91.7
Morrison EA (1992)	USA	16	Lavage	100*	100*	100*	100*
Suwannarurk K	Thailand	250	Lavage	12.5	97.5	33.3	91.3

*reviewer calculation

yielded respectable results, as seen in Table 4.

We believe that our investigation could not reproduce the same level of sensitivity due to limitation in patient education. Women in United States, Netherlands, Canada and Taiwan have high self esteem, good self and sexual awareness. Reproductive health and sex education are available as a part of young adult education in these countries. The Thais have double standards when it comes to sexuality. It is widely known in Thailand that good women are supposed to keep their virginity till the matrimony and be passive about their own sexual life. If women know too much, their husbands would condemn them to be promiscuous and dirty. With better self confidence, women will feel OK to get to know their own reproductive organs. Then they can feel and touch their genital area without any mental stigma.

Larsen et al studied Norwegian women's attitude toward gynecological examination. Many reported unpleasant feelings (Larsen et al., 1997). However, they endured the examination because they believed that it could discover hidden diseases. With this knowledge they became good participants during the examination. Thai patients in general had unjustified fear about discovering any hidden diseases (unpublished interview). They thought not knowing about the disease was better than knowing that they harbored any illness.

Thai patients are actually afraid of loss of control of their well being once any threatening disease is found. In Thailand doctors are highly respected. They normally carry out treatment with full authorization from patients. This common conduct makes patients feel helpless. This is also the case with cancer screening. Our group is finding ways to reduce the screening barriers in the hard-to-reach group (which is the majority of our patients) so that precancerous and early cancerous cases can be detected as early as possible.

Sensitivity of self-vaginal douching in Thai patients under this study yielded an unacceptable sensitivity level. We believe that one of the factors contributing to this result was because of poor participants' education and the Thai women's low value toward their sexual empowerment. As a result, sensitivity and other parameters such as specificity, PPV, and NPV were affected. However, when Chi square treatment was applied to false negative cases (- HPV with + Pap result) using data from this current data and data from physician collected sample of the same author (Suwannarurk et al., 2009), at 8.71 and 8.57 percent respectively it showed no significant different at $p = 0.959$. In layman's term there is no significant difference between physician or self collecting sample in HPV false negative sample. We believe better patient empowerment thru health education, especially reproductive health education, will allow Thai women to have more self esteem and self confidence. When this condition is achieved, self vaginal douching may be used as a sample collection method like in other developed countries.

Correctly anticipating and predicting abnormal Pap smear and abnormal cervical histopathological result is difficult so that the objective of avoiding additional more expensive cyto/histological tests can be achieved. When self vaginal douching was used for high-risk HPV DNA detection, prediction of abnormal Pap smear sensitivity was 12.5% (Table 3). However, high specificity (97.4%) and high negative predictive value (91.3%) were obtained.

When self vaginal douching was used for high-risk HPV DNA detection, prediction of CIN2+ sensitivity was still low (25.0%). Negative predictive value was reasonable at 72.7%. The amount of affected subjects in this study was too small for any conclusion at this point.

The Thais in general, are not well aware of internal anatomical detail. High school subject in science teaches them the correct information. However, people who demonstrate knowledge about reproductive subjects are viewed as bad kids and not winning approval from the adults. This is an issue and a dilemma for later learning about reproductive health. People in the western hemisphere and developed Asian Nations do not have to cope with a similar prejudice.

From this study, the self vaginal douching for high-risk HPV DNA testing cannot replace the conventional method of cervical cancer screening (Pap smear) in Thailand under the present condition. However, high negative predictive value pointed out that if collection method could be improved, self vaginal douching collecting method could be useful. Self collecting method by collection of urine sample is planned as an alternative test of the new self screening concept.

