Hindawi Publishing Corporation
Obstetrics and Gynecology International
Volume 2016, Article ID 6801491, 8 pages
http://dx.doi.org/10.1155/2016/6801491

Research Article

Comparison of Human Papillomavirus Detection in Urine and Cervical Samples Using High-Risk HPV DNA Testing in Northern Thailand

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Received 19 July 2016; Revised 15 November 2016; Accepted 30 November 2016

Academic Editor: Robert Coleman

Objective. To evaluate the performance of high-risk human papillomavirus (HPV) DNA testing in urine samples compared to that of cervical sample testing in Northern Thailand. *Methods*. Paired urine and cervical samples were collected during the follow-up of women with a previous positive HPV test. HPV testing was performed using the Cobas 4800 HPV Test. Linear Array assay was used for genotyping in selected cases. *Results*. Paired urine and cervical samples were obtained from 168 women. Of 123 paired samples with valid results, agreement in the detection of high-risk HPV DNA was present in 106 cases (86.2%), with a kappa statistic of 0.65 (substantial agreement). Using the cervical HPV results as a reference, the sensitivity of urine HPV testing was 68.6% (24/35) and the specificity 93.2% (82/88). For the detection of histologic high-grade squamous intraepithelial lesion or worse (HSIL+), the sensitivity of urine HPV testing was 80.0% (4/5) and the specificity 78.0% (92/118). *Conclusion*. Although urine HPV testing had a rather low sensitivity for HPV detection, its sensitivity for histologic HSIL+ detection was high. For clinical use of urine HPV testing, standardization of specimen collection and processing techniques or application of a more sensitive test, especially in the detection of HPV52 and HPV58, is necessary.

1. Introduction

Cervical cancer is a major health concern in women in developing countries. Cervical cytology has been the main strategy used in cervical cancer screening and prevention. However, establishing an effective cytology screening program with good quality control is difficult in low-resource settings. Recently, HPV DNA testing has gained increasing acceptance as an alternative screening method to cytology [1]. However, these screening methods require trained personnel to perform the pelvic examination and cervical sample collection.

Coverage of screening is also an important issue as this is usually low in developing countries, mainly due to the shortage of the infrastructure to support the screening program. Even in developed countries, the coverage of screening is not perfect as there is still a certain proportion of women who do not participate in the screening program [2]. One of the factors that contribute to this problem is the physical or psychological discomfort of women regarding pelvic examination. A screening test with a simple and noninvasive collection method is one of the solutions to this problem, and application of HPV testing has now been investigated in other types of self-collected samples such as vaginal samples and urine [2, 3]. HPV detection in urine specimens has been introduced to be another possible method for cervical cancer screening [4]. Collection of urine samples is easy and noninvasive and is more acceptable among women compared to other self-sampling methods [5]. In a recent meta-analytical study, the detection of high-risk HPV in urine samples was found to have high accuracy when compared with the detection in cervical samples, with a pooled sensitivity of 77%

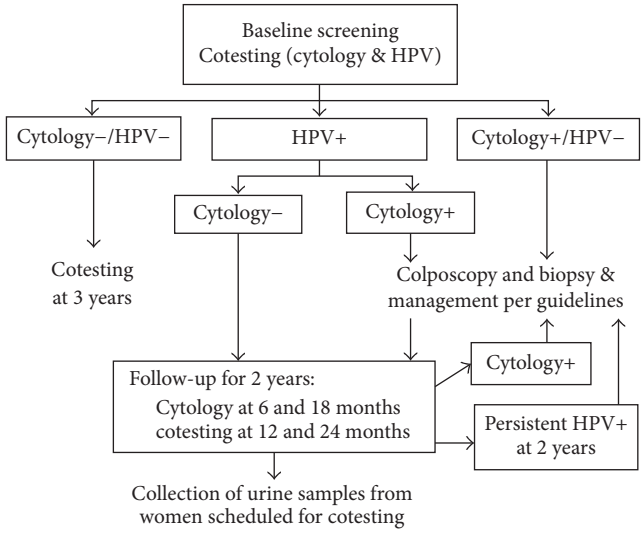

Figure 1: A flowchart describing the study population.

and specificity of 88% [2]. However, a rather small number of studies evaluated the clinical performance of urine-based HPV detection in the prediction of cervical precancerous lesions and cancer [2, 6]. There is also limited information regarding the use of clinically validated high-risk HPV DNA testing in urine specimens [5, 7–9].

