ORIGINAL RESEARCH

Cobas® 4800 HPV test is accurate for detecting high risk Human Papillomavirus from urine samples at dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia

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ABSTRACT

Objective: To find the accuracy, sensitivity, specificity, as well as predicted values, both positive and negative, of urine samples using Cobas® 4800 in detecting high risk Human Papillomavirus.

Materials and Methods: This study was a cross-sectional study with a total of 72 samples taken from medical records of hrHPV DNA examination with Cobas® 4800 in 2017-2020. Study subjects were called for re-examination of urine samples and cervical samples using Cobas® 4800. Samples with positive hrHPV DNA in the cervix, urine, or both were examined for cervical fluid-based cytology (LBC). Data were analyzed using Chi-square.

Results: Overall, 84.72% agreement was detected through specimens of urine and cervical mucus tested of hrHPV DNA with Cobas® 4800. In all samples, a significant rate of concordance detection of hrHPV DNA with Cobas® 4800 was reported ($\kappa = 0.62; 95\% IC: 39.84$). In this population, in determining the presence of hrHPV DNA in cervical and urine specimens, it was found that the sensitivity, specificity, positive predictive value and negative predictive value were respectively 87.5% (95% IC: 64.97–97%), 84% (95% IC: 72.91%), 60.9% (95% IC: 40.8–77.8%), and 96% (95% IC: 86.3–98.9%).

Conclusion: The presence of hrHPV infection in the cervix can be determined by detecting hrHPV DNA in the urine. According to these findings, urine samples subjected to the Cobas® 4800 HPV test may be helpful for the clinical treatment of HPV infection.

INTRODUCTION

Cervical cancer has the highest prevalence and it is the fourth leading cause of death from all cancers, as well as the high risk human papillomavirus (hrHPV) is a virus that poses a high risk of infection, considered the main etiology of cervical cancer.¹ According to WHO, one of the goals of the worldwide as a technique for expediting the eradication cervical carcinoma, a global health-related services concern, is intended for 70% of women to have been screened at the age of 35 and then again at the age of 45 by 2030.² The hrHPV DNA test has to be the most sensitive screening procedure for CIN 2+ sensitively value of 98.3% and a specificity of 85.3–86.2%.³

According to Indonesia's 2018 health profile data, women who have screened for cervical cancer with acetate visual inspection are 7.34%.⁴ However, this low achievement is due to these screening methods need a pelvic examination, which is invasive, painful for the patient, time-consuming for healthcare professionals,
and difficult to do in settings with limited resources. Therefore, the development of non-invasive self-sample collection techniques might potentially have the benefit of raising the acceptability of the screening processes. Cytology-based screening is being replaced by primary screening or cotesting based on the human papillomavirus (HPV). For this, the use of urine, which is simple to collect, would be beneficial. Furthermore, molecular techniques to identify DNA in urine samples are already regularly used to diagnose prevalent sexually transmitted diseases including Chlamydia trachomatis and Neisseria gonorrhoeae.7

HPV does not have a preference for the urinary system, in contrast to its preference for the cervix and other anogenital anatomical regions 8,11. Therefore, the presence of HPV in urine is very certainly due to secondary cervix exfoliation or other anogenital lesions. The purpose of this study was to assess urine testing for high risk HPV in a group with a high prevalence of the disease. The clinical effectiveness of self urine-based sampling was also assessed in comparison to cervical sampling, and the results were compared to the presence of cervical disease as determined by histology. The Cobas® 4800 HPV test, an FDA-approved real-time PCR assay created for the simultaneous genotyping of HPV-16 and HPV-18 and the detection of high-risk HPV (HR-HPV), was utilized for this purpose 12,13.

MATERIALS AND METHODS

This study used a cross-sectional design of a diagnostic test to compare urine hrHPV DNA to cervical hrHPV DNA preparations which is the gold standard that carried out at the Kencana Women Health Cluster from January 2020 to December 2020, at dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Patients aged 30-60 years were enrolled on this study. Pregnant women and those who had given birth within the past three months of the examination date were excluded. The patient was recalled for cervical and urine hrHPV DNA examinations on the same day and a cytology examination with liquid-based cytology (LBC) was performed if the results of the hrHPV examination were positive, either in cervical swabs, urine, or both.

Cervical samples were taken using a cervical brush by the doctors and deposited within five days, in a Real-time polymerase chain Cell Cobas® medium bottle (Roche Diagnostic). Cervical specimens taken for PCR cell collection at Cobas® media are stable for up to 6 months at 2–30°C for use with the Cobas® 4800 test for HPV testing.5 In sterile containers, we collected urine specimens identically dated as the pelvic examination and delivered to a laboratory for further processing within five days. A minimum of each urine sample should be diluted to a volume of twenty milliliters. was centrifuged for 10 minutes at 4000 x g. The solid pellet was resuspended in water following centrifugation five milliliters supernatant into a vial of PCR collect media. The Cobas® 4800 test was used to process urine samples, despite the fact that it still hasn’t been verified for use in specimens of this kind 12,13.

