ANTICANCER RESEARCH *37*: 4183-4187 (2017)
doi:10.21873/anticanres.11807

Vaginal and Urine Self-sampling Compared to Cervical Sampling for HPV-testing with the Cobas 4800 HPV Test

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Abstract. *Background/Aim: To compare human papillomavirus (HPV) DNA detection in self-collected vaginal and urine samples with clinician-taken cervical samples in relation to histology. Materials and Methods: Self-collected vaginal, urine and clinician-taken cervical samples were analyzed from 218 women with the Cobas 4800 HPV test (Roche Molecular Diagnostics). Results: The sensitivity for detection of HPV in the vaginal self-sampling test was 96.4% and in urine was 83.9% relative to detection by clinician-taken cervical sample. The vaginal self-sampling and the clinician-taken HPV tests had the same sensitivity of 92.8% (95% confidence interval=86.3-96.8%) and specificity for detection of high-grade squamous intraepithelial lesion (HSIL) and adenocarcinoma in situ (AIS). Detection in urine samples had a sensitivity of 76.3% (95% confidence interval=67.9-84.2%) for HSIL/AIS. Conclusion: The Cobas 4800 HPV test detects high-grade pre-cancerous cervical lesions in self-collected vaginal samples with the same high sensitivity as in clinician-taken cervical samples.*

The Swedish national screening program for cervical dysplasia covers approximately 80% of women in the screening ages of 23 to 65 years. The well-organized midwife-driven cervical screening program is a cost-effective method for detection of cervical dysplasia and to prevent cervical cancer. However, almost half of the cervical cancer cases in Sweden develop in women who do not attend the screening program, or in women above 65 years of age. Cervical cancer in women above the age of 65 years is discovered at advanced stages of the disease and their prognosis is poor (1).

Persistent infection with high-risk human papilloma virus (hr-HPV) is the main reason for more than 99% of cervical intraepithelial neoplasias, as well as for invasive cervical cancer. Vaginal swabs or urine analyses for presence of genital hr-HPV infection may be used to find individuals with hr-HPV in the cervix and increased risk for cervical cancer. In a large meta-analysis, Arbyn *et al*. stated that in screening programs using signal-based assays, sampling by a clinician should be recommended, while HPV testing on self-samples can be suggested to reach women not participating in these (2). Polymerase chain reaction (PCR)-based HPV tests may be used for routine screening after carefully planned pilot studies assessing feasibility, compliance and logistics.

Some studies have used urine sampling as a non-invasive alternative for HPV detection for screening purposes. Urine sampling has also been proposed as an alternative for monitoring HPV prevalence in females to determine the early effect of HPV vaccination in the targeted cohorts (3-7).

The purpose of this study was to investigate the sensitivity and accuracy of hr-HPV DNA detection in self-collected urine and vaginal swabs compared to clinician-taken cervical samples using the Cobas® 4800 HPV test (Roche Molecular Diagnostics, Pleasanton, CA, USA).

Materials and Methods

Women aged 19 to 71 years with an abnormal cervical smear in the screening program or with symptoms were invited to the Outpatient Colposcopy Clinic at regional hospitals in Kristianstad and Helsingborg from April 2015 to June 2016. Samples from 218 women, from whom informed consent was obtained, were analyzed. An oral and written instruction was given to the study participants before taking firstly the self-collected urine sample and then the vaginal samples. The women were asked to urinate without washing first stream urine into a plastic cup and to place a swab (Cobas® PCR Female Swab Sample Kit; Roche Molecular Diagnostics) 6-10 cm into the vagina and rotate it 360

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Key Words: HPV analysis, vaginal smear, cytology, self-collected vaginal smear.

