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Original article

Comparison of initial stream urine samples and cervical samples for detection of human papillomavirus

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ABSTRACT

Background: Uterine cervical cancer is a treatable and preventable cancer. Medical efforts to reduce rates of cervical cancer focus on the promotion of human papillomavirus (HPV) vaccination and the promotion of routine cervical cancer screening done by cervical cytology and cervical HPV testing. Urine-based HPV testing would be simple and noninvasive approach to screen for cervical cancer.
Methods: Two biospecimens (clinician-taken sample from cervix and initial stream urine sample) were provided from a total of 240 healthy women attending for cancer screening provided for HPV testing. We have assessed the HPV detection rates among cervical samples and pellet fraction of urine samples using HPV test (Anyplex™ II HPV28 Detection kit, Seegene, Korea).
Results: Among 240 samples screened, HPV prevalence was 42.9% in pellet fractions of urine samples. The agreement between the two kinds of samples was 98.4%, k = 0.792. Discordant results were observed in 27 cases; 5 were positive only by urine samples and 22 were positive only by smear samples. Sensitivity and specificity for all HPV DNA in pellet fractions of urine using cervical samples as reference was 68.4% and 99.9%.
Conclusions: Comparing methodologies of collection of samples for HPV detection, they showed the higher agreements for almost genotypes between cervical samples and pellet fractions of urine samples. These results suggest that urine could be a good noninvasive tool to monitor HPV infection in women. Additional research in a larger and general screening population would be needed.

1. Introduction

Uterine cervical cancer is a treatable and preventable cancer. Medical efforts to reduce rates of cervical cancer focus on the promotion of human papillomavirus (HPV) vaccination and the promotion of routine cervical cancer screening done by cervical cytology and cervical HPV testing. Molecular and epidemiologic studies have clearly demonstrated that persistent infection with HPV is a risk factor for the development of cervical intraepithelial lesions and invasive carcinoma [1,2].

To date, 170 PV types have been identified [3], and about 40 of them infect the human anogenital tract [4]. The genital HPVs are classified into high-risk and low-risk types based on their association with uterine cervical cancer [5,6]. Among the high risk types detected most frequently in uterine cervical cancer, HPV-16, 18, 31, 33, 35, 45, 52, 58, 39, 51, 56, 59 are classified as carcinogens of group 1 by the International Agency for Research on Cancer (IARC, Lyon) [7].

Current uterine cervical cancer screening strategies in Japan include cytology or co-testing cytology plus testing for high-risk HPV which both require pelvic examination by trained medical personnel. Then, single genotyping is important to study the carcinogenic potential of HPV types and improve the triage of HPV positive women by single type risk stratification [8], and to follow-up persistent infections.

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Of note, HPV screening coverage remains low [9]. In the United States, an estimated 56% of incident invasive cervical cancer is due to insufficient screening [10]. Then, self-collection of samples for high-risk HPV testing can be performed outside a health facility to increase ease of and access to screening uptake [11], and has been found highly acceptable in different populations [12]. Urine collection for high-risk HPV detection could provide an especially simple, non-invasive method for screening women reluctant to undergo a pelvic examination.

Therefore, in this study, we have assessed the HPV detection rates among cervical samples and pellet fraction of urine samples. Results were compared according to the sensitivity and specificity of the two kinds of samples, genotype inclusivity, and detection of multiple genotypes.

2. Material and methods

2.1. Samples collection, DNA extraction and genotyping

Two biospecimens (clinician-taken sample from cervix and initial stream urine sample) were provided from a total of 240 healthy women attending for cancer screening provided for HPV testing, and sent to the laboratory, Aichi Medical University Hospital, Aichi, Japan, from January to October 2015, were included in this study. The age of the women from whom the samples were collected ranged from 19 to 58 years old, mean age 32.2 years, median 31 years. Cervical cells were collected by swab and stored according to the manufacturer's instructions until analysis. Total DNA was extracted using the GeneAll® Ribospin™ vRD (GeneAll Biotechnology, Seoul, Korea) with manual following the manufacturer's instructions. Two aliquots of the extracted DNA from smear and urine samples were used to detect HPV genotypings with Anyplex™ II HPV28 detection kit [13,14].

