Original Article

A Pilot Study of Anal Cytology and Anal Human Papilloma Virus (HPV) Infection in Women with High-risk Cervical HPV Infection

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Background: Anus is in a pelvic region close to cervix and may be a reservoir for human papilloma virus [HPV] infection. Hence, anal HPV infection may have similar result of cervical origin and could probably be used for cervical cancer screening.

Objective: To evaluate the concordance of anal HPV and cervical HPV infection and anal cytology in women with high-risk cervical HPV infection.

Materials and Methods: Between July 2011 and May 2012, we enrolled 38 Thai women, 20 to 70 years of age, with high-risk cervical HPV infection who consented to the study. We evaluated the concordance of anal HPV typing using a LINEAR ARRAY® HPV Genotyping test that can detect 12 types of high-risk HPV and 25 types of non-high-risk HPV and anal cytology using BD Sure Path Pap test kit.

Results: The mean age was 39.5 years, (range, 22 to 61 years) and the mean age of sexual activity onset was 22.8 years. Seven participants (18.4%) were menopausal, and 30/38 (78.9%) participants were sexually active. Overall HPV DNA was detected in 19/38 (0.04%) anal samplings, and 12/38 participants (31.6%) had at least one high-risk HPV genotype. Type specific (at least one type) match of high-risk HPV accounted for 11/38 (28.9%). The HPV genotypes distinguished in anal samples were predominantly HPV 16 (6 cases); HPV 6, 58, 61, and 84 (2 cases each); and HPV 18, 33, 39, 40, 45, 51, 53, 56, 60, 68, 72, 73, 81, and IS39 (1 each). Cervical HPV was detected without concurrent anal HPV detection. All anal cytology results were negative.

Conclusion: Low rates of cervical and anal high-risk HPV concordance in this study (approximately 30%) suggest that anal HPV test cannot be used as a substitute for cervical HPV test in cervical cancer screening program.

Keywords: Anal cytology; Human papilloma virus [HPV]; Linear array; Concordance rate; Cervical cancer

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According to GLOBOCAN 2012, cervical cancer is the fourth most common female carcinoma worldwide but the second most common female carcinoma in Thailand, and cervical cancer screening can reduce cervical cancer incidence and death from cervical carcinoma[1]. High-risk human papillomavirus [HPV] types especially HPV type 16, 18 are now recognized as a causal factor of cervical cancer[2]. Anal cancer is uncommon in the general population, with rates of 1 and 2 cases per 100,000 per year[3]; however, high-risk human papilloma virus [HPV], predominantly HPV type 16, causes more than 80% of squamous cell anal cancers[4]. These two cancers share similarity including site (pelvic region), cause [HPV] and predominately squamous cell type.

In 2011, Calore et al. reported 49 abnormal cervical cytology patients who underwent anal swab testing within 1 week of cervical cytology. The anal swab results revealed anal squamous intraepithelial lesions in 29 patients (29/49, 59.2%), supporting the hypothesis that anal mucosa maybe an HPV reservoir[5]. Heraclio et al reported that 324 abnormal cervical colposcopic patients had 102 abnormal anal cytological findings (31.5%), which were composed of low-grade anal lesions in 62 patients (19.1%), high-grade lesions in 10 patients (3.1%), and atypical squamous cells of undetermined significance in 30 patients (9.3%)[6]. Valari et al reported that in 235 female patients who underwent colposcopy, the results revealed HPV DNA, high-risk HPV DNA, and high-risk mRNA in anal smears for 45%, 31%, and 8% of the patients, respectively, and in cervical smears for 56%, 39%, and 25% of the patients, respectively. The concordance of HPV DNA compared with cervical testing in the study by Valari et al was 74%[7]. However, limited studies have used LINEAR ARRAY® HPV genotyping test (Roche Molecular Systems Inc., Pleasanton, CA, USA), which has a high sensitivity for HPV testing and can detect 12 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, as well as 25 non high-risk HPV types including HPV 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 68, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108 in anal HPV testing. Linear array test can increase the quantity of DNA by polymerase chain reaction techniques, and increase test specificity using a hybridization probe technique with a genotyping probe that is specific for each HPV type.