degrees 3-4 times before putting the swab into the tube provided. The urine was transferred to the provided urine tube [Cobas® PCR Urine Sample Kit] by the woman or an assistant nurse without centrifugal processing. The gynecologist collected a cervical sample [Cobas® PCR Female Swab Sample Kit] and a liquid-based cytology (LBC) specimen from the cervix before colposcopy and biopsy or loop electrosurgical excision procedure (LEEP) conization. The LBC was collected by a plastic device (Cervex-Brush® Combi; Rovers Medical Devices, Oss, the Netherlands), scraping cells from the cervix and transferring them into Thin Prep preservCyt Solution (Hologic Inc., Marlborough, MA, USA). All samples were stored at room temperature (18-24˚C) and the samples for HPV analyses were sent to the Department of Clinical Microbiology in Jönköping, while the cytological and histopathological evaluations were performed at the Laboratories of Cytology and Pathology in Lund, Helsingborg and Kristianstad.

The gynecologist performed a colposcopy and based on the clinical situation, an additional cervical biopsy or LEEP conization was performed with local anesthesia. In order to compare the hr-HPV test results with cytology and histological biopsies, the results from the histopathological review was primarily used when such a sample was taken. The two-tiered terminology system with high-grade squamous intraepithelial lesion (HSIL) and low-grade squamous intraepithelial lesion (LSIL) according to the LAST Consensus Conference was used (8).

All samples were transported and analyzed within 3 weeks using the Cobas 4800 HPV Amplification/Detection Kit on the Cobas 4800 System according to instructions from the manufacturer (Roche Molecular Diagnostics). DNA extraction was performed in the Cobas x 480 instrument. The specimens were digested under denaturing conditions at elevated temperatures and then lysed in the presence of a chaotropic reagent. PCR amplification of target DNA sequences was performed by using both HPV- and β-globin-specific complementary primer pairs. The detection of amplified DNA (amplicon) was performed during thermal cycling using oligonucleotide probes labeled with four different fluorescent dyes. Fourteen hr-HPV types (namely HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68), and β-globin were detected. The concurrent extraction, amplification and detection of β-globin was used for monitoring the entire test process.

The study was approved by the Regional Ethics Board in Lund (Reference number DNR 390:2013).

Statistical analyses. The tests were based on the binomial distribution and the exact confidence intervals (CI) are given. Concordance between tests were assessed using Kappa statistics (Cohen's kappa). According to the power analysis with α=5% and power of 90%, 188 patients were required in order to be able to find a 10% difference or more.

All comparisons were two-sided and *p*-values of less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS (PASW) version 22.0.0 (IBM Corp., Armonk, NY, USA) and Omnistat (SBU, Trelleborg, Sweden).

Results

The mean age of the 218 patients included was 35.2 years (SD=10.8 years; range=19-71 years). All urine samples (n=218), the vaginal (n=215) and cervical swabs (n=215) obtained sufficient DNA for HPV analysis. Histopathological specimens were obtained for 199 patients and cytology analyzed in 101 patients.

The sensitivity for detection of HPV in the vaginal self-sampling test was 96.4% and in the urine test was 83.9% relative to detection by clinician-taken cervical sample (Table I). The kappa value between the vaginal self-sampling test and the clinician-taken sample was 0.82 (95% CI=0.73-0.91; $p<0.001$), while that between urine and clinician-taken sample was 0.58 (95% CI=0.46-0.70; $p<0.001$). The vaginal self-sampling and the clinician-taken HPV tests had the same sensitivity for detection of HSIL and AIS in histological samples of 92.8%, as well as similar specificity (Table II). The urine sample had lower sensitivity (76.3%) for detection of HSIL/AIS. The negative likelihood for HSIL/AIS in the vaginal self-sampling test and the clinician-taken sample was 0.20, while that for the urine sample was 0.58. The sensitivity in use of cytology samples for detection of HSIL/AIS in histology was 66.7%, with specificity 93.0% and negative likelihood of 0.36. No histological sample showed invasive cancer.