Anyplex™ II HPV28 detection kit detects simultaneously 19 high-risk HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58,59, 66, 68, 69, 73, 82) and 9 low-risk HPVs (6, 11, 40, 42, 43,44, 54, 61, 70), which allows the screening and identification of the most clinically relevant HPV types. The inclusion of internal control allows check the entire process from DNA extraction to PCR amplification. A negative control and three positive controls provided by the manufacturer are included in each PCR run as requested. The study was conducted according to the indications of the Ethical Committee.

2.2. Statistical analysis

Statistics was performed with SPSS statistics. Agreement of HPV typing results between paired cases was evaluated with the Cohen's kappa statistics and their uneven distribution evaluated with McNemar's test. The trend of association between viral load (single and multiple infections) and discordance was evaluated with the Chi-square test and Fisher's exact test for trend. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Detection of HPV genotypes in urine and smear samples

Of the 240 samples of urine samples and smear samples, respectively, screened, 98 (40.8%) were concordantly screened by samples as HPV positive, and 115 (47.9%) as HPV negative (Table 1). Thus, the total number of positive samples combined was 103 in urine samples. Of them, 58 (56.3%) were single infections (positive at two kids of samples for the same genotype; positive at only urine sample; or positive at two kinds of samples but for different genotype), and 45 (43.7%) multiple infections (i.e., positive at two kinds of samples for different genotypes or positive at only urine samples for two or more genotypes). The discordant samples were 27 (11.3%, 5 positive by urine samples and 22 positive by smear samples).

Table 1
Detection of HPV in smear and urine.

Smear samples	Urine results		Total
	Negative	Positive	
Negative	115	5	120
Positive	22	98	120
Total	137	103	240

Overall the agreement between the two tests was 98.4% with k = 0.792 (strong). Among the 103 positive urine samples, 20/38 (52.6%) single infections were fully concordant while 25 (10.4%) samples (including single and multiple infections) were completely discordant. Most of the samples (60; 25%) tested by urine samples and smear samples gave partial discordant results. The agreement between genotypes comparing the two kinds of samples is reported in Table 2. A lower number of high-risk (173/229) and low-risk HPV types (43/75) in urine samples were detected compared with smear samples, Table 2 ($p < 0.05$). However, there was a high percentage of agreement concerning HPV 16 (99.6%, k = 0.969), HPV 18 (99.22%, k = 0.905), and HPV 31, 33, 45, 51, 58, 59 and 66 showed more than 99.0% agreement in the high-risk group, and HPV genotype 11 in the low-risk group (99.2%, k = 0.829). The agreement interpretation was perfect for HPV genotype 45, near perfect for HPV 16, 18, 31, 33, 39, 51, 52, 53, 58, 59, 66, 11, 61, strong for HPV 35, 68, 69, 42, 44 and poor for HPV 56, 73, 82, 6, 40, 43, 54, 70. The agreement interpretation after the kappa statistics was omitted for HPV 26 since they were present at a very low frequency. Sensitivity and specificity for all HPV genotypes in urine using smear samples as reference was 68.4% and 99.9%. Sensitivity and specificity for high-risk HPV genotypes in urine using smear samples as reference was 74.7% and 99.9%. And sensitivity and specificity for low-risk HPV genotypes in urine using smear samples as reference was lower than that of high-risk HPV genotypes (49.3% and 99.7%) (Table 2).

All genotypes found statistically significant by McNemar's test, were analyzed for the agreement with urine and smear samples on the basis of single/multiple infections (Table 3). No HPV genotype was found significantly discordant in relation to the infection status of multiple infections.

4. Discussion

Due to the HPV screening coverage remains low [9], an estimated frequency of incident invasive cervical cancer is still high [10]. Previous study predicted that positive test results are 15 times more likely to occur in HPV infected women than in non-infected women [15]. Hence, single genotyping is important to design preventive strategies, to study the carcinogenic potential of HPV types and improve the triage of HPV positive woman by single type risk esterification, and to follow-up persistent infections [8]. Then, self-collection of samples for high-risk HPV testing can be performed outside a health facility to increase ease of and access to screening uptake [11] due to non-invasive, easily accessible [16]. In fact, urine testing has been successful for the detection of common sexually transmitted infections, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* [17]. Therefore, we thought that urine collection for high-risk HPV detection could provide an especially simple non-invasive method for screening women reluctant to undergo a pelvic examination.