The objective of this study is to evaluate the application of high-risk HPV testing in urine samples compared to that of cervical sample testing and to evaluate its performance in the prediction for histologic high-grade squamous intraepithelial lesion or worse (HSIL+) in women in Northern Thailand.

2. Methods

The study was approved by the institutional Ethics Committee of the Faculty of Medicine, Chiang Mai University. The study population comprised women who were resident in 3 prefectures (Sankumpang, Mae-on, and Sarapee) of Chiang Mai, Northern Thailand. The inclusion criteria were women who had previous positive HPV test results in the population-based cervical cancer screening program and were scheduled for follow-up between April and July 2015, either due to negative cytology and colposcopy or due to the presence of previous cervical epithelial lesions (including women who were under surveillance following conization or loop electrosurgical excisional procedure (LEEP)). A flowchart describing the selection of the study population is shown in Figure 1. Written informed consent was obtained from all participants. These women had a 2-year risk of less than 10% for histologic HSIL+ based on previous studies in the same region [10, 11]. Data of HPV genotyping results of previous cervical samples from these women were retrieved from the database.

During the follow-up visit, paired urine and cervical samples were collected at the local clinics. From each woman, a urine sample was collected before pelvic examination. Between 5 mL and 50 mL of first-stream urine was collected in a sterile container. The sample was kept in an ice-cold container immediately after the collection. After pelvic examination, the first cervical sample was collected using an Ayre spatula to prepare a conventional Pap smear for cytologic examination. The second cervical sample for HPV analysis was collected using a Cytobrush and was transferred into 5 mL of phosphate-buffered saline. Both urine and cervical samples were kept in an ice-cold container during transfer to the Department of Pathology, Chiang Mai University, for HPV testing/genotyping.

In the laboratory, the urine samples were centrifuged at 2,500 rpm, 4°C for 15 minutes. After discarding the supernatant until the final volume of 3 mL was reached, the sample was resuspended, and 2 mL of this was used for HPV testing and the remaining 1 mL for pellet preparation. For HPV analysis of the cervical sample, 2 mL was used for HPV testing and the remaining 3 mL for pellet preparation. For pellet preparation, the samples were centrifuged at 1,500 rpm, 4°C for 10 minutes. After the supernatant was discarded, the pellets were stored at −20°C.

High-risk HPV DNA testing on both cervical and urine samples was performed using the Cobas 4800 HPV Test (Roche Diagnostics, Indianapolis, Indiana, USA) which is designed to detect 14 high-risk HPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68), with further genotype specification for HPV16 and HPV18. When neither beta-globin (an internal control for the presence of adequate human DNA) nor HPV was detected, the test result was classified as invalid.

Women who had positive or abnormal cytology (at least atypical squamous cells of undetermined significance) in concurrent Pap smears were referred for colposcopy and were managed further as per standard guidelines [12]. In cases with histologic HSIL+ detection in colposcopy-directed biopsy or subsequent conization specimens, further HPV genotyping was carried out using cervical sample pellets. Genotyping was also performed in cases with HPV-positive cervical samples/HPV-negative or invalid urine samples. The samples were screened using PCR amplification using primers MY09/MY11 located within the HPV L1 gene. The samples that were negative with primers MY09/MY11 were reamplified using primers GP5+/GP6+. The presence of coamplified 199-bp fragment of a beta-globin gene served as an internal standard for DNA quality and quantity of samples. Only the samples that were PCR-positive were further processed for genotyping. HPV genotyping was performed using the Linear Array HPV Genotyping Test (Roche Molecular System, Inc., Branchburg, NJ, USA) according to the manufacturer's protocol.

The data were analyzed using STATA version 11 (StataCorp LP, College Station, TX, USA). Agreement of HPV test results between urine and cervical samples was assessed using the kappa statistic. Accuracy values of urine HPV testing were calculated using positive cervical HPV testing and histologic HSIL+ as references. Differences of the results were tested using Fisher's Exact test. A p value < 0.05 was considered to be statistically significant.