Discussion

Our results display very high concordance between self-sampling vaginal swab and clinician-taken cervical samples for detection of hr-HPV. The self-sampling vaginal and clinician-taken cervical HPV testing were also equally sensitive for detection of HSIL/AIS.

Even though a previous publication reported a lower sensitivity for hr-HPV testing from vaginal self-samples compared to clinician-taken cervical samples, our results indicate comparable clinical performance (2). Variation of sensitivity and specificity values might be due to the use different sampling devices and different HPV testing methods. We used the Cobas 4800 HPV test platform together with the recommended sampling devices, and all HPV samples were run on the same PCR amplification equipment. Thus, the HPV test method was equivalent for both vaginal, urine and cervical samples and the sampling devices optimized for the process. Studies performed without PCR amplification, *i.e.* the Hybrid Capture 2 HPV test, have shown lower sensitivity in detecting HPV in vaginal self-sampling specimens (2). Such lower clinical sensitivity of HPV testing of self-samples may be due to the load of hr-HPV DNA in the vagina being at a level beneath the detection threshold of the assay but which can be detected by more analytically sensitive PCR tests. HPV tests with PCR amplification with the GP5+/6+ primers or the Abbott RT hr-HPV Test were shown to have sensitivity and specificity on vaginal self-samples *versus* clinician-taken samples similar to those found in this study (2).

The sensitivity for vaginal self-sampling for detection of HSIL/AIS in histological samples was 93%, which is very high and corresponds exactly to the sensitivity value obtained for the cervical clinician-taken samples. In

Table I. *Results of human papilloma virus tests – Self-sampled specimens from urine and vagina compared to clinician-taken sample from the cervix.*

Method	Clinician-taken sample from cervix, n			Sensitivity	95% CI	Specificity	95% CI likelihood	Negative	95% CI
	Positive	Negative	Total						
Vaginal self-sampling									
Positive	160	7	167	96.4%	93.5-99.2%	85.1%	74.9-95.3%	0.04%	0.02-0.09%
Negative	6	40	46						
Urine									
Positive	141	8	149	83.9%	78.4-89.5%	83%	72.2-93.7%	0.19%	0.13-0.28%
Negative	27	39	66						

CI: Confidence interval.

Table II. *Sensitivity, specificity and negative predictive value of high-risk human papilloma virus (hr-HPV) testing on cervical, vaginal and urine samples for detection of atypical squamous cells of undetermined significance (ASUS), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL)/adenocarcinoma in situ (AIS) in histological specimens.*

Method	Histology, n			HSIL/AIS							
	Normal, ASUS or LSIL	HSIL/ AIS	Total	Sensitivity (%)	95% CI	Specificity (%)	95% CI	NPV (%)	95% CI	Negative likelihood	95% CI
Vaginal self- sampling											
Positive	55	103	158	92.8	86.3-96.8	35.3	25.2-46.4	79	64.5-88.6	0.2	0.10-0.42
Negative	30	8	38								
Urine											
Positive	52	86	138	76.8	67.9-84.2	40.2	29.9-51.3	57.4	46.9-67.3	0.58	0.38-0.88
Negative	35	26	61								
Clinician-taken											
Positive	55	103	158	92.8	86.3-96.8	35.3	25.2-46.4	79	64.5-88.6	0.2	0.10-0.42
Negative	30	8	38								

CI: Confidence interval; NPV: negative predictive value.

comparison to cytology, the vaginal self-sampling HPV test had significantly higher sensitivity but with lower specificity. A meta-analysis showed that the pooled sensitivity of HPV testing for self-samples was 76% (95% CI=69-82%) for cervical intra-epithelial neoplasm grade 2 (CIN2) or worse and 84% (95% CI=72-92%) for CIN3 or worse and that the range in articles on primary screening was between 51% and 93% for CIN2 or worse and between 63% and 94% for CIN3 or worse (2). The negative likelihood ratios of HSIL in our study were exactly the same in the vaginal self-sampling test and the clinician-taken sample (*i.e.* 0.20), indicating that the risk of missing a HSIL is very low for both the vaginal self-sampling test and the clinician-taken sample.

