RESEARCH ARTICLE

Experience of Combined Liquid Based Cervical Cytology and High-Risk HPV mRNA for Cervical Cancer Screening in Thammasat University Hospital

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Abstract

Background: Cervical cancer is the second most common of malignancy found in Thai women. Human papillomavirus (HPV) infection is a major cause. The objective of the present study was to evaluate the prevalence of HPV infection and association with abnormal cervical cytology in Thai women. Materials and Methods: This study was conducted at the Gynecologic Clinic, Thammasat University, Pathum Thani, Thailand. A total of 2,144 cases who underwent annual cervical cancer screening by co-testing (liquid based cytology and HPV testing, DNA versus mRNA) during the priod from July 2013 to June 2016 were recruited in this study. Results: Prevalence of positive high risk (HR) HPV DNA and mRNA test were 19.7 and 8.4%, respectively with a statistically significant difference. Majority of cases of abnormal cytology in this study were atypical squamous cells of undetermined significance (ASC-US). In patients with ASC-US, positive HR HPV DNA was greater than in the mRNA group (10.1 and 4.5%, p<0.001). Nonetheless, there was no significant difference in participants with cervical intraepithelial neoplasia (CIN). HPV mRNA test had slightly lower sensitivity but higher negative predictive value (NPV) than the DNA test to detect abnormal cytology during cervical cancer screening (p<0.001). Both HPV test (DNA and mRNA) had equally efficacy to detect high grade precancerous lesion or higher (CIN 2+). Conclusions: Prevalence of HR HPV DNA and mRNA were 19.7 and 8.4 percent, respectively. NPV of HPV mRNA was higher than DNA test. Both tests had equal efficacy to detect CIN 2+ with sensitivity and specificity of 63% vs 55.7% and 83% vs 92%, respectively.

Keywords: Cervical cancer - screening - human papillomavirus - liquid-based cytology - mRNA

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Introduction

Cervical cancer is the second most common type of malignancy found in Thai women, only preceded by breast cancer (IARC 2015). Early detection of cervical cancer can decrease its incidence. Implementation of an effective organized screening in the target population is highly desirable by providing coverage of adequate screening intervals. It can also provide an effective and reliable means for investigating women with abnormal screening results. Definite diagnosis will insure that appropriate measure can be given to the patient in timely manner.

It is now understood that human papillomavirus (HPV) infection is a major cause of cervical cancer (Thomas et al., 2014). Only a minor fraction of all HPV infection will progress to precancerous lesions and cancer. Over 200 different HPV genotypes are described and classified to high risk (HR) and low risk (LR) group. The most often found high risk strains are HPV 16, 18, and 45 (Li et al., 2013; Zhao et al., 2014; Dickson et al., 2013; Anderson et al., 2012; Ucakar et al., 2012). Many commercial kits are available for HR HPV detection.

Cervical cytology is a worldwide tool for cervical cancer screening. This is undertaken either via conventional Papanicolaou (Pap) smear or liquid-based cytology (LBC). There are currently two methods of HPV testing, namely cell marker detection and HPV genome.

Abnormal cell markers detection primarily look for protein p16 and Ki67. These proteins are found on HR HPV infected cervical epithelium (Thomas et al., 2014). HPV genome detection can be undertaken by viral DNA or mRNA detection. DNA is a more stable biomolecule than mRNA. However DNA detection yields positive result indiscriminately on both past and active infection. Detection of mRNA represents only an active infection. Therefore mRNA could provide a far superior mean to

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HPV infection screening. Very few studies investigated the prevalence of HPV mRNA and its effectiveness as a prognostic factor for cervical cancer (Luttmer et al., 2015). The focus in this study was to determine the usefulness of HPV mRNA testing in cervical cancer screening and its future clinical implications.

Materials and Methods

This retrospective study was conducted in Department of Obstetrics and Gynecology, Thammasat University, Pathum Thani, Thailand. The study was approved by the Ethics Committee of Faculty of Medicine Thammasat University. The criteria included patients who underwent LBC and HPV testing at Gynecologic outpatient unit between July 2013 and June 2016. Patients who had history of gynecologic malignancy were excluded from this study.

Cervical cytology was performed by liquid based technic defined as Bethesda system 2001 terminology. It is classified as a negative for intraepithelial lesion or malignancy (NILM), atypical squamous cell of undetermined significant (ASC-US), atypical squamous cell cannot exclude high grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and cancer.

Cervical samples obtained from July 2013 to August 2014 and underwent HPV DNA detection. HPV testing was performed to detect HPV DNA (Cervista®, Hologic, Inc, Marlborough, MA, USA). Semi-automated process HPV type 16 and 18 assays were performed in samples with positive HR HPV results.

