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Human papillomavirus DNA testing with the urine sample is not yet available: the accuracy of two distinct kits

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SUMMARY

OBJECTIVE: The aim of this study was to assess the results and efficiency of two real-time polymerase chain reaction procedures for detecting human papillomavirus utilizing urine samples.

METHODS: This study comprised 151 patients who had previously tested positive for human papillomavirus in their cervical samples. Two different commercial real-time polymerase chain reaction techniques were used for identification and genotyping human papillomavirus in urine specimens. The urine samples of 151 patients were evaluated via the Roche Cobas test, and the urine samples of 91 patients were also evaluated via the Qiagen test.

RESULTS: The overall consistency of urine and cervical swab specimens for the identification of human papillomavirus in Roche Cobas and Qiagen tests were 44.8 and 44%, respectively. The rates of positive human papillomavirus results from urine samples were 57 and 70.3%, respectively. The overall concordance among Roche Cobas and Qiagen tests utilizing urine samples for human papillomavirus type 16/18 was 84.3% with a kappa value of 0.675, and for other high-risk-human papillomavirus, it was 75.60% with a kappa value of 0.535. Roche Cobas showed high concordance with Qiagen test.

CONCLUSION: human papillomavirus positivity was not detected in all urine samples. It is still inappropriate to recommend the use of urine liquid biopsy for the accurate and reliable detection of human papillomavirus. Due to the lack of a standardized tool, the utilization of urine samples as a screening human papillomavirus test remains a challenge.

KEYWORDS: Pap smear. Human papillomavirus. Real time PCR. Urine collections.

INTRODUCTION

Screening programs should have been standardized, practical, sufficient, effective, and acceptable for the target population. Cervical neoplasm is the fourth common cancer, causing deaths in females of poor- and moderate-income countries[1]. Scientific and demographic studies have obviously shown that persistent HPV infection is a risk factor for the occurrence of both pre-invasive cervical disorders and invasive carcinoma[2]. HPV detection for cervical cancer screening has different benefits compared with that for cytology-based screening, which comprises heightened sensitivity and improved diagnostic repeatability in many environments, but it is an invasive method[2]. The utilization of urine liquid biopsy as self-collection of specimens for HPV detection has been demonstrated to be very suitable in various cultures due to many reasons, such as the ability to apply samples outside the health center, being a noninvasive method, and the facility to access and increase the screening uptake[3-5]. However, due to the lack of a standardized tool, the utilization of urine samples for screening HPV test remains a challenge.

The purpose of this trial was to determine the efficiency of HPV determination in urine samples of the patients who had prior HPV-positive results in the cervical swab specimens.

METHODS

Study design and characterization of participants

The Department of Gynecologic Oncology at the Gazi University Faculty of Medicine Hospital carried out this prospective investigation. A total of 151 patients with previous HPV DNA-positive results in the cervical swab specimens that were taken by clinicians between January 2019 and January 2021 were considered in this trial. Besides, the demographic data such as age, body mass index (BMI), the number of pregnancy and parity, the route of parturition, first coitus age, the history of smoking and using of oral contraceptives (OC), and the pathological findings in cervical cytology were also evaluated. None of them had undergone hysterectomy or received prior

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treatment for cervical disorders or cancer. They had no history of HIV or other sexually transmitted infections and were not pregnant at the time of trial.

Detection of human papillomavirus DNA in cervical swab and urine specimens

Cervical swab specimens

Cervical specimens were gathered by an gynecologist oncologist via a cervical swab, stored in a PCR Cell Cobas medium vial (produced by Roche Diagnostics, USA), and sent to the laboratory. Cervical swabs are steady at 2–8°C for testing with the Cobas 4800 HPV test kit (Roche Cobas 4800 HPV Test Package Insert 2010). Real-time PCR technology is integrated with completely automated specimen preparation in the Cobas 4800 platform. The test is created to isolate, replicate, and identify a wide range of high-risk HPV (HR-HPV) genotypes. The test can identify 14 HR-HPV genotypes in a single assay by presenting individual scores for high-risk genotypes: HPV 16 and HPV 18 and other HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)[6].