3. Results

Paired urine and cervical samples were obtained from 168 women. All participants were native Thai women. The mean age was 45.8 ± SD 8.1 years. The rate of invalid testing was higher in urine samples (45 of 168; 26.8%) than in cervical samples (1 of 168; 0.6%) ($p < 0.001$). One woman had invalid HPV test results in both cervical and urine samples, and this case was excluded, resulting in a total of 167 paired samples for further analysis; 123 of these (73.7%) had valid test results in both urine and cervical samples. High-risk HPV infection was detected in 45 cervical samples (26.9%) and in 30 of 123 valid urine samples (24.4%). Between urine samples, there was no significant difference in the mean age or in the location of sample collection between the groups with invalid and valid test results or negative and positive HPV testing.

Comparison of HPV test results between urine and cervical samples is shown in Table 1. Of the 44 urine samples with invalid HPV testing, 10 samples had HPV-positive paired cervical samples. Of the 123 paired samples with valid test results, 24 were HPV-positive in both samples, 11 in cervical samples only, and 6 in urine samples only and 82 were HPV-negative in both samples. Agreement in the detection of high-risk HPV DNA was present in 106 out of 123 women (86.2%), with a kappa statistic of 0.65 (95% confidence interval (CI) 0.47–0.82), which is consistent with substantial agreement. Using HPV detection in cervical samples as a reference for calculation of the accuracy of valid urine HPV results in 123 samples; the sensitivity was 68.6% (95% CI; 50.7–83.2%); specificity was 93.2% (95% CI; 85.8–97.5%); positive predictive value was 80.0% (95% CI; 61.4–92.3%); and negative predictive value was 88.2% (95% CI; 79.8–94.0%).

The concurrent cytology results were abnormal in 22 women, and subsequent colposcopy or conization (LEEP) identified 7 cases of histologic HSIL+. None of the women with abnormal cytology had HPV-positive urine/HPV-negative cervical samples. The association of urine HPV results and the detection of histologic HSIL+ is shown in Table 1. When the detection of histologic HSIL+ was used as a reference, the accuracy of valid urine HPV results included a sensitivity of 80.0% (95% CI; 28.4–99.5%), specificity of 78.0% (95% CI; 69.4–85.1%), positive predictive value of 13.3% (95% CI; 3.8–30.7%), and negative predictive value of 98.9% (95% CI; 94.2–100%). Of the cervical samples, histologic HSIL+ was detected in 7 out of 45 HPV-positive patients, whereas no HSIL+ was detected in 122 HPV-negative patients. The accuracy values of cervical HPV results in the prediction of histologic HSIL+ were sensitivity 100% (95% CI; 59.0–100%), specificity 76.3% (95% CI; 68.9–82.6%), positive predictive value 15.6% (95% CI; 6.5–29.5%), and negative predictive value 100% (95% CI; 97.0–100%).

Table 2 shows the comparison of the HPV genotype results between urine and cervical samples provided by the Cobas 4800 HPV Test and a comparison with the previous HPV genotyping results in cervical samples (using the Linear Array assay). In the group of 24 women with HPV-positive paired samples, full agreement between urine and cervical samples was observed in 22 out of 24 cases (91.7%). Agreement of Cobas HPV results of paired samples with the genotyping results of previous cervical samples was observed in 16 (72.7%) out of 22 cases (2 cases with an indeterminate original genotype were excluded). Of 7 cases with HPV16 in the previous cervical samples, HPV16 was present in 5 cervical samples (71.4%) and in 4 urine samples (57.1%). In one case, both urine and cervical samples were positive for HPV16, whereas the genotyping result in the previous cervical sample was HPV52. In 11 cases with HPV-positive cervical samples only, all (100%) showed genotypic agreement with original cervical samples, whereas 4 out of 6 cases (66.7%) with HPV-positive urine samples only had such genotype agreement. Overall agreement of Cobas HPV results in any of the current samples (urine or cervical) with the previous high-risk genotypes in cervical samples was observed in 75.6% for full agreement and 85.4% for at least partial agreement.