In a single system, the Cobas® 4800 system combines completely Real-time PCR technique enables automated sample preparation. In addition to hrHPV genotypes, the test will identify globin gene of human cells. In a single tube, amplification and detection of polymerase chain reaction took place, with probes containing four separate dyes that act as reporters tracking the multiplex reaction’s distinct targets. There were four different dyes: Reporter dye 1 looked at the hrHPV pool contains 12 human papillomavirus (HPV) targets (HPV-31, -33, -35; 39; 45; 51), while 2 and 3 dyes looked at the two forms of HPV are HPV type 16 and HPV type 18. Dye 4 examined the b-globin protein to determine the adequacy of the cells, their extraction, and amplification. The examination was conducted according to the producer’s recommendations for the extraction of DNA with the Cobas® X 480 and real-time PCR with the Cobas® Z 480 12,13.

Sensitivity, PPV (positive predictive value), specificity, NPV (negative predictive value) are things terms used to describe how accurate a prediction of hrHPV detection in urine specimens were measured as percentages with confidence intervals of 95% in comparison to cervical sample detection (gold standard) (95% CI). The Kappa statistic (Cohen’s Kappa,) was utilized to ascertain if test concordance was "poor" (κ=0), "slight" (0.01<κ<0.20), "fair" (0.21<κ<0.40), "moderate" (0.41<κ<0.60), "substantial" (0.61<κ<0.80), "nearly perfect" (0.81<κ<1), or "perfect" (κ=1). Differences between paired proportions were calculated using the McNemar’s test. P <0.05 was utilized as the significative level.14

RESULTS AND DISCUSSION

The b-globin gene was detected in every 72 cervix and urinary specimens used for this investigation. Preliminary data from 2017-2020 tests showed that 42 women had previously tested positive for hrHPV DNA, while 30 women had previously tested negative. A prevalence of 34.7% was found in the 25 women who a positive test result hrHPV utilizing a minimum of one of the two specimens examined. In urine samples, the prevalence of hrHPV carcinogenic kinds was 31.9 %, while in cervical samples, it was 22.2% (Table 1).
There was an overall concordance of 84.72% between the identification of hrHPV in specimens of urine and cervical secretions. The identification of hrHPV DNA in both samples had a high concordance rate ($\kappa = 0.62; 95\%$ IC: 39–84). For both types of samples, there were 11 disparities in the results. In two of them, hrHPV was detected in the cervix but not in the urine. However, nine positive samples were obtained for hrHPV in the urine but negative in the cervix. Overall, for detecting of hrHPV DNA extracted from urine compared to cervical samples, sensitivity, PPV (positive predictive value), specificity, and NPV (negative predictive value) were 87.5% (95\% IC: 64–96), 83.9% (95\% IC: 72–91), 95.9% (95\% IC: 86–99), and 60.9% (95\% IC: 41–78), respectively. For the pool of HR-genotypes, the prevalence of hrHPV samples of urine was higher compared to cervical samples (without HPV16 and HPV 18).

Table 2 compares the sensitivity, PPV (positive predictive value), specificity and NPV (negative predictive value) of particular PCR analysis (HPV type 16, HPV type 18, and another hrHPV) comparing those found in urine specimens to those found in cervical specimens. The sensitivity of any hrHPV genome was 84.62\% (95\% IC = 58–96), and the Cohen’s Kappa was moderate.

Twenty-five women received cytological results, with 17 (68\%) being negative for intraepithelial lesions, 5 (20\%) having LSIL (Low Grade Squamous Intraepithelial Lesions), 2 (8\%) having ASCH (Atypical Squamous Cells cannot exclude HSIL), and 1 (4\%) having ASCUS (Atypical Squamous Cells of Uncertain Significance). To find out the factors that influence the results of the urine DNA hrHPV examination, a relationship test was carried out between the factors that might affect the examination results (age, education, and parity) with the suitability of the urine DNA hrHPV results to the cervix as shown in Table 4.

Table 3. hrHPV infection prevalence cervical and urinary examinations sample method compared to cytology results
Several pieces of data suggest that using self-collected urine as a more HPV testing options than cervical samples could be beneficial. First, HPV genotypes in urine and cervix lesions tissue specimens agreed significantly. Urine contaminated with exfoliated cervical cells infected with HPV is known to rise as the severity of cervical lesions increases. Thus, HPV presence in urine requires constant monitoring resembles an HPV infection in the cervix.  