Urine samples

The first stream urine samples of the HPV-positive patients were collected in a sterile cup and sent to the laboratory. A minimum of 20 mL of every urine specimen was separated by centrifugation at 4000×g for 10 min. After vortexing, the concrete pellet was redissolved in a volume of 5 mL of supernatant and relocated to one phial of PCR collection medium. After this procedure, urine specimens were analyzed with real-time PCR techniques. Two different commercial kits (Roche Cobas and Qiagen) were employed for the determination of HPV DNA in urine specimens. Qiagen could detect 14 HR-HPV genotypes (16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68)[6,7]. DNA extraction from urine samples was carried out in an automatic device (EZ1, Qiagen, Germany) using the EZ1 Virus Mini Kit. Extracted DNA samples were studied with two different commercial real-time PCR kits on a real-time PCR device (Rotor-Gene Q, Qiagen, Germany). Four different florescent dyes (green), Joe (yellow), Rox (orange), and Cy5 (red) channels were used for the determination of HPV genotypes.

Statistical analysis

SPSS version 21.0 was used to conduct the statistical analysis (SPSS Inc., Chicago, IL, USA). Descriptive statistical analysis was quantified for the overall population, in addition to both the urinary HPV-positive and -negative groups. The sensitivity of HPV determination in urine specimens, checked for the identification in cervical samples, was computed as percentages. Chi-square test for competition pairs (McNemar test) was used to crosscheck the efficiency of the two types of samples concerning the determination of HPV types. The kappa correlation coefficient was computed for the correlation analysis. Outcomes were considered statistically significant at a $p<0.05$.

Ethical approval and informed consent

The Ethics Committee of the Faculty of Medicine of Gazi University consented to the research (date: 16.10.2018, decision number: 795). Written informed permission was obtained after all participants received information about the study. This research was carried out in accordance with the Helsinki Declaration principles.

RESULTS

In this research, the median age of the cases was 40 years (ranged from 23 to 60), and most of them were between 30 and 50 years old (71.5%). The demographic features of patients are listed in Table 1. There were 151 and 91 urine samples examined with the Roche Cobas and Qiagen tests, but 17 and 7 of the study population were excluded from each test due to an invalid result, respectively. Therefore, 134 and 84 urine samples

Table 1. Distribution of human papillomavirus genotypes, histopathological results, and demographic data of human papillomavirus-positive patients in cervical samples.

	n, %
Age (median/min–max) (years)	40 (8/23–66)
Nulliparous/ birth child	30 (19.9)/121 (80.1)
Route of parturition (VD/C-S)	75 (49.7)/46 (30.5)
BMI (average)	29.23 (21–53)
First coitus age (min–max) (years)	20 (14–38)
Smoking status (none/active smoking)	81 (53.6)/70 (46.3)
Using oral contraceptive status (−)/(+)	120 (79.5)/31 (20.5)
HPV type	
16/18 OHR	44 (29.1)/15 (9.9)/58 (38.4)
16+OHR/18+OHR/16+18+OHR	27 (17.9)/6 (4)/1 (0.7)
Cervical cytology	
Malignancy(−)/ASCUS/ASC-H	80 (53%)/30 (19.9%)/3 (2%)
LSIL/HSIL	33 (21.9%)/5 (3.3%)
Total	151

VD: vaginal delivery; C-S: cesarean section; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesions; HSIL: high-grade squamous intraepithelial lesions; ASC-H: atypical squamous cells (cannot exclude HSIL).

were studied by Roche Cobas and Qiagen tests, respectively, and,then compared.

The frequencies of HPV DNA in urine samples that were studied using Roche Cobas and Qiagen kits were 57 and 70.3%, respectively. The sensitivities of Roche Cobas and Qiagen tests were 64.2 and 76.2%, respectively. The overall consistency between urine and cervical swab specimens for the identification of HPV in Roche Cobas and Qiagen tests was 44.8% and 44%, with a kappa value of 0.321 and 0.314, respectively. The overall accuracy to detect HPV DNA, between Roche Cobas and Qiagen tests, was 77.3%, with a kappa value of 0.504 (p<0.001).

The highest prevalent HPV subtype was OHR, with 38.4% rate in cervical samples (Table 1). Furthermore, in urine samples, the highest rates belong to OHR-HPV types in both Roche Cobas and Qiagen tests (27.2 and 16.6%) (Figure 1).