Thammasat University Hospital had changed all its HPV testing to mRNA testing in June 2014. This test was performed by using commercial kit (Aptima®, Hologic, Inc, Marlborough, MA, USA). It is a fully-automated process detecting HPV type 16/18/45. Patient who had positive HR HPV with ASC-US cytology or abnormal cytology at least LSIL underwent colposcopic directed biopsy. Histologic finding were classified as normal, low grade (CIN 1; cervical intraepithelial neoplasia), high grade precancerous lesion (CIN 2/3) and cancer.

Descriptive statistic were used for demographic data. SPSS Statistics version 17 (SPSS Inc., Chicago, USA) was used for all statistical analysis. Chi-square or Fisher's exact test, where appropriated, was used to compare the difference between groups. A p-value of less than 0.05 was consider significant.

Results

A total of 2,144 women underwent cervical cancer screening at Thammasat University Hospital. The period of study was between July 2013 and June 2016. There were 782 cases in HPV DNA testing group (group A) and 1,362 cases in HPV mRNA testing group (group B). Age of both groups were not statistically significant difference at p= 0.109. There were more cases of positive HR HPV DNA (19.7%, 154/ 782) than mRNA (8.4%, 114/1,362) with statistically significant difference at $p \leq 0.001$. There were more cases of abnormal cytology mostly as ASC-

Table 1. Age, Cytology Results, Diagnosis and HR-HPV Detection for Women in the Study

	HR-HPV						p-value
	DNA (n=782)*			mRNA (n=1,362)*			
	positive	negative	total	positive	negative	total	
Age (yr)							0.109
<30	6(0.77)	15(1.92)	21	9(0.66)	40(2.94)	49	
>30	148(18.93)	613(78.38)	761	105(7.71)	1,208(88.69)	1,313	
Cytology							<0.001
NILM	104(13.30)	599(76.60)	703	80(5.87)	1,221(89.65)	1,301	
Abnormal	50(6.39)	29(3.71)	79	34(2.50)	27(1.98)	61	
Atypical							
ASC-US	20(2.56)	24(3.07)	44	6(0.44)	15(1.10)	21	
ASC-H	5(0.64)	1(0.13)	6	3(0.22)	2(0.15)	5	
Others	2(0.26)	0(0)	2	1(0.07)	6(0.44)	7	
LSIL	5(0.64)	3(0.38)	8	17(1.25)	5(0.37)	22	
HSIL	15(1.91)	1(0.13)	16	7(0.51)	0(0)	7	
Cancer	3(0.38)	0(0)	3	0(0)	0(0)	0	
Diagnosis							0.059
<CIN 1	139(17.77)	628(80.31)	767	102(7.49)	1247(91.56)	1349	
Normal	46(5.88)	628(80.31)	674	21(1.54)	1240(91.04)	1261	
Cervicitis	0(0)	0(0)	0	1(0.07)	0(0)	1	
CIN 1	13(1.66)	0(0)	13	11(0.81)	4(0.29)	15	
Post pone	80(10.23)	0(0)	80	69(5.07)	3(0.22)	72	
CIN 2+	15(1.91)	0(0)	15	12(0.88)	1(0.07)	13	
CIN 2	5(0.64)	0(0)	5	1(0.07)	0(0)	1	
CIN 3	8(1.02)	0(0)	8	8(0.59)	0(0)	8	
Cancer	2(0.26)	0(0)	2	3(0.22)	1(0.07)	4	

*n(%), HPV HR: high risk human papilloma virus, DNA: Deoxyribonucleic acid, mRNA: Messenger Ribonucleic acid, NILM: negative for intraepithelial lesion or malignancy, ASC-US: atypical squamous cell of undetermined, ASC-H: atypical squamous cell cannot exclude high grade lesion, LSIL: low grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia, post pone: post pone to colposcopy

US; in group A (10.1%, 79/782) than group B (4.5%, 61/1,362), p< 0.001. In patients with LSIL, there were 22 cases with positive HR HPV. Five cases had positive HR HPV DNA while 17 cases had positive HR HPV mRNA, p <0.001. Nevertheless, there was no statistically significant difference between positive HR in both groups of participants with HSIL.

In 28 cases of CIN I, there were 13 and 11 cases with positive HR HPV DNA and positive HR HPV mRNA, respectively. There were 4 cases of CIN I with negative HR HPV mRNA.

HR HPV genotype of 268 cases were determined. Among 154 cases in DNA group, genotype 16, 18, 16&18 and non 16/18 were 37, 3.6, 1.3 and 58.1 percent, respectively. Another 114 cases of mRNA group had smaller positive percentage than that of DNA group. Percentage of genotype 16, 18/45 and non 16, 18/45 were 14, 3.5 and 82.5, respectively.