When urine HPV analyses were compared to HPV tests obtained from cervical material, the sensitivity ranged from 53-100% and the specificity from 67% to 100% (2, 9). Our urine sample results regarding sensitivity and specificity values are similar to an earlier study comparing HPV testing in urine specimen to cervical samples (10). We observed that the sensitivity for HSIL/AIS 77% was lower than for the vaginal self-samples. The Cobas 4800 assay cut-off of PCR cycles needed to detect hrHPV DNA (Ct values) was applied according to the instructions from the manufacturer but adjustment of the cut-off for urine samples may enhance sensitivity if urine samples are used in future studies (11). The negative predictive value for HSIL/AIS was 60% in this study which is too low for use in a screening program but may be acceptable if used to analyze the effects of HPV vaccination in a cohort (12).

Previous studies have shown that women prefer self-sampling and that self-sampling is an option for increasing the attendance and the compliance rate for cervical cancer prevention programs (13). We have found that the most

common reasons for not attending cervical screening were "uncomfortable with vaginal examination", "lack of time" and "experience of unfriendly health workers" (14), all of which are eliminated with vaginal self-sampling. None of the women in this study reported that vaginal self-sampling was uncomfortable. The swab sample kit was easy to use and the women did not report any difficulties or problems with the written and figure instructions, probably due to a widespread use of tampons for menstrual bleeding. A previous article has shown that vaginal HPV sampling is not dependent on menstrual cycle, sexual intercourse or timing of sampling (15). Both the urine and the swab kits are stable for up to 12 months, allowing use of regular mail for delivery to the laboratory.

In Sweden, the costs of a clinician- and midwife-taken cervical HPV sample is at least €40 (County of Skåne) plus the costs of the HPV analysis. The cost of the self-sampling vaginal devices, logistics and transport (mail) of the sample is less than 1/10th that for a clinician-taken sample. However, the positive predictive values for self-samples and clinician-taken HPV samples are usually lower than 10% in low-risk and medium-risk populations, explaining why there is a need for an additional triage test, *e.g.* cytology or a repeated HPV test, before referral to colposcopy for diagnostic work-up or treatment in women with positive vaginal HPV test results.

Immune-suppressed individuals have a higher risk of persistent HPV infections and development of cytological pathologies leading to cervical cancer (16). These high risk women may benefit from more frequent self-sampling HPV tests, which are easier and more cost-effective to perform than the procedure used in the regular screening program.

In this study, the women served as their own controls, thereby limiting biases. However, in the general population, HSIL is estimated to be present in about 1-2% of women as opposed to our high-risk population. A recent study in a screening population with a prevalence of approximately 15% of women with hr-HPV showed results equivalent to ours in a high prevalence population (11) but further studies are needed to confirm these promising results for detection of HSIL with vaginal self-sampling. Since the cervical screening program does not reach one quarter of the women in certain areas; there is a large demand for evaluation of self-test alternatives in relation to standard cytological screening. Vaginal self-samples seem to be a suitable, cost-effective and attractive alternative in the screening population, especially in order to reach non-attendees. This self-sampling method will be further evaluated in an up-coming study inviting non-attendees to participate.

Conflicts of Interest

Roche provided the sample kits and did not charge for the HPV analyses performed at the Laboratory in Jönköping. Roche did not have any influence on the study design, statistical analyzes or article writing.

None of the Authors have declared any conflict of interest in regard to this study.

Acknowledgements

The study was supported by the Swedish Cancer Foundation (CAN 2013-630). The Authors would like to thank the personnel in Molecular Biology, Department of Clinical Microbiology, County Hospital Ryhov, Jönköping, and especially Anette Nilsson Bowers.

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Received May 30, 2017
Revised June 19, 2017
Accepted June 21, 2017