Table 2
HPV genotype-specific comparison with urine and smear samples.

Genotype	Risk	No. of samples for each result				% agreement	Total no. positive	% positive agreement	Kappa data			Two-tailed McNemar's *p* value
		−/−	+/−	−/+	+/+				κ	SD	Int.	
16	High	220	1	0	17	99.6	18	94.4	0.969	0.030	NP	0.317
18	High	226	1	1	10	99.2	12	83.3	0.905	0.067	NP	1.000
26	High	234	4	0	0	98.3	4	0.0	0.000	0.000	NA	0.046
31	High	221	1	0	16	99.6	17	94.1	0.967	0.032	NP	0.317
33	High	227	2	0	9	99.2	11	81.8	0.896	0.073	NP	0.157
35	High	231	3	0	4	98.7	7	57.1	0.721	0.154	ST	0.083
39	High	224	4	0	10	98.3	14	71.4	0.825	0.086	NP	0.046
45	High	232	0	0	6	100.0	6	100.0	1.000	0.000	PE	NA
51	High	228	2	0	8	99.2	10	80.0	0.885	0.081	NP	0.157
52	High	206	5	1	26	97.5	32	81.3	0.882	0.047	NP	0.103
53	High	221	3	0	14	98.7	17	82.4	0.897	0.059	NP	0.083
56	High	234	3	0	1	98.7	4	25.0	0.396	0.276	PO	0.083
58	High	216	2	0	20	99.2	22	90.9	0.948	0.037	NP	0.157
59	High	231	2	0	5	99.2	7	71.4	0.829	0.119	NP	0.157
66	High	229	2	0	7	99.2	9	77.8	0.871	0.090	NP	0.157
68	High	225	5	0	8	97.9	13	61.5	0.752	0.106	ST	0.025
69	High	231	3	0	4	98.7	7	57.1	0.721	0.154	ST	0.083
73	High	227	7	0	4	97.1	11	36.4	0.522	0.156	PO	0.008
82	High	228	8	0	2	96.6	10	20.0	0.324	0.173	PO	0.005
6	Low	229	3	3	3	97.5	9	33.3	0.487	0.180	PO	1.000
11	Low	231	0	2	5	99.2	7	71.4	0.829	0.119	NP	0.157
40	Low	230	5	0	3	97.9	8	37.5	0.537	0.182	PO	0.025
42	Low	228	4	0	6	98.3	10	60.0	0.742	0.124	ST	0.046
43	Low	231	4	0	3	98.3	7	42.9	0.593	0.184	PO	0.046
44	Low	232	3	0	3	98.7	6	50.0	0.661	0.183	ST	0.083
54	Low	222	10	1	5	95.4	16	31.3	0.457	0.136	PO	0.007
61	Low	227	3	0	8	98.7	11	72.7	0.836	0.093	NP	0.083
70	Low	231	6	0	1	97.5	7	14.3	0.244	0.200	PO	0.014
Total genotype sample combinations												
High and low risk		6352	96	8	208	98.4	312	66.7	0.792	0.020	ST	<0.001
High risk		4291	58	2	171	98.7	231	74.0	0.844	0.020	NP	<0.001
Low risk		2061	38	6	37	97.9	81	45.7	0.617	0.053	ST	<0.001

Smear/Urine. Two cases were detection error. PE: perfect ($\kappa = 1$), NP: near perfect ($0.8 \leqq \kappa < 1$), ST: strong ($0.6 \leqq \kappa < 0.8$), PO: poor $\kappa < 0.6$, NA: not applicable.

Table 3
Distribution of HPV genotype in single and multiple infections.

Genotype	Risk	No. of cases				*p* value
		Single infection		Multiple infections		
		Discordant	Concordant	Discordant	Concordant	
26	High	0	0	3	0	1.000
39	High	0	2	3	9	1.000
68	High	1	0	4	8	0.385
73	High	5	0	3	3	0.182
82	High	1	2	7	0	0.067
42	Low	2	1	2	5	0.500
43	Low	0	0	4	3	1.000
54	Low	1	1	9	5	1.000
70	Low	1	0	5	1	1.000

Fisher's exact test, assessing whether discordance is associated with the infection status.

