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Original Article

Concentrated urine as an alternative to cervical smear samples enabling easy screening of HPV in large populations

Neval Yurttutan Uyar [a], Harika Bodur [b], Merve Olcen Erdem [c], Irem Ayse Kanneci Altinisik [c], Ece Aksoy [d,e], Tuba Polat [d,e], Figen Demir [f], Osman Acar [g], Boran Aksakal [g], Gulnur Alizade [g], Narmin Nadirova [g], Alara Apa [g], Ozge Can [h], Tanil Kocagoz [a,d,*]

[a] Department of Medical Microbiology, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[b] Acibadem Altunizade Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkiye
[c] Anatolia Geneworks, Sultanbeyli, Istanbul, Turkiye
[d] Department of Medical Biotechnology, Institute of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[e] Bio-T Biotechnology Solutions and Production, Teknopark Istanbul, Istanbul, Turkiye
[f] Department of Public Health, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[g] School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[h] Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye

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ABSTRACT

Background: Human Papillomavirus (HPV) infection is a leading cause of cervical cancer, necessitating effective screening methods, particularly in large populations and resource limited settings. Current cervical smear-based screening faces challenges related to accessibility, invasiveness, and patient compliance. This study investigated the feasibility of using concentrated urine samples as a noninvasive alternative for HPV detection.

Methods: First-void urine samples from 126 patients were collected alongside cervical swabs. A biological fluid concentrator, MyMagiCon®, was used to concentrate the urine samples before HPV detection via RT-PCR.

Results: The results demonstrated substantial agreement (Fleiss' kappa = 0.796, $p < 0.0001$) between HPV detection in concentrated urine samples and cervical smear samples. Concentrated urine samples showed a 17 % increase in HPV detection compared to unconcentrated urine.

Conclusions: This noninvasive and novel approach offers significant advantages in terms of accessibility and patient acceptance, potentially improving screening coverage and early detection rates, especially in underserved populations. Further research is needed to validate these findings in larger, more diverse populations and optimize the methodology for enhanced sensitivity and specificity, but the findings suggest concentrated urine-based HPV testing holds considerable promise as a cost-effective, accessible screening strategy in preventing cervical cancer.

Introduction

Human Papillomavirus (HPV) is one of the most prevalent sexually transmitted infections globally and a critical factor in the pathogenesis of cervical cancer, responsible for approximately 99 % of cases [1–4]. People infected by high-risk HPV types are prone to developing precancerous lesions, which can progress to cancer [5]. Early detection of high-risk HPV types in cervical smear samples and treatment of dysplasia at precancerous stage, lowers the risk for developing cancer below 1 % and mortality rate from cervical cancer below 0.5 %. Therefore, screening for HPV in large populations is the key for the prevention of cervical cancer [6].

Despite significant advances in vaccination and screening programs, cervical cancer remains a major public health challenge, particularly in low- and middle-income countries where access to healthcare resources can be limited [7]. Traditionally, HPV screening relies on cervical cytology and HPV DNA testing using samples collected from the cervix, procedures that are effective but also invasive. These methods require a clinical setting for sample

* Corresponding author at: Department of Medical Microbiology, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye.
E-mail address: tanilkocagoz@gmail.com (T. Kocagoz).

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collection, which can pose barriers in terms of accessibility, privacy concerns, and cultural acceptability, potentially leading to lower participation rates in regular screening programs [8]. Traditional screening methods, primarily based on Pap smears and HPV DNA testing from cervical samples, have proven effective in detecting precancerous lesions and preventing cervical cancer. However, these methods, in certain settings, may present obstacles in terms of accessibility, acceptability, and patient compliance, especially in underserved populations [9].

The current widely used screening method for HPV is PCR that requires cervical smear sampling, which is accomplished by gynecological examination. In rural areas, a lack of hospital facilities and a shortage of medical personnel are the main issues interfering with the screening process. Even women, who have access to hospital facilities can be hesitant to participate in screening, since taking vaginal samples is kind of an invasive procedure. Some additional factors that may discourage women from getting the test done for screening purposes, other than socioeconomical status, are embarrassment of genital examination, fear or anxiety about the procedure's potential for discomfort and pain. Regarding the importance of early HPV detection, development of self-sampling methods is a key issue to screen large populations for prevention of cervical cancer [10].