Of the 7 cases with histologic HSIL+ detected, 3 cases were positive for HPV16 in both urine and cervical samples. The remaining 4 cases were positive for non-HPV16/18 high-risk genotypes in cervical samples with variable urine HPV results including invalid (2 cases), negative (1 case), and non-HPV16/18 high-risk genotype (1 case). The high-risk genotypes detected by the Linear Array assay in the current cervical samples showed a correlation with Cobas HPV results and were also present in the previous cervical samples (HPV16 in 3 cases, HPV 52 in 2 cases, and HPV58 in 2 cases).

Table 3 illustrates 21 cases with HPV-positive cervical samples but with invalid or negative urine results (10 and 11 cases, resp.). In 3 of these 21 cases (14.3%), histologic HSIL+ was detected. Using the Linear Array assay for genotyping in current cervical samples, HPV52 was identified in 6 out of 21 cases (28.6%) and in 2 cases of histologic HSIL+. In another 2 cases with HPV58-positive cervical samples, one had histologic HSIL+.

4. Discussion

As the method of urine collection is noninvasive and self-sampling, urine appears to be the most convenient and acceptable type of specimen for HPV testing [5]. The high sensitivity and specificity of urine HPV testing with reference to cervical HPV testing found in the meta-analytical study suggest that high-risk HPV DNA testing in urine has the potential for use in the case of cervical cancer screening [2]. The detection of HPV DNA in urine samples was found to have a similar operative characteristic to that of cervical samples for the detection of abnormal cervical cytology [4], supporting the use of self-collected urine samples in cervical cancer screening. In previous PCR-based HPV genotyping studies, the rates of overall concordance in the identification of high-risk HPV genotypes between urine and cervical samples were between 79 and 80% [13, 14]. In this study, there was substantial agreement between urine and cervical samples in the detection of high-risk HPV DNA using the Cobas test.

Discordance of Cobas genotype results between urine and cervical samples was found in 2 out of 24 (8.3%) HPV-positive

Table 1: Comparison of Cobas HPV results in urine samples with cervical samples and histology.

HPV result in urine samples	HPV result in cervical samples		Detection of histologic HSIL+	
	Number of positive cases	Number of negative cases	Number of positive cases	Number of negative cases
Positive (%, $n = 30$)	24 (80.0)	6 (20.0)	4 (13.3)	26 (86.7)
Negative (%, $n = 93$)	11 (11.8)	82 (88.2)	1 (1.1)	92 (98.9)
Invalid (%, $n = 44$)	10 (22.7)	34 (77.3)	2 (4.5)	42 (95.5)
Total (%, $n = 167$)	45 (26.9)	122 (73.1)	7 (4.2)	160 (95.8)

HSIL+: high-grade squamous intraepithelial lesion or worse.

Table 2: Results of Cobas HPV Test and previous genotyping in 41 women with HPV-positive urine and/or cervical samples.

Number of Cobas-positive case(s), total $n = 41$	Urine Cobas result	Cervical Cobas result	Agreement of Cobas results between paired samples	Linear Array genotype(s) in previous cervical sample	Agreement of any Cobas result with previous genotyping
Positive in both samples ($n = 24$)					
3	HPV16	HPV16	Yes	HPV16	Yes
1	HPV16	HPV16	Yes	HPV16/HPV39/ HPV58/HPV59	Partial
1	HPV16	HPV16	Yes	HPV52	No
1	HR	HPV16	No	HPV39	Partial
1	HR	HPV16, HR	Partial	HPV16	Partial
1	HR	HR	Yes	HPV16	No
1	HR	HR	Yes	HPV33	Yes
2	HR	HR	Yes	HPV39	Yes
2	HR	HR	Yes	HPV52	Yes
1	HR	HR	Yes	HPV58	Yes
1	HR	HR	Yes	HPV16/HPV52	Partial
1	HR	HR	Yes	HPV31/HPV39	Yes
4	HR	HR	Yes	HPV39/HPV68	Yes
1	HR	HR	Yes	HPV52/HPV68	Yes
1	HR	HR	Yes	HPV56/HPV66	Yes
2	HR	HR	Yes	Indeterminate	—
Positive cervical samples only ($n = 11$)					
1	Neg	HPV16	No	HPV16	Yes
1	Neg	HPV18, HR	No	HPV18/HPV31	Yes
1	Neg	HR	No	HPV45	Yes
1	Neg	HR	No	HPV51	Yes
2	Neg	HR	No	HPV52	Yes
2	Neg	HR	No	HPV58	Yes
3	Neg	HR	No	HPV39/HPV68	Yes
Positive urine samples only ($n = 6$)					
1	HPV16	Neg	No	Indeterminate	No
1	HR	Neg	No	HPV16	No
1	HR	Neg	No	HPV35	Yes
1	HR	Neg	No	HPV51	Yes
1	HR	Neg	No	HPV39	Yes
1	HR	Neg	No	HPV39/HPV59	Yes