This study was a pilot study to evaluate the concordance of anal HPV typing using a linear probe genotyping assay compared with cervical HPV. A high concordance rate for anal HPV detected in this study could indicate that anal HPV testing may be a useful method for cervical cancer screening in patients who are not willing to have per vagina cervical HPV test examination. Anal cytology was also performed to evaluate potential cytological abnormality.

Materials and Methods

Patient samples

The protocol of this research was reviewed and approved by the Ethics Committee for Human Research, Chulabhorn Research Institute (EC No. 023/2554). Written informed consent was obtained from all participants. The study was conducted from July 2011 to May 2012 at Chulabhorn Hospital, Bangkok, Thailand. Forty participants from cervical cancer screening project were planned and recruited for this study[8]. In this project, all patients with abnormal cervical cancer screening results either cytology or HPV, colposcopy with biopsy were performed. Inclusion criteria were Thai women, age 20 to 70-year-old with cervical high-risk HPV infection. We excluded 1 case with a history of cancer in the last 5 years and other case whose anal specimen collection occurred more than 14 days after than the most recent pelvic examination for cervical HPV testing and cytology.

Anal specimen collection was obtained using BD SurePath™ Pap Test (BD Diagnostics-Tripath, Burlington, NC, USA) for anus area, rotated 360 degrees for three rounds clockwise, then three rounds counterclockwise, together with a Dacron swab moistened with sterile water in the day performing colposcopy. The swab was inserted into the anus up to the dentate line (1.5 to 2.0 cm from the anal margin) and rotated 360 degrees for three rounds clockwise, then three rounds counter clockwise. The swab was then placed in a BD SurePath 10 ml collection vial and sent to pathology laboratory.

Anal cytology

Anal specimens for cytology interpretation were processed according to BD SurePath™ Pap Test protocol (as same as cervical cytology). Anal cytology results were interpreted per Bethesda 2001 system as cervical cytology[9].

HPV genotyping

Residual anal specimens after cytology processing were centrifuged, and then the cell pellets were taken for further DNA extraction using a QIAamp kit. LINEAR ARRAY® HPV genotyping test (Roche Molecular Systems Inc., Pleasanton, CA, USA) was

performed according to the kit protocol. In brief, 450-bp fragments from the L1 region of the virus were first amplified by polymerase chain reaction [PCR] of target DNA, followed by hybridization using a reverse line blot system for simultaneous detection of up to 37 HPV genotypes. For PCR amplification, each 100 µL reaction contained 50 µL working master mix and 50 µL of DNA sample. Amplification was performed in an Applied Biosystems GeneAmp PCR System. After that, the HPV and the β-globin amplicon were chemically denatured to form single stranded DNA by addition of 100 µL Denaturation Solution. Aliquots 100 µL of denatured amplicon were then transferred to the appropriate well of typing tray containing hybridization buffer and single LINEAR ARRAY HPV Genotyping Strip coated with HPV and β-globin probe lines. The biotin-labeled amplicon was hybridized to the oligonucleotide probes only if the amplicon contained the matching sequence of complementary probe. For colorimetric determination, a blue colored complex precipitated at the probe positions where hybridization occurred. Then, the linear array HPV genotyping strip was read visually by comparing the pattern of blue lines to the linear array HPV genotyping test reference guide.

Statistical analysis

Demographic data, such as personal, sexual, and medical data, were collected by interview and questionnaire. Independent sample t-test was used to compare the means between two unrelated groups, Fisher's exact test and exact test were used to associate between anal intercourse. The binomial test was used to test the alternative hypothesis of a one-sided test, with a hypothesis test proportion greater than 0.8, and significance level of 0.05.