In response to these challenges, there is a growing interest in developing noninvasive screening methods that can encourage broader participation and facilitate early detection of HPV infections. One promising approach is urine-based sampling for HPV testing, which offers a noninvasive alternative that could complement existing cervical screening programs. Urine samples can be collected easily and privately, making the process more acceptable to people, who might be hesitant to participate in more invasive procedures. This characteristic is particularly beneficial in expanding the outreach of HPV screening to underserved populations and enhancing compliance among individuals who might otherwise miss out on testing [11]. Urine sampling offers numerous advantages, including noninvasiveness, ease of collection, and the potential for broader reach, particularly among populations hesitant to undergo traditional pelvic examinations [6,10]. Although, having lower sensitivity, urine samples can present a considerable portion of HPV positive samples, making them a viable alternative for screening. Recent studies have underscored the potential of urine-based HPV testing, demonstrating its feasibility and accuracy in detecting high-risk HPV types responsible for cervical carcinoma [12]. HPV detection in urine samples has shown promising results regarding sensitivity and specificity compared to those achieved with traditional cervical samples [13]. Moreover, the use of self-collected urine samples can reduce the logistical burdens associated with organizing and conducting large-scale screening initiatives, thereby lowering healthcare costs and improving overall efficiency in resource limited environments [14]. Therefore, it is of paramount importance to develop superior ways of utilizing urine samples in HPV testing, especially by increasing the sensitivity of tests to reach the level of traditional smear sampling.

The potential of urine-based HPV testing not only enhances screening accessibility but also aligns with the goals of public health initiatives aimed at reducing cervical cancer incidence globally. Furthermore, integrating urine screening into existing healthcare frameworks may facilitate broader screening outreach and improve overall participation rates, particularly in low-resource settings, where traditional screening methods face logistical challenges.

In this study, we have investigated the efficiency of detecting HPV in urine samples, after concentrating by a biological fluid concentrator, *MyMagiCon®*, compared to cervical smear sampling. The polymer in a filter bag removes small molecules quickly from solutions. The elastic polymer beads swell quickly by absorbing water and other small molecules, concentrating microorganisms, and macromolecules. Microorganisms are concentrated if they are in intact form. Even if the organisms are lysed and their nucleic acids and antigens are released in the solution, these are also concentrated. Molecules larger than approximately 0.5 kDa stay outside the polymer beads, while small molecules that can penetrate the pores of the polymer meshes, are removed. Thus, *MyMagiCon®* concentrates the microorganisms and their macromolecules. During Covid19 pandemic, *MyMagiCon®* was used for concentrating gargle and mouthwash, which enabled the detection of SARS-CoV-2 by PCR with higher sensitivity in gargle and mouthwash compared to nasopharyngeal swab samples. This eliminated the disadvantages of nasopharyngeal swab sampling, which is a painful process and poses discomfort for patients and enabled better compliance [15].

If HPV can be detected from self-collected urine samples with a sensitivity comparable to smear sampling, this may make a prominent impact in early detection of HPV in large populations and in prevention of cervical cancer.

Materials and methods

Ethical approval was obtained from Acibadem University Ethical Committee (Approval no: 2022-06/33). All volunteers signed informed consent before giving urine samples. Three different sample types were analyzed for the presence of HPV using the Bosphore RT-PCR HPV test kit (Anatolia Geneworks, Istanbul, Turkiye) according to the manufacturer's instructions. A total of 378 samples were analyzed for the detection of HPV. This corresponds to 126 patient samples. First void urine samples have been collected from patients who have come to the hospital to give cervical smear samples and 20 mL was used for concentration with MyMagiCon®. All volunteers between 25 and 65 years old were included in the study. The study was carried out for one year between June 2022 and May 2023. Samples were stored at 4–8 °C until analysis within one day. On the day of analysis, the urine samples were concentrated using MyMagiCon® kits according to the manufacturer's instructions. MyMagiCon® is used as a concentrator for biological fluids (MyMagiCon UB-125, Bio-T Biotechnology Solutions and Production Co.). It absorbs molecules less than 0.5 kDa so that in the remainder concentrate there will be less volume, in which HPV gets concentrated. Briefly, 20 mL of urine sample was put in the concentration tube. Then, urine concentrator pouch was inserted into the tube, cap was secured, and the tube was inverted a few times to soak the whole pouch in the urine. Urine sample was concentrated in 5 min and the concentrate was collected for further PCR analysis.