HR: non-HPV16/HPV18 high-risk genotypes.

Table 3: High-risk HPV genotypes in women with Cobas-positive cervical samples but invalid/negative urine samples.

Case number	Linear Array genotype(s) in previous cervical sample	Cobas result in current cervical sample	Linear Array genotype(s) in current cervical sample
Invalid urine results ($n = 10$)			
1	HPV16	HR	HPV39
2	HPV16/HPV51	HPV16	HPV16
3	HPV18/HPV31	HR	HPV31
4	HPV39/HPV68	HR	HPV51
5	HPV39/HPV59/HPV68	HR	HPV39/HPV51/HPV68
6*	HPV52	HR	HPV52
7*	HPV52	HR	HPV52
8	HPV52	HR	HPV52
9	HPV52	HR	HPV16/HPV52
10	HPV56/HPV66	HR	HPV56
Negative urine results ($n = 11$)			
1	HPV16	HPV16	HPV16
2	HPV18/HPV31	HPV18, HR	HPV18/HPV31
3	HPV39/HPV68	HR	HPV39/HPV68
4	HPV39/HPV68	HR	HPV39/HPV68
5	HPV39/HPV68	HR	PCR-negative
6*	HPV39/HPV58/HPV68	HR	HPV58
7	HPV45	HR	HPV45
8	HPV51	HR	HPV51
9	HPV52	HR	HPV52
10	HPV52	HR	HPV52
11	HPV58	HR	HPV58

HR: non-HPV16/HPV18 high-risk genotypes.
*With subsequent detection of histologic high-grade squamous intraepithelial lesion or worse.

paired samples in this study (Table 2). In a previous study by Burroni et al. [13], 13 of 33 (39.4%) paired cervical and urine samples which were positive for high-risk HPV had partial or complete discordance in PCR-based genotyping results. An explanation for such discordance is that HPV-infected cells in urine may exfoliate from the cervix, other anogenital regions, or possibly the urethra, resulting in a possibility that the HPV infection in the more distal parts may be independent of the cervix or caused by different HPV genotypes [13, 14]. Discordance between the Cobas genotype results and Linear Array genotyping results in the same cervical samples was observed in one case (Table 3; invalid urine results, case number 9). In this case, HPV16 that coexisted with HPV52 was not detected by the Cobas test. In a previous study comparing the results between Cobas test and Linear Array assay, genotyping agreement was reported in 90.0% of cervical samples [15].

In most previous studies, the study populations comprised women who attended gynecology or colposcopy clinics, and variable PCR methods were used in HPV detection and/or genotyping [2, 6]. HPV detection in urine samples was usually compared with HPV positivity in cervical samples with or without cytological correlation. However, only a few studies have evaluated the use of clinically validated high-risk HPV tests in urine samples in the detection of histologic HSIL+ [5, 7–9].

The applications of validated high-risk HPV testing in urine samples from women with abnormal cytology have been reported in 3 previous studies [5, 7, 9]. Sellors et al. [5] compared HPV detection using Hybrid Capture 2 between cervical samples, vulvar samples, vaginal swab samples, and urine samples (the latter three sample types being self-collected) from 200 women. The detection rate of high-risk HPV was the lowest in urine samples (44.8%) in contrast to