One of HSIL case from 16 cases in DNA group had negative HR HPV while all cases of HSIL in mRNA group had positive result. Approximate 80 percent of positive HR HPV in DNA (12/15) and mRNA (5/7) group had positive genotype of non 16/18. All CIN 2/3 in this study had positive HR HPV result. One third of CIN 2/3 had positive genotype 16/18 and shown in table 2. There were six cases of cancer in this study. Four and two cases of cervical and endometrial cancer were their final diagnosis, respectively.

Table 2. Cytology Results, Diagnosis and Type of HR-HPV detection for Women in the Study

	HR-HPV positive						
	DNA(n=154)				mRNA(n=114)		
	T16	T18	T16/18	T non 16/18	T16	T18/45	T non 16 18/45
Cytology							
NILM	42(27.27)	2(1.30)	1(0.65)	59(38.31)	9(7.89)	2(1.75)	70(61.40)
LSIL	0(0)	0(0)	1(0.65)	4(2.60)	1(0.88)	0(0)	15(13.16)
HSIL	3(1.95)	0(0)	0(0)	12(7.79)	2(1.75)	0(0)	5(4.39)
Atypical	10(6.49)	1(0.65)	0(0)	16(10.39)	4(3.51)	1(0.88)	5(4.39)
ASCUS	6(3.70)	1(0.65)	0(0)	13(8.44)	2(1.75)	1(0.88)	3(2.63)
ASCH	4(2.60)	0(0)	0(0)	1(0.65)	2(1.75)	0(0)	1(0.88)
Atypical endometrium	0(0)	0(0)	0(0)	2(1.30)	0(0)	0(0)	1(0.88)
Cancer	2(1.30)	0(0)	0(0)	1(0.65)	0(0)	0(0)	0(0)
Diagnosis							
Normal	11(7.14)	0(0)	1(0.65)	33(21.43)	6(5.26)	0(0)	16(14.34)
Cervicitis	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
CIN 1	9(5.84)	0(0)	0(0)	4(2.60)	2(1.75)	0(0)	8(7.02)
CIN 2	2(1.30)	1(0.65)	0(0)	3(1.95)	0(0)	0(0)	1(0.88)
CIN 3	2(1.30)	0(0)	0(0)	6(3.70)	4(3.51)	0(0)	4(3.51)
Cancer	2(1.30)	0(0)	0(0)	0(0)	1(0.88)	0(0)	2(1.75)
Post pone	31(20.13)	2(1.30)	1(0.65)	46(29.87)	2(1.75)	3(2.63)	64(56.14)

*n(%),HR-HPV: high risk human papilloma virus, DNA: Deoxyribonucleic acid, mRNA: Messenger Ribonucleic acid, NILM: negative for intraepithelial lesion or malignancy, ASC-US: atypical squamous cell of undetermined, ASC-H: atypical squamous cell cannot exclude high grade squamous intraepithelial lesion, LSIL: low grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia, post pone: post pone to colposcopy, T: type of HR HPV

Table 3. Comparison of HR HPV Prevalence in Screening Setting In Various Countries

Study	Year	Country	N	HPV	
				Method	Prevalence
n (%)					
Present study	2016	Thailand	782	DNA	154(19.7)
			1,362	mRNA	114(8.4)
Tangjitgamol	2016	Thailand	4,428	DNA	295(6.7)
Zeng	2016	China	51,345	DNA	13,349(26)
Maggino	2016	Italy	23,211	mRNA	1,620(7)
Westre*	2016	Norway	564	mRNA	165(30.3)
				DNA	105(18.6)
Sarah	2016	Denmark	5,022	DNA(HC2)	1,024(20.4)
				DNA(PCR)	1,345(26.8)
				mRNA	838(16.7)
Kantathavorn	2015	Thailand	5,906	DNA	376(6.4)
Laowahutanont	2015	Thailand	2,897	DNA	270(9.3)
Chen	2015	China	1,978	DNA	291(14.7)
Zhao	2014	China	3,640	DNA	924(15.3)
Li	2013	China	8,156	DNA	755(20.7)
Anderson**	2013	Ireland	5,712	DNA	(16.6)
Dickson**	2013	USA	309,471	DNA	(11.9)
Ucakar	2012	Slovenia	4,431	DNA	502(11.3)
Suwannarurk***	2009	Thailand	263	DNA	88(33.5)

*prevalence in abnormal cytology ≥ ASC-US, **reported as percent in total population, ***previous study from this institute

Table 4. Comparison of HPV Test for Detection of Abnormal Cervical Cytology and CIN 2+*

		Cervical cytology					Diagnosis				
		DNA		mRNA		p value	DNA		mRNA		p value
		Abn**	NILM	pos	NILM		CIN 2+	<CIN 1	CIN 2+	<CIN 1	
HPV test	pos	50	104	34	80	0.645	15	59	12	33	0.833
	neg	29	599	27	1,221	0.003	0	628	1	1,247	0.478
	sum	79	703	61	1,301	<0.001	15	687	13	1,280	0.059
Sensitivity		63.3		55.7			100		92.3		
Specificity		85.2		93.9			91.4		97.4		
PPV		32.5		29.8			20.3		26.7		
NPV		95.4		97.8			100		99.9		
Accuracy		50.1		34.1			15.1		12		

*CIN 2+, CIN 2/3 and cancer, **cervical cytology abnormal

Accuracy of HPV testing for detection of abnormal cervical cytology and CIN 2+ was shown in Table 4. Sensitivity of HPV DNA testing was slightly greater than mRNA. While specificity of HPV mRNA was better than HPV DNA.