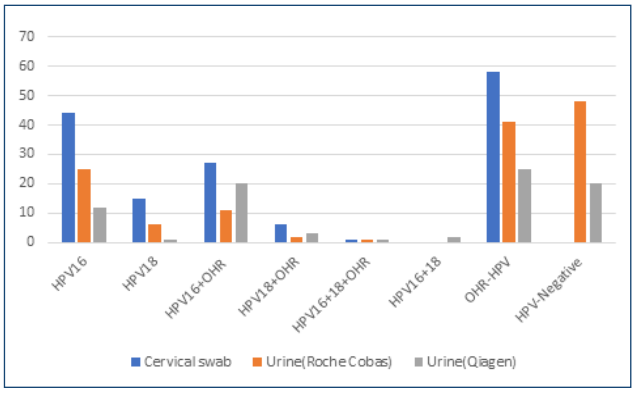

Figure 1. Distribution of human papillomavirus genotypes in cervical swab and urine samples with the result of two different tests.

The accuracy among the Roche Cobas and Qiagen tests utilizing urine samples for HPV type 16/18 was 84.3% with a kappa value of 0.675, and that for OHR-HPV type was 75.60% with a kappa value of 0.535 (Table 2). There was no statistical significance in urine samples of HPV-positive and HPV-negative patients between analysis via Roche Cobas and Qiagen tests in terms of demographic features such as age (p=0.354 and p=0.914), parity status (p=0.565 and p=0.976), BMI (p=0.850 and p=0.967), smoking behaviors (p=0.542 and p=0.075), using oral contraceptives (p=0.159 and p=0.376), and first coitus age (p=0.656 and p=0.319).

DISCUSSION

The most essential strategy for eradicating cervical cancer is to screen women by an HPV test with cervical cytology samples[3]. Unfortunately, many women ignored these programs or continued many years without being screened because of different reasons, such as difficulty to access medical centers in developing countries, busy work schedule, time constraints, and socio-economic anxiety in developed countries. Furthermore, obstacles to testing include absence of information, personal choice, anxiety, shame, and honesty in the health care system. Besides, the detection of HPV with cervical screening has brought some limitations for single, sexually inactive women and adolescents who do not wish to have a vaginal examination[5,8]. Due to all these unfavorable reasons, new strategies are

Table 2. Accuracy in human papillomavirus DNA detection results between Roche Cobas and Qiagen tests using urine samples.

HPV genotypes		Qiagen HPV(+)	QiagenHPV(−)	Diagnostic accuracy	Kappa value	95%CI kappa value	p-value
16 and/or 18	Roche HPV(+)	27	1	84.30%	0.675	0.409–0.941	p<0.001
	Roche HPV(−)	7	16				
OHR	Roche HPV(+)	12	0	75.60%	0.535	0.250–0.820	p<0.001
	Roche HPV(−)	9	16				
		16 and/or 18(+)	OHR(+)				
16 and/or 18	Roche HPV(+)	27	0	92.80%	0.837	0.538–1.136	*p<0.001
OHR	Roche HPV(−)	3	12				

*McNemar test, p<0.05.

needed to facilitate participation in cervical cancer screening. The utilization of urine as liquid biopsy for the detection of HPV has been demonstrated to be extremely favorable because of similarly strong association between urinary and cervical HPV DNA, easy collection of samples, and relatively high suitability for women[9-11].