Results

Anal specimens were obtained from 38 patients who had high-risk cervical HPV detection from July 2011 to May 2012. Patients' demographic and sexual data are shown in Table 1. The mean age was 39.5 years (range, 22 to 61 years) and the mean age of first sexual activity onset was 22.8 years (range, 15 to 30 years). Seven patients were menopausal (18.4%). All patients had sexual experiences. Sixteen of the patients (42.1%) had multiple sexual partners; 30 were currently engaged in sexual activity with men: 30 vaginally, 13 via external body touch, 10 receiving oral sex, and one rectally. Only one patient in the study engaged in rectal intercourse, and her anal HPV result was negative. The most common frequency of sexual activity was once to four times per month for 17 patients (56.7%). Circumcision was reported for 10.5% of patients' partners. Twenty-four women had never used a condom during sexual activity (63.2%) and 14 women sometimes used a condom during sexual intercourse (36.8%). Thirty-four patients (89.5%) cleaned their external genitalia following every sexual encounter; four patients (10.5%) sometimes cleaned their external genitalia after sexual intercourse; and 25 (65.8%) were screened for cervical cytology. Thirteen subjects (34.2%) were never screened for cervical cytology. Patients with cervical HPV did not always have positive anal HPV detection.

Based on our results, the younger mean age associated with overall anal HPV infection (42.8 vs. 36.1 years, $p = 0.038$). There was also no association between anal intercourse, age of onset sexual activity, menopause, sexual activity, multiple sexual partners, frequency of sexual activity, circumcision, condom use during sexual activity, cleaning external genitalia after sexual activity, or previous cervical cytology screening, and overall anal HPV detection (Table 1).

Table 2 shows that HPV DNA was detected in 19 of 38 (50%) anal samples and 12/38 women (31.6%) had at least one high-risk HPV genotype. Type-specific (at least one type) match of high-risk HPV accounted for 11/38 (28.9%). The HPV genotype distinguished in anal samples was mostly HPV 16 (6 cases); HPV 6, 58, 61, and 84 were found in two cases each; HPV 18, 33, 39, 40, 45, 51, 53, 56, 60, 68, 72, 73, 81, and IS39 were found in one case each.

Cervical cytology abnormalities were found in 3 women (7.9%), including two ASC-US (atypical squamous cell of undetermined significant) and one LSIL (low-grade squamous intraepithelial lesions) but all anal cytology results were negative. Final pathology results revealed cervical intraepithelial neoplasia [CIN] 3 (7.9 %, n = 3), CIN2 (2.6%, n = 1), CIN1 (21.1%, n = 8), and HPV infection (68.4%, n = 26). Severity of cervical disease was not associated with HPV concordance rate.

Discussion

This study was a pilot study to evaluate anal HPV testing and anal cytology results in 38 consented women with cervical high-risk HPV infection at Chulabhorn Hospital during an 11 month period. Concurrent anal HPV and cervical high-risk HPV infection rates were 31.6%, $p = 0.062$, respectively (Figure. 1A). Concurrent type-specific, at least one high-risk type, of anal and cervical HPV infections were observed in 28.9% ($p = 0.122$), as shown in Fig.1B. This