the rate in cervical samples (98.3%) which was the highest. The clinical sensitivity of urine HPV testing in the detection of histologic HSIL+ was only 44.8% compared with the 98.3% sensitivity of cervical testing, although the specificity of urine testing was higher (69.7% versus 52.1%) [5]. Bernal et al. [7] compared the performance of the Cobas test in paired urine and cervical samples from 125 women. A high rate of agreement in high-risk HPV detection between urine and cervical samples was reported (88.0%), similar to the finding in the present study (86.2%). Urine samples showed a high sensitivity in the detection of high-risk HPV (90.5%) and histologic HSIL (95.0%), while the specificity was 85.0% and 52.4%, respectively [7]. Stanczuk et al. [9] compared HPV detection using the Cobas test between cervical samples, vaginal samples, and urine samples (the latter two sample types being self-collected) in 100 women. The sensitivity for HPV detection in urine samples was 84.5% when compared with cervical samples, and the specificity was 87.5% [9]. For the detection of histologic HSIL+, the sensitivity in urine samples was 80.0% which is comparable to that of the present study. The sensitivity of urine samples was lower than that of cervical and vaginal samples (92.3% each), but the specificity (22.8%) was higher than that of vaginal (11.4%) and cervical samples (8.5%) [9].

To our knowledge, the application of urine-based high-risk HPV testing in primary cervical cancer screening has been reported in only one study carried by Stanczuk et al. [8]. In the study population of 5,318 women, the Cobas HPV-positive rate was lower in urine samples (11.6%) than in cervical samples (14.7%). In that study, the sensitivity for the detection of histologic HSIL+ was much lower in urine samples (63.1%) compared with cervical and self-collected vaginal samples (97.7% and 94.6%, resp.), although the specificity in urine samples was only slightly higher (89.8% versus 87.3% and 85.4%, resp.) [8]. The sensitivity of urine HPV testing in HSIL+ detection was also lower than that of liquid-based cervical cytology (75.4%). These results suggest that the sensitivity of urine HPV testing needed to be improved if it is to be used as a primary screening method [8]. In another population-based study from a rural area in India, only 5 out of 1,305 women (0.4%) were positive for HPV DNA using PCR detection [16]. However, the study did not include cervical HPV detection to serve as a reference for HPV prevalence in the population.

Compared with these previous studies using Cobas HPV tests on urine samples, the clinical performance of urine HPV testing in the detection of histologic HSIL+ in this study (sensitivity 80.0%, specificity 78.0%) was within the reported range (sensitivity 63–95%, specificity 23–90%) [7–9]. However, it should be noted that the reliability of the sensitivity and specificity values in this study is limited by the very few cases with histologic HSIL+.

The lower sensitivity of HPV testing in urine samples compared with that of cervical samples may be related to the low cellular or HPV DNA yield in urine [6]. The low cellular yield of urine may also be a cause of invalid urine results in this study, which made up 26.8% of the samples. However, in the cases with valid results, the accuracy of the clinical detection of histologic HSIL+ was high. Among 21 cases with HPV-positive cervical samples but invalid or negative urine samples, 3 women (14.3%) had histologic HSIL+ and these patients had HPV52 or HPV58 detected in cervical samples.

The volume of urine used for HPV analysis may be one of the several possible factors affecting the cellular yield [13]. In previous studies, a wide range of initial urine volume was used in the preparation for HPV testing, that is, from less than 1 mL to over 50 mL [13]. Another 2 studies used at least 20 ml of urine for Cobas HPV testing, and there were no invalid results in a total of 225 urine samples in these studies [7, 9]. Burroni et al. [13] used an initial urine volume of 60 mL for the preparation of 2 pellets for HPV genotyping analysis, and all but one of 216 urine samples (99.5%) in the study contained a positive internal control for human DNA. These findings suggest that at least 20–30 mL volume of urine may be required for the presence of sufficient DNA to enable valid testing. In the present study, the volume of urine sample ranged from 5 to 50 mL. One weakness in this study was the lack of complete urine volume records, which limited the analysis for the association between urine volume and invalid test results. Regarding urine collection time, first-void urine samples (first urination of the day) were found to be associated with better HPV detection performance compared with random or midstream urine samples, possibly due to the higher level of DNA [2]. However, a recent study showed no significant difference in HPV detection rate in samples of varying collection times [17], and another study did not find a correlation between an HPV viral load and the number of cells in urine samples [18]. As the methods (e.g., sample collection and processing, HPV detection technique) and the results in previous studies varied considerably, standardization of the methods for specimen collection and processing will be an important step for further studies in urine HPV testing [2].