To assess the test agreement among the screening tests, we calculated Cohen's kappa and Fleiss' kappa. Fleiss' kappa, also known as Cohen's kappa for multiple raters, is a statistical measure of interrater reliability or agreement when assessing categorical items with multiple raters or observers. It is an extension of Cohen's kappa, which is used for two raters (screening tests for this study).

Fleiss' kappa is a widely accepted measure of agreement for multiple raters (Fleiss, 1971). The three screening tests independently assessed a total of 126 categorical items (positive or negative) related to HPV. The interpretation of Cohen's and Fleiss' kappa values is as follows: values less than 0.20 indicate poor agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement, and 0.81–1.00 almost perfect agreement [16].

Cohen's kappa values were calculated using SPPS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp.). Fleiss' kappa was calculated by Python using an in-house code.

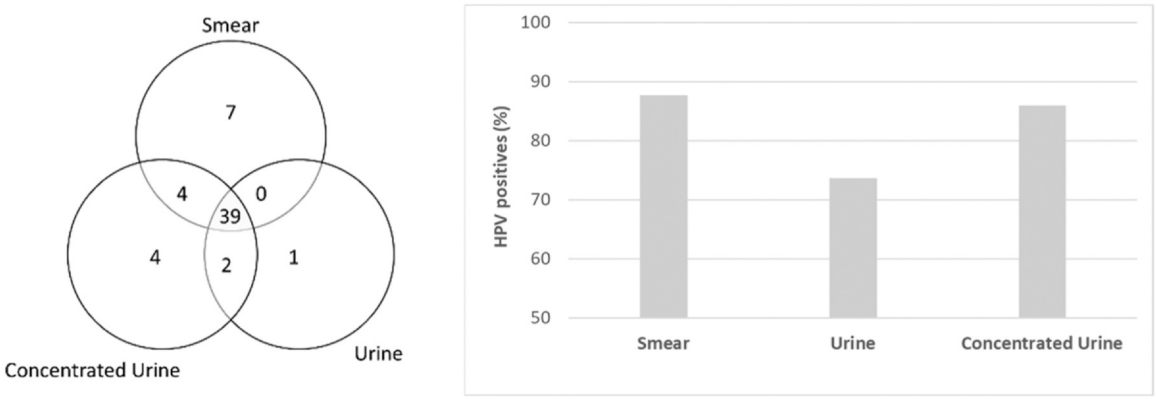

Fig. 1. Detection of HPV by RT-PCR in cervical smear samples, in urine samples before and after concentration by MyMagiCon® UB-125.

Results

In 57 samples, HPV was identified in at least one of the three sample types. The viral DNA was detected in 50 (87.7 %) cervical smear samples, in 42 (73.7 %) urine samples before concentration, and in 49 (86 %) after concentration among the total RT-PCR-positive patients (Fig. 1). In 7 patients, cervical swab samples, which were positive for HPV, were negative in their concentrated urine samples. 5 of these samples yielded C_t values in subtyping of HPV, suggesting that these are not false positives. On the other hand, HPV was positive in 6 concentrated urine samples, which were negative in cervical swab samples of the same patients. 5 of these samples yielded C_t values in subtyping of HPV, suggesting that these are not false positives. Concentration of samples increased the number of samples in which HPV DNA was detected by approximately 17 %. The effect of concentration on the detection of HPV DNA is shown in Fig. 1. There were no cases of cervical cancer among the study participants. One patient was diagnosed with cervical intraepithelial neoplasia (CIN 1) and HPV16 was detected in both the cervical smear and concentrated urine sample of this patient.