Table 1. Demographic and sexual data of 38 cervical high-risk HPV positive women

Factors	Total n (%)	Anal HPV (overall)		*p*-value
		Negative (n = 19)	Positive (n = 19)	
Age (years)				
Mean ± SD (min-max)	39.5±10.2 (22 to 6	2.8±10.8 (26 to 61)	36.1±8.4 (22 to 55)	0.0381*
Age of onset sexual activity (years)				
Mean ± SD (min-max)	22.8±4.3 (15 to 30)	21.9±4.0 (16 to 30)	23.7±4.5 (15 to 30)	0.2031
Menopause	7 (18.4)	6 (31.6)	1 (5.3)	0.0902
Sexual experiences	38 (100.0)	19 (100.0)	19 (100.0)	NA
Multiple sexual partner	16 (42.1)	9 (47.4)	7 (36.8)	0.7432
Sexual activity	30 (78.9)	15 (78.9)	15 (78.9)	1.0002
Sexual activity with men in the present+ (multiple choice)	30	15	15	NA
Vaginal route	30 (100.0)	15 (100.0)	15 (100.0)	
Rectal route	1 (3.3)	1 (6.7)	0 (0.0)	
External body touch	13 (43.3)	6 (40.0)	7 (46.7)	
Oral route to the patient	10 (33.3)	6 (40.0)	4 (26.7)	
Frequency of sexual activity	30	15	15	0.8422
At least everyday	1 (3.3)	1 (6.7)	0 (0.0)	
2 to 5 times per week	10 (33.3)	4 (26.7)	6 (40.0)	
1 to 4 times per month	17 (56.7)	9 (60.0)	8 (53.3)	
Least than 1 times per month	2 (6.7)	1 (6.7)	1 (6.7)	
Circumcision				1.0002
Yes	4 (10.5)	2 (10.5)	2 (10.5)	
No	20 (52.6)	10 (52.6)	10 (52.6)	
Unknown	14 (36.8)	7 (36.8)	7 (36.8)	
Condom use during sexual activity sexual intercourse				0.7372
Never	24 (63.2)	11 (57.9)	13 (68.4)	
Sometime	14 (36.8)	8 (42.1)	6 (31.6)	
Cleaned their external genitalia after				0.6042
Sometime	4 (10.5)	3 (15.8)	1 (5.3)	
Every	34 (89.5)	16 (84.2)	18 (94.7)	
Screened for cervical cytology				1.0002
Never	13 (34.2)	7 (36.8)	6 (31.6)	
Ever	25 (65.8)	12 (63.2)	13 (68.4)	

n = number; SD = standard deviation; [1] independent sample t-test, [2] Fisher's exact test, * $p<0.05$, NA = not available

number was higher than 13% (178 of 1,363) of women from a study by Hernandez et al, but was lower than 74% from Valari et al[7,10].

Nevertheless, the concordance rate of about 30% was too low for substituting anal HPV testing for cervical HPV testing.

The strength of this study was the use of linear array for anal HPV detection, which has a high sensitivity for HPV detection but concordance rate between cervical and anal HPV results is still low. Technical development is needed to increase concordance rate. Due to a small sample size related to this being a pilot study, this study could definitely not define predictor for association between cervical and anus HPV infection. Nevertheless, younger mean age (36.1 vs. 42.8 years, $p = 0.038$) was the only predictor for anal HPV infection.

During anal cytology specimen preparation, no obstacle was detected. Feces were washed out by media solution of liquid based cytology. Cytologic interpretation using Bethesda system for anal cytology was as feasible for pathologists as cervical smear.

However, in this study, all anal cytology results were negative.

Table 2. Cervical and anal HPV/cytology data and final pathology results (cervix biopsies) of 389 participants

ID	Cervical HPV	Anal HPV	Cervical Pap	Anal Pap	Pathology results
P01	18	-	ASC-US	Negative	CIN1
P02	16	-	Negative	Negative	CIN3
P03	58	-	Negative	Negative	CIN1
P04	16	16	Negative	Negative	HPV
P05	58, 62	-	Negative	Negative	CIN1
P06	16, 68, 84	16, 84	Negative	Negative	HPV
P07	31, 84	84	Negative	Negative	HPV
P08	59	56	ASC-US	Negative	CIN1
P09	16, 62	-	Negative	Negative	HPV
P10	18, 72	-	Negative	Negative	HPV
P11	16, 68	68	Negative	Negative	CIN1
P12	33, 61	61	LSIL	Negative	CIN3
P13	58, 81, 83	58	Negative	Negative	HPV
P14	16	16	Negative	Negative	HPV
P15	40, 53, 56, 58, 66, CP6108	53, 60	Negative	Negative	HPV
P16	33, 70	33	Negative	Negative	HPV
P17	16	-	Negative	Negative	HPV
P18	6, 18, 84	6	Negative	Negative	HPV
P19	52	-	Negative	Negative	HPV
P20	16	-	Negative	Negative	HPV
P21	58	58	Negative	Negative	HPV
P22	52, 72	-	Negative	Negative	HPV
P23	59	-	Negative	Negative	HPV
P24	52	-	Negative	Negative	HPV
P25	33	-	Negative	Negative	CIN1
P26	39, 72, 84	-	Negative	Negative	CIN1
P27	18, 53	40	Negative	Negative	HPV
P28	6, 45, 61	6, 45, 61, 73, 81	Negative	Negative	HPV
P29	52	-	Negative	Negative	CIN1
P30	52	-	Negative	Negative	HPV
P31	31	-	Negative	Negative	CIN2
P32	16, 18, 54, 72	16, 18, 72	Negative	Negative	HPV
P33	39	39	Negative	Negative	HPV
P34	16	I5, 39	Negative	Negative	HPV
P35	39	-	Negative	Negative	HPV
P36	16, 51	16, 51	Negative	Negative	CIN3
P37	58	-	Negative	Negative	HPV
P38	16, 62	16	Negative	Negative	HPV