HPV DNA testing in urine samples presents some obstacles because of so many variables, such as urine collection method, storage situations, centrifugation process, and DNA isolation or amplification procedure[10]. Currently, there are various HPV genotyping methods for identifying DNA, such as PCR, real-time PCR, restriction fragment length polymorphism (RFLP), hybrid capture, and linear array[9]. In this investigation, we compared the abilities of the Cobas 4800 HPV test and the Qiagen test to identify HPV DNA in urine samples. These two real-time PCR assays have a variety of benefits over existing HPV genotyping and/or detection bioassays. The outcomes can be acquired nearly 4–6 weeks after receiving the cervical samples in these assays. The overall agreement between urine and cervical swab samples for the detection of HPV in Roche Cobas and Qiagen tests was 44.8% and 44%, with a kappa of 0.321 and 0.314, respectively, in this study. Bernal et al., determined 88% agreement between urine and cervical samples. Bernal et al., applied the PCR method with Roche Cobas 4800 HPV test on matched cervical and first voided urine in 125 patients between the ages of 21 and 65 years[12]. In addition, Hagihara et al., reported that the concurrence among the urine and cervical samples was 98.4%, with a kappa of 0.792. Hagihara et al., detected DNA by the PCR method with the Anyplex™ HPV28 kit on synchronous cervical and urine samples in 240 patients between the ages of 19 and 58 years[9]. High correlation was observed in both studies with simultaneous HPV DNA detection in cervical and urine samples[9]. In this study, urine samples were collected from HPV-positive patients in cervical swab when the patients came to get information about their cervical cytology results. The reason of low sensitivity to detect HPV in urine samples is that the time interval between the collection time of urine samples and cervical cytology was longer. During this period, the patients may be in the recovering period. The overall concordance among the Roche Cobas and Qiagen tests utilizing urine samples for HPV type 16/18 was 84.3% with a kappa value of 0.675, and that for OHR-HPV was 75.60% with a kappa value of 0.535. Roche Cobas showed high concordance with Qiagen test. However, three samples that were detected as type 16 and/or 18 by the Qiagen test were detected as OHR-HPV by the Roche Cobas test (Table 2). Lim et al., demonstrated a similar agreement to detect HPV 16/18 between Roche Cobas and Abbott (relative sensitivities: 79.2% and 81.8%) in their study[13]. In this and Lim's studies, we determined that different kits did not influence the HPV detection rate in urine samples determined by the PCR technique.

There are also some confusions in the description of first void urine, frequently thought to be the first urine of the day[11]. In our study, first void urine was defined as the collection of the initial urine (not midstream urine) at any time of the day. Recent studies demonstrated significantly higher level of HPV DNA in the first part of void urine than in the subsequent part[14]. Furthermore, recent studies determined that there was no significant impact on time of collection between morning and later during the day[15,16]. However, we had no information about the interval time of two urinations in terms of viral DNA accumulation in our cases, which is the study's limitation. When the interval between two urinations is long, the rate of detecting excess HPV DNA may increase due to the increased accumulation of infected cells in the cervical discharge.

In addition, the collection device affects the ratio of the HPV detection level. Pattyn J. demonstrated that the HPV concentrations were observed higher in the Colli-Pee® device than the standard urine cup. During transport, storage, and pre-analytical processing steps, the collection of a nucleic acid preservative for urine samples provides effective and accurate results as it prevents degradation of cell-associated and cell-free DNA by nucleases[15]. Another reason for low sensitivity in our study is that we collected the urine samples in a sterile urine cup without any preservative medium.

In this study, no statistical significant differences were observed in terms of age, parity, types of parturition, body mass index, smoking status, first coitus age, or contraceptive method when comparing patients with a positive HPV to those with a negative result in urine samples which were analyzed by Roche Cobas and Qiagen tests similar to the study by Nicolau et al.[17].

CONCLUSION

The detection rate of HPV in urine specimens is lower than that in cervical samples. Many factors such as the urine collection methods, storage situations, centrifugation process, DNA extraction, or amplification techniques may be responsible for the low DNA level in urine samples. Beside this, Roche Cobas and Qiagen tests showed high concordant results, including genotyping. An approach for testing HPV using urine samples is not yet available.