A few studies have been evaluated with alternative sample of cervical smears, while acceptability of urine HPV testing with other detection method has already been shown in previous study [16]. However, previous studies have evaluated sensitivity and specificity of some detection method with limited number of HPV genotypes, while there have been some publications with same idea. Our study investigated 19 high-risk HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) and 9 low-risk HPVs (6, 11, 40, 42, 43,44, 54, 61, 70), which allows the screening and identification of the most clinically relevant HPV types with Anyplex™ II HPV28. Consequently, our study shows that detection of HPV genotypes in urine has a good accuracy for the presence of cervical HPV. Additionally, sensitivity was moderate for detection of any HPV, especially for high-risk HPV (Table 3). The specificity for detection of HPV in urine was especially high for any HPV.

Of note, our study suggested that testing urine samples could be used for the testing HPV infection. In particular, the high specificity provoke important changes in the likelihood of infection for a woman with a positive test result. This is a major strength of the testing method, as false positive results would lead to women undergoing unnecessary invasive investigations, including cytology, colposcopy, or biopsy, to prove lack of disease. This would generate increased anxiety and costs, which could be reduced by urine testing. The high specificity of this test makes this scenario less likely and could thereby increase trust and uptake.

However, our results must be interpreted with caution for several reasons when we think that in the case of cervical cancer screening with urine sample. Because, patient important outcomes must be considered include acceptability of testing, prediction of CIN or invasive cancer, management of positive test results, and safe intervals for testing between negative test results. Firstly, sensitivity was not as high as specificity. The consequences of overestimation are especially important as they can lead to unacceptable morbidity and mortality. False negative results would lead to missing cases of precancerous or cancerous lesions, and false positive results would lead to over-investigation and anxiety. As the consequences of false negative results are serious because this implies missing uterine cervical precancer and cancer, the test could be done more frequently than current screening methods. This would improve the chances of minimizing false negative results.

Additionally, our results suggested that urine samples could be used as first HPV screening test, instead of smear samples, since urine sample revealed the sensitivity was not as high as specificity, compared to smear samples. Urine test may not be suitable for initial screening due to the lower sensitivity with single urine sample test. However we still think urine sample test has strength, because it is invasive test and can be improve its usability by repeating the test, such as a screening procedure for HIV. The test could be done more frequently and using smear samples as screening test when the HPV screening test with urine was negative but the skeptical for the result. This would improve the chances of minimizing false negative results.

Secondly, our study compared the accuracy of detecting HPV in urine samples to cervical samples, so that cervical testing for HPV is being considered as invasive and inconvenient. However, HPV detection in urine samples has ranged considerably among studies, likely due to lack of standardization of urine collection and handling and different HPV extraction and amplification techniques [18,19]. Hence, understanding variations in HPV detection in urine by sample collection method tested and the cost are essential for developing urine collection and processing procedures for future screening amplification, while our study used initial stream urine sample.

Finally, we did not evaluated cervical morphology data including PAP smears and/or colposcopy. For that reason, our study did not consistency check between uterine sample data and cervical morphology data in this study. Hence, further evaluation would be needed. Additionally, we have not found any method for the detection of the source of HPV. These methods are requested to conduct further investigation for discrimination between the persistent infection in urinary tract or contamination of virginal secretions when urine sample would be used for clinical practice.

In conclusion, our study demonstrates the accuracy of detection of HPV in urine for the presence of cervical HPV. Comparing methodologies of collection of samples for HPV detection, they showed the higher agreements for almost genotypes between cervical samples and pellet fractions of urine samples. When cervical testing for HPV is sought, these results suggest that urine could be a good noninvasive tool to monitor HPV infection in women and urine based testing should be an acceptable alternative to increase coverage for subgroups that are hard to reach. However, results must be interpreted with caution owing to variation between individual studies for participant characteristics, lack of standardized methods of urine testing, and the surrogate nature of cervical HPV for cervical disease. Additional research in a larger and general screening population would be needed.

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Competing interests

None declared.

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