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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.