Discussion

This was a retrospective study. Subjects were women who underwent cervical cancer screening at Gynecologic Clinic, Thammasat University Hospital. Around ten percent of routine screening were co-testing (LBC and HPV testing). Data was collected from hospital computerized record. There were two methods of HPV testing; DNA or mRNA. Most studies from previous literature in Thailand came from HPV DNA analysis (Suwannarurk et al., 2009; Kantathavorn et al., 2015; Tangjitgamol et al., 2016; Laowahutanont et al., 2015). The present study was based on data from HPV DNA and mRNA analysis. The first and second half of this study were DNA and mRNA method, respectively.

Prevalence of HR HPV infection from China were found between 14.7-26 percent (Li et al., 2013; Zhao et al., 2014; Chen et al., 2015; Zeng et al., 2016). HR HPV prevalence from other region were summarized in Table 3. It ranged between 7-26.8 percent (Anderson et al., 2012; Dickson et al., 2013; Ucakar et al., 2012; Maggino et al., 2016; Sarah et al., 2016).

Prevalence of HPV from previous study in Thailand varied between 6.4-33.5 percent (Suwannarurk et al., 2009; Kantathavorn et al., 2015; Tangjitgamol et al., 2016; Laowahutanont et al., 2015). Prevalence of HPV from DNA method in this study was 19.7 percent. Most of the previous works were performed in hospital based populations in Bangkok. The present study was based on population in Pathum Thani province located in Northern suburb of Bangkok. People in this area either were referred to us by their primary or secondary health care providers or came in by themselves from North, Central and North-East region of Thailand. They represented a different population from those resided in Bangkok. Our subjects consisted of a mixed high and low socio-economic status.

Data from the first half of this study came from DNA based analysis while the later came from mRNA analysis. Prevalence of HPV by DNA and mRNA methods in this study were 19.7 and 8.4 percent, respectively. The result in this study showed higher prevalence of HPV from DNA testing than those of mRNA method. Theoretically, DNA was more durable than mRNA (Thomas et al., 2014). DNA persisted for a long time. It was suitable for HPV prevalence study. Positive HPV test from DNA method did not represent the activity of current HPV infection. The mRNA was a temporally template for HPV DNA synthesis. It was spontaneously disappeared after the end of HPV DNA synthesis. It was not surprising that prevalence of HPV from mRNA method was less than DNA method from representative population of the same demographic region.

Positive mRNA HPV testing reflected the just-in-time activity of HPV infection. The clinical application of HPV testing by mRNA method should be used for detection of active disease of precancerous lesion and follow up after treatment. This was the first study of HPV prevalence by mRNA technic in Thailand. This investigation supported the other studies that mRNA testing represented a more useful clinical data representing the active HPV infection.

Goal of cervical cancer screening was to detect precancerous or early cancerous lesion. The high sensitivity of the HPV DNA testing led high false positive frequency. Low sensitivity of cytology test also led to high false negative frequency. In the past, the solution for the low sensitivity of this test was to perform more frequent screening.

In this study, sensitivity of HPV DNA testing for abnormal cytology detection was higher than HPV mRNA test with statistical significant (63.3% & 55.5%, $p<0.001$). Negative predictive value (NPV) of HPV mRNA test was higher than DNA technic (97.8% & 95.4%, $p<0.001$). The good screening test should have high NPV to guarantee that it had low silent disease possibility. The HPV mRNA test in this study met the good quality of screening test criteria. Both HPV test (DNA & mRNA) had equal potency to detect CIN 2+. This study indicated that HPV mRNA test had slightly lower sensitivity but higher NPV than DNA technic.

In the present study, most of precancerous lesion contained HR HPV genotype. The power of this study cannot determine the difference between CIN prevalence.

Our finding from this study, HR HPV prevalence from mRNA method was lower than that of the DNA method. Prevalence of precancerous lesion from both technics was not different.

In conclusion, HPV detection from co-testing was compared to data from previous literature. The prevalence

of HPV testing by mRNA method in this study was 8.4 percent. The abnormality of test either cervical cytology or HPV positivity required further investigation. Colposcopic directed biopsy was a subsequent investigation after positivity screening result. Cost effectiveness analysis was needed for guideline development in the future.

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