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AUTHORS' CONTRIBUTIONS

MAO: Conceptualization, Methodology, Writing – review & editing. **FK:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **OE:** Data curation, Writing – review & editing. **GB:** Methodology, Project administration, Supervision, Validation, Writing – review & editing.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33. https://doi.org/10.3322/caac.21708
2. Kyrgiou M, Arbyn M, Bergeron C, Bosch FX, Dillner J, Jit M, et al. Cervical screening: ESGO-EFC position paper of the European Society of Gynaecologic Oncology (ESGO) and the European Federation of Colposcopy (EFC). Br J Cancer. 2020;123(4):510-7. https://doi.org/10.1038/s41416-020-0920-9
3. Ørnskov D, Jochumsen K, Steiner PH, Grunnet IM, Lykkebo AW, Waldstrøm M. Clinical performance and acceptability of self-collected vaginal and urine samples compared with clinician-taken cervical samples for HPV testing among women referred for colposcopy. A cross-sectional study. BMJ Open. 2021;11(3):e041512. https://doi.org/10.1136/bmjopen-2020-041512
4. Lorenzi AT, Fregnani JHT, Dockter J, Fitzgerald K, Strohecker E, Eaton B, et al. High-risk human papillomavirus detection in urine samples from a referral population with cervical biopsy-proven high-Grade lesions. J Low Genit Tract Dis. 2018;22(1):17-20. https://doi.org/10.1097/LGT.0000000000000352
5. Daponte A, Michail G, Daponte AI, Daponte N, Valasoulis G. Urine HPV in the context of genital and cervical cancer screening-an update of current literature. Cancers (Basel). 2021;13(7):1640. https://doi.org/10.3390/cancers13071640
6. Roche Molecular Systems 2010. Cobas 4800 HPV test, for in vitro diagnostic use: package insert. Pleasanton (CA): Roche Molecular Systems; 2020.
7. QIAscreen. HPV PCR test instructions for use (handbook). 2019. Available from: www.qiagen.com
8. Ducancelle A, Legrand MC, Pivert A, Veillon P, Guillou-Guillemette H, Brux MA, et al. Interest of human papillomavirus DNA quantification and genotyping in paired cervical and urine samples to detect cervical lesions. Arch Gynecol Obstet. 2014;290(2):299-308. https://doi.org/10.1007/s00404-014-3191-y
9. Hagihara M, Yamagishi Y, Izumi K, Miyazaki N, Suzuki T, Kato H, et al. Comparison of initial stream urine samples and cervical samples for detection of human papillomavirus. J Infect Chemother. 2016;22(8):559-62. https://doi.org/10.1016/j.jiac.2016.05.009
10. Lorenzi AT, Fregnani JHT, Dockter J, Fitzgerald K, Strohecker E, Eaton B, et al. High-risk human papillomavirus detection in urine samples from a referral population with cervical biopsy-proven high-grade lesions. J Low Genit Tract Dis. 2018;22(1):17-20. https://doi.org/10.1097/LGT.0000000000000352
11. Nilyanimit P, Chansaenroj J, Karalak A, Laowahutanont P, Junyangdikul P, Poovorawan Y. Comparison of human papillomavirus (HPV) detection in urine and cervical swab samples using the HPV GenoArray diagnostic assay. Peer J. 2017;5:5:e3910. https://doi.org/10.7717/peerj.3910
12. Bernal S, Palomares JC, Artura A, Parra M, Cabezas JL, Robles A, et al. Comparison of urine and cervical samples for detecting human papillomavirus (HPV) with the Cobas 4800 HPV test. J Clin Virol. 2014;61(4):548-52. https://doi.org/10.1016/j.jcv.2014.10.001
13. Lim MC, Lee DH, Hwang SH, Hwang NR, Lee B, Shin HY, et al. Comparison of the abbott real time high risk HPV test and the roche cobas 4800 HPV test using urine samples. J Virol Methods. 2017;243:74-9. https://doi.org/10.1016/j.jviromet.2017.01.020
14. Bober P, Firment P, Sabo J. Diagnostic test accuracy of first-void urine human papillomaviruses for presence cervical HPV in women: systematic review and meta-analysis. Int J Environ Res Public Health. 2021;18(24):13314. https://doi.org/10.3390/ijerph182413314
15. Pattyn J, Keer S, Biesmans S, Ieven M, Vanderborght C, Beyers K, et al. Human papillomavirus detection in urine: Effect of a first-void urine collection device and timing of collection. J Virol Methods. 2019;264:23-30. https://doi.org/10.1016/j.jviromet.2018.11.008
16. Smith JS, Smith JB, Faherty BR, Isaacson SC, Marais AC, Fakner A, et al. Effect of urine preservative on HPV testing results among urine samples. In: HPV 2017. Cape Town; 2017.
17. Nicolau P, Mancebo G, Agramunt S, Solé-Sedeño JM, Bellosillo B, Muset MM, et al. Urine human papillomavirus prevalence in women with high-grade cervical lesions. Eur J Obstet Gynecol Reprod Biol. 2014;183:12-5. https://doi.org/10.1016/j.ejogrb.2014.10.005