Urine sample for the diagnosis of oncogenic HPV has been studied in several research. Because various factors, such as the urine sample technique, conditions of storing, centrifuging, and extracting DNA, and HPV DNA test, might adversely influence HPV DNA detection, HPV DNA testing in the urine has a variety of limits applicable to this purpose.  

We examined 72 matched cervix and urine specimens from women who have been referred to the unit of gynecology by community physicians for examination a Pap smear that is abnormal or VIA screening outcomes. Due to the fact that the research was done on a select sample a group of women previously possessed an abnormality VIA or pap smear test, HPV infection was quite prevalent (>50%), as expected. Urine had a higher prevalence (31.9%) than cervical specimens (22.2%). The presence of hrHPV-DNA from epithelial tissues of the urethra and vulva, which are similarly vulnerable to hrHPV infection, could be one explanation. In 11 samples, there was a discrepancy, with two samples having positive hrHPV in the cervix but negative in urine sample and nine specimens having positive hrHPV in the urine but negative in the cervix. These 11 samples had normal cervical cytology examinations using liquid-based cytology (LBC). Some allegations that may occur include the possibility of a faster regression in the cervix than in the urine. Another possibility is that there was previous sexual contact so that hrHPV transmission occurred, while the cervix had not yet had an infection process. In this group, the possibility of primary hrHPV infection originating from the urinary tract requires further investigation. Some literature mentions the possibility of hrHPV infection as a cause of urinary tract cancer, the most common is bladder cancer (transitional cell cancer), around 22%.  

We discovered a significant concordance rate of HR-HPV DNA detection and an overall agreement of 84.72%, (κ=0.62; 95% IC: 0.39–0.84). The sensitivity was 87.5% and specificity 83.9%. According to Pathak et al. that HPV infection detection in urine had a pooling sensitivity of 87% and specificity of 94%, while hrHPV had a 77% sensitivity, 88% specificity in a meta-analysis and systematic review on precision of urine HPV testing for cervical HPV infection. The HPV types 16 and 18 detection through urine exhibited a combined sensitivity and specificity of 73% and 98%, respectively. When urine samples were obtained as first voiding pee instead of random or midstream urine samples, meta-regression revealed a 22-fold increase in overall accuracy.  

The sensitivity of first voiding urine is significantly higher than random or intermediate urine samples, resulting in a considerable difference in accuracy. The results of this study were better than those of Oliveira et al. (sensitivity of 50.0% with a Kappa value of 0.55), Tranberg et al (sensitivity of 63.9% with a Kappa value of 0.66), sensitivity of 51,6% with a Kappa value of 0.55 with CLART assay, and Sahasrabuddhe et al (κ=0.55).  

These findings contrast with those of Bernal et al (sensitivity 88%, Kappa value 0,76), Ostensson et al (with Abbot RealTime sensitivity 33-100%, Kappa ranged from 0,36 to 0,85), and Ornskov et al (with Abbot RealTime sensitivity 33-100%, Kappa ranged from 0,36 to 0,85), and Ornskov et al (with Abbot RealTime sensitivity 33-100%, Kappa ranged (sensitivity 93-97%, Kappa value 0,66-0,77). The sensitivity of 12 hrHPV other than HPV types 16 and 18 was calculated to be 84.62%. Because there were no samples with a single infection with HPV type 16 and
just one specimen having only one infection of HPV type 18, it was not done for HPV types 16 and 18. Using bivariate analysis on the variables in the research subjects, the researchers looked for factors that could influence the outcome of a urine hrHPV DNA examination. The investigation found no research variables in terms of age, education level, or parity that are connected to the outcomes of the HPV DNA urine screening. With respect to alternative HPV detection and/or genotyping assays, we chose the Cobas® 4800 HPV test for our investigation. This real-time PCR-based assay test offers a number of benefits. First off, the test simultaneously gives partial HPV genotyping for HPV-16 and HPV-18, in addition to reporting the existence of HR HPV genotypes. Second, since the test has been modified for primary specimens, it is highly automated and simple to use. Finally, findings are available around 4 hours after receiving the material.

CONCLUSION

The findings of this research encourage the use of urine in conjunction Cobas® 4800 HPV testing among women who have cervical lesions that are more severe. Before using urine/ Cobas® 4800 as a diagnostic test, it should be validated by others. More research is needed to show equivalent performance in a group undergoing initial screening. If these are findings are validated, urine collection may be used within populations that contain limited availability of healthcare when, for example a pelvic exam is not practical. Additionally, when molecular methods are used in conjunction with initial tests for primary cervical cancer screening, urine samples may be a suitable substitute for cervical specimens. For vaccination trials, disease surveillance, and epidemiological investigations, urine could also be utilized to evaluate HPV prevalence. 

DISCLOSURES

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Conflict of interest

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