In conclusion, this investigation looked into the accuracy of self vaginal douching for HPV detection using cases from Thammasat University Hospital. Overall HPV infection was 3.6%. Sensitivity, specificity, PPV and NPV were 12.5%, 97.5%, 33.3% and 91.3%, respectively. While the gold standard for cervical cancer screening is abnormal cytology detection via Pap smear, therefore it is necessary to compare cytological results with the PCR based HPV detection. Sensitivity of self-vaginal douching in Thai patients under this study was low. Self-vaginal douching cannot be used in HPV screening to replace cervical cancer screening in Thailand at the current time. Better patient empowerment thru health education will allow Thai women to have more self esteem and self confidence about their body. When this condition is achieved, self-vaginal douching may be used as a sample collection method for HPV study.

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References

Chandeying V, Garland SM, Tabrizi SN (2006). Prevalence and typing of human papilloma virus (HPV) among female sex workers and outpatient women in southern Thailand. *Sex Hlth*, **3**, 11-4.

Chen FC, Shaw SW, Cheng PJ, et al (2008). Diagnosis of human papillomavirus infection by abnormal cervical cytology is highly reproducible after vaginal douching. *Taiwan J Obstet Gynecol*, **47**, 412-6.

Coutlée F, Hankins C, Lapointe N (1997). Comparison between vaginal tampon and cervicovaginal lavage specimen collection for detection of human papillomavirus DNA by the polymerase chain reaction. The Canadian Women's HIV study group. *J Med Virol*, **51**, 42-7.

Dannecker C, Siebert U, Thaler CJ, et al (2004). Primary cervical cancer screening by self-sampling of human papillomavirus DNA in internal medicine outpatient clinics. *Ann Oncol*, **15**, 863-9.

Daponte A, Tsezou A, Oikonomou P, et al (2008). Use of real-time PCR to detect human papillomavirus-16 viral loads in vaginal and urine self-sampled specimens. *Clin Microbiol Infect*, **14**, 619-21.

Deerasamee S, Srivatanakul P (1999). Cervix Uteri. In 'Cancer in Thailand Vol. 11 1992-1994', Eds Deerasamee S, Martin N, Sontipong S, et al. IARC, Lyon pp 56-9.

Franceschi S, Cuzick J, Herrero R, et al (2009). EUROGIN 2008 roadmap on cervical cancer prevention. *Int J Cancer*, **125**, 2246-55.

Larsen M, Oldeide CC, Malterud K (1997). Not so bad after all…Women's experiences of pelvic examinations. *Fam Pract*, **15**, 148-52.

Lorenzato FR, Singer A, Ho L, et al (2002). Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. *Am J Obstet Gynecol*, **186**, 962-8.

Morrison EA, Goldberg GL, Hagan RJ, et al (1992). Self-administered home cervicovaginal lavage: a novel tool for the clinical-epidemiologic investigation of genital human papillomavirus infections. *Am J Obstet Gynecol*, **167**, 104-7.

Nobbenhuis MA, Helmerhorst TJ, van den Bruld AJ, et al (2002). Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. *J Clin Pathol*, **55**, 435-9.

Ogilvie G, Krajden M, Maginley J, et al (2007). Feasibility of self-collection of specimens for human papillomavirus testing in hard-to-reach women. *CMAJ*, **177**, 480-3.

Reowchopisakul K, Linasmita V, Srivannaboon S, et al (1993). Cytologic evaluation of smears obtained by self-collection vaginal tampon. *J Med Assoc Thai*, **76**, 260-3.

Saslow D, Runowicz CD, Solomon D, et al (2002). American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin*, **52**, 342-62.

Suwannarurk K, Sueblinvong T, Sermboon A, et al (2004). Knowledge and attitudes in pap smear screening among women at antenatal and postpartum clinic at Thammasat university hospital. *Thammasat Med J*, **1**, 11-9.

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

Wright TC Jr, Massad LS, Dunton CJ, et al (2007). 2006 American society for colposcopy and cervical pathology-sponsored consensus conference. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening test. *Amj J Gynecol*, **197**, 346-55.