Another possible factor that could affect the sensitivity of the HPV test in this study may be the viral detection threshold of the Cobas test. The Cobas test has a different analytical sensitivity or limit of detection for different HPV genotypes, and the viral detection thresholds vary from 150 copies/mL for HPV45 or 300 copies/mL for HPV16/18 to 2,400 copies/mL for HPV52 [19]. In the present study, HPV52 was found in almost 30% of cases with HPV-positive cervical samples/invalid or HPV-negative urine samples, and HPV52 was detected in cervical samples in 2 out of 3 cases with histologic HSIL+ in this group. HPV52 has been shown to be a major genotype associated with HSIL+ in Thailand and Eastern Asia [20, 21], which is different from the prevalence in the Western population. It should also be noted that another case of histologic HSIL+, with negative urine HPV testing, had the genotype HPV58, whose analytical sensitivity in the Cobas test was 600 copies/mL.

The presence of cases with HPV-positive urine samples/HPV-negative cervical samples in this study (6 of 123 cases with valid testing or 4.9%) was within the previously reported range in the studies which used high-risk HPV testing (from 1% to 7.2% of cases) [5, 7, 9]. This finding may be explained by the presence of HPV infection in the anogenital regions other than the cervix as previously discussed [13, 14]. It is uncertain whether a variation in the

collection process (e.g., cleaning of external genitalia) could limit the inclusion of HPV-infected cells from different sites in urine samples [6].

This study was limited by a small number of cases and a higher than expected rate of invalid test results as discussed above. In addition, there was some verification bias in the detection of histologic HSIL+ in this study because only the cases with concurrent abnormal cytology were referred for colposcopy.

5. Conclusion

Although urine HPV testing had a lower sensitivity for high-risk HPV detection compared with that of cervical samples, the clinical sensitivity for histologic HSIL+ detection was high in the cases with valid test results. To facilitate the possible clinical use of HPV testing in urine samples, standardization of specimen collection and processing techniques or application of a more sensitive test, especially in the detection of HPV52 and HPV58, is necessary.

Disclosure

The data in this paper has been presented in part at the XXXI International Congress of the International Academy of Pathology and 28th Congress of the European Society of Pathology, Cologne, Germany [22].

Competing Interests

The authors declare that there is no conflict of interests.

Acknowledgments

This study was supported by (1) the National Research University Project, under Thailand's Office of the Higher Education Commission, and (2) the Faculty of Medicine, Chiang Mai University.

References

[1] W. K. Huh, K. A. Ault, D. Chelmow et al., "Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance," *Gynecologic Oncology*, vol. 136, no. 2, pp. 178–182, 2015.

[2] N. Pathak, J. Dodds, J. Zamora, and K. Khan, "Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis," *British Medical Journal*, vol. 349, Article ID g5264, 2014.

[3] M. Arbyn, F. Verdoodt, P. J. F. Snijders et al., "Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis," *The Lancet Oncology*, vol. 15, no. 2, pp. 172–183, 2014.

[4] M. Munoz, M. Camargo, S. C. S.-D. Leon et al., "Classical molecular tests using urine samples as a potential screening tool for human papillomavirus detection in human immunodeficiency virus-infected women," *Journal of Clinical Microbiology*, vol. 51, no. 11, pp. 3688–3693, 2013.

[5] J. W. Sellors, A. T. Lorincz, J. B. Mahony et al., "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," *Canadian Medical Association Journal*, vol. 163, no. 5, pp. 513–518, 2000.

[6] A. Sehgal, S. Gupta, A. Parashari, P. Sodhani, and V. Singh, "Urine HPV-DNA detection for cervical cancer screening: prospects and prejudices," *Journal of Obstetrics and Gynaecology*, vol. 29, no. 7, pp. 583–589, 2009.

[7] S. Bernal, J. C. Palomares, A. Artura et al., "Comparison of urine and cervical samples for detecting human papillomavirus (HPV) with the Cobas 4800 HPV test," *Journal of Clinical Virology*, vol. 61, no. 4, pp. 548–552, 2014.