HPV = Human papillomavirus; - = negative result; ID = identification; P = patient; CIN = cervical intraepithelial neoplasia; ASC-US = atypical squamous cell of undetermined significant; LSIL = low-grade squamous intraepithelial lesions

Lacking patients with negative cervical test results made sensitivity and specificity calculations impossible.

Further studies in a larger population recruiting subjects with negative cervical HPV results are warranted to confirm the results of these preliminary results.

Moreover, the overall concordance of the anal HPV and cervical methods was 33.0%, which was not high enough to support the use of anal HPV tests to replace conventional HPV cervical screening. Other techniques should be investigated to identify non-invasive tests that show a better correlation with cervical results. Due to small sample size related to this being

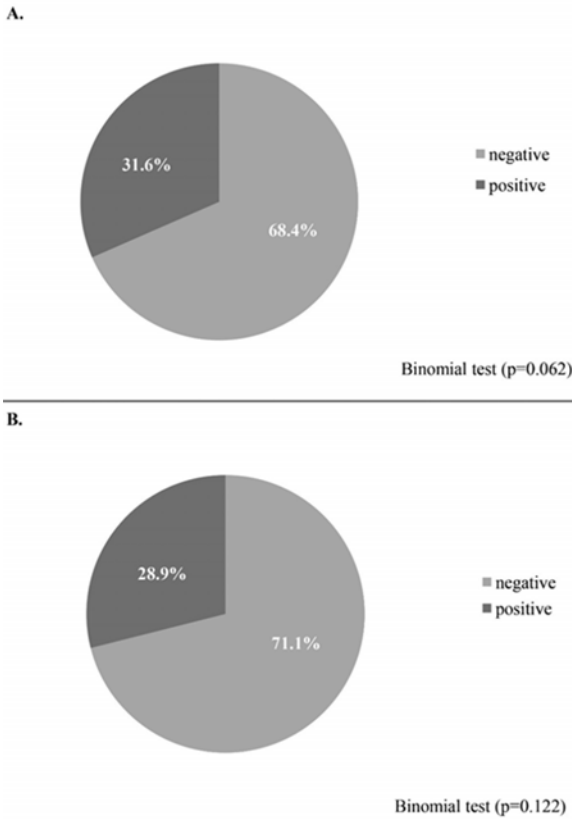

Figure 1. A) Percentage of cervicalx and anal high-risk HPV matches positive. B) Percentage of cervical and anal high-risk HPV matches at least one type.

a pilot study, this study cannot find predictor for association between cervical and anus HPV infection.

Conclusion

The overall concordance of the anal high-risk HPV and cervical HPV results was 31.6%, which was not high enough to support the use of anal HPV tests to replace conventional HPV cervical screening. Other techniques should be investigated to identify non-invasive tests that show a better correlation with cervical results.

What is already known on this topic?

Previous studies showed that anal HPV results may be related with cervical HPV status. The concordance rate in some studies is quite high and may be used as cervical cancer screening method.

What this study adds?

This was the first study that used Linear array HPV genotyping test to compare anal HPV status with cervical HPV result. However, the low concordance rate was found (about 30%); therefore, anal HPV testing ~~is~~ may not be a good alternative nor replacement for cervical HPV testing for women who wish to participate in cervical cancer screening program.

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Potential conflicts of interest

The authors declare no conflict of interest.

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