[8] G. Stanczuk, G. Baxter, H. Currie et al., "Clinical validation of hrHPV testing on vaginal and urine self-samples in primary cervical screening (cross-sectional results from the Papillomavirus Dumfries and Galloway—PaVDaG study)," *BMJ Open*, vol. 6, no. 4, Article ID e010660, 2016.

[9] G. A. Stanczuk, H. Currie, G. Baxter et al., "Cobas 4800 HPV detection in the cervical, vaginal and urine samples of women with high-grade CIN before and after treatment," *Journal of Clinical Pathology*, vol. 68, no. 7, pp. 567–570, 2015.

[10] S. Khunamornpong, J. Settakorn, K. Sukpan et al., "Application of HPV DNA testing in follow-up after loop electrosurgical excision procedures in Northern Thailand," *Asian Pacific Journal of Cancer Prevention*, vol. 16, no. 14, pp. 6093–6097, 2015.

[11] S. Siriaunkgul, J. Settakorn, K. Sukpan et al., "Population-based cervical cancer screening using high-risk HPV DNA test and liquid-based cytology in northern Thailand," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 16, pp. 6837–6842, 2014.

[12] L. S. Massad, M. H. Einstein, W. K. Huh et al., "2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors," *Journal of Lower Genital Tract Disease*, vol. 17, no. 5, pp. S1–S27, 2013.

[13] E. Burroni, P. Bonanni, C. Sani et al., "Human papillomavirus prevalence in paired urine and cervical samples in women invited for cervical cancer screening," *Journal of Medical Virology*, vol. 87, no. 3, pp. 508–515, 2015.

[14] V. V. Sahasrabuddhe, P. E. Gravitt, S. T. Dunn et al., "Comparison of human papillomavirus detections in urine, vulvar, and cervical samples from women attending a colposcopy clinic," *Journal of Clinical Microbiology*, vol. 52, no. 1, pp. 187–192, 2014.

[15] J. C. Gage, M. Sadorra, B. J. Lamere et al., "Comparison of the cobas human papillomavirus (HPV) test with the hybrid capture 2 and linear array HPV DNA tests," *Journal of Clinical Microbiology*, vol. 50, no. 1, pp. 61–65, 2012.

[16] S. Sabeena, P. Bhat, V. Kamath et al., "Detection of genital HPV infection using urine samples: a population based study in India," *Asian Pacific Journal of Cancer Prevention*, vol. 17, no. 3, pp. 1083–1088, 2016.

[17] V. Senkomago, A. C. Des Marais, L. Rahangdale, C. R. T. Vibat, M. G. Erlander, and J. S. Smith, "Comparison of urine specimen collection times and testing fractions for the detection of high-risk human papillomavirus and high-grade cervical precancer," *Journal of Clinical Virology*, vol. 74, pp. 26–31, 2016.

[18] C. Payan, A. Ducancelle, M. H. Aboubaker et al., "Human papillomavirus quantification in urine and cervical samples by using the Mx4000 and LightCycler general real-time PCR systems," *Journal of Clinical Microbiology*, vol. 45, no. 3, pp. 897–901, 2007.

[19] A. Rao, S. Young, H. Erlich et al., "Development and characterization of the cobas human papillomavirus test," *Journal of Clinical Microbiology*, vol. 51, no. 5, pp. 1478–1484, 2013.

[20] Y.-J. Chang, H.-C. Chen, B.-H. Lee et al., "Unique variants of human papillomavirus genotypes 52 and 58 and risk of cervical neoplasia," *International Journal of Cancer*, vol. 129, no. 4, pp. 965–973, 2011.

[21] J. Chansaenroj, P. Junyangdikul, T. Chinchai et al., "Large scale study of HPV genotypes in cervical cancer and different cytological cervical specimens in Thailand," *Journal of Medical Virology*, vol. 86, no. 4, pp. 601–607, 2014.

[22] S. Khunamornpong, J. Settakorn, K. Sukpan, S. Lekawanvijit, and S. Siriaunkgul, "Comparison of Human Papillomavirus (HPV) detection in urine and cervical samples using high-risk HPV DNA testing in Northern Thailand," *Virchows Archiv*, vol. 469, supplement 1, p. S281, 2016, abstract E-PS-07-031.