Fleiss' kappa was calculated as 0.796 with a p value of 0.0001. This suggests that the overall agreement of the results yielded from three sample types were substantial. When individual tests were investigated Cohen's kappa values were calculated along with the corresponding p values. When the cervical swab and concentrated urine samples were compared Cohen's kappa value was calculated as 0.784 ($p < 0.001$), which means there is substantial agreement between cervical swab and concentrated urine samples. Further, urine versus concentrated urine results were almost in good agreement yielding Cohen's kappa value of 0.846 ($p < 0.001$).

Since three different sample types yield results statistically in good agreement, they can be used interchangeably. Clinical significance of concentrated urine samples seems to be valuable especially in screening large populations. Our results also suggest that HPV vaccination does not have any meaningful relation on patients' samples being positive or negative. A total of 21 patients included in this study had been vaccinated, 9 of which came out HPV positive that is also statistically independent of previous HPV history.

More detailed clinical study on HPV screening is crucial to validate our initial clinical study and to implement this non-invasive, practical, cheaper and what seems to be almost equally sensitive urine concentration technology.

Discussion

This study investigated the efficacy of detecting Human Papillomavirus (HPV) in urine samples concentrated using MyMagiCon®, compared to standard cervical smear sampling. Our findings demonstrate a high degree of concordance between HPV detection in concentrated urine samples and cervical smear samples, suggesting that concentrated urine samples represent a viable alternative for large-scale HPV screening programs.

Evidence supporting the validity of urine-based HPV testing is continuously accumulating. Several studies have explored the efficacy of urine as a specimen for HPV detection, reporting promising sensitivity and specificity results comparable to cervical samples [17,18]. According to a recent clinical study results, high risk HPV could be detected in self-collected urine with similar sensitivity and specificity compared to clinician collected smear samples for CIN2+ cases [19]. Yang et al., have reported an overall agreement of 77.1 % between genital and urinary HPV DNA test results along with even higher agreements for HPV16 type (96.02 %) [20]. Lefeuvre et al., proposed that urinary self-sampling may be an alternative especially for women who are reluctant to have a Pap smear [11].

The substantial agreement (Fleiss' kappa = 0.796, $p < 0.0001$) observed across the three sample types (cervical smear, unconcentrated urine, and concentrated urine) analyzed in this study highlights the reliability and consistency of our results. The high Cohen's kappa values between cervical swabs and concentrated urine samples (0.784, $p < 0.001$) and between unconcentrated and concentrated urine samples (0.846, $p < 0.001$) further support the interchangeability of these sample types for HPV detection. The increased detection rate of HPV (approximately 17 %) in concentrated urine samples compared to unconcentrated urine samples underlines the importance of sample preparation using MyMagiCon® for enhancing sensitivity.

The noninvasive nature of urine collection offers significant advantages over traditional cervical smear sampling, particularly in addressing accessibility and acceptability challenges commonly encountered in HPV screening programs and among populations that are hesitant to go through invasive procedures. The ease of self-sampling improves patient compliance and reduces the logistical burdens associated with large scale screening programs. This is consistent with findings from other studies demonstrating the feasibility and accuracy of urine-based HPV testing [2,11,18,20]. The higher sensitivity achieved in concentrated urine samples using urine concentration technology further enhances the utility of this approach, potentially alleviating the lower sensitivity previously reported for some urine-based tests.

Our study was done using first void urine samples collected at any time during the day when the patients came to hospital for gynecological examination and cervical smear sampling. This may be a major disadvantage for the sensitivity of HPV detection from urine. Since HPV and HPV infected epithelial cells are expected to accumulate in vagina during the night, they are washed out in the first

void urine in the morning. Obtaining first void urine in the morning may increase the sensitivity of HPV detection which may further be augmented by concentration with MyMagiCon®. In addition, it is expected to have more HPV in cervical cancer cases. Therefore, the sensitivity of detecting HPV may be higher in urine samples from patients with cervical cancer, which is of significant importance for detecting early stage cervical cancer. In one of the patients CIN1 was detected along with HPV16 in cervical smear sample. It was also possible to detect HPV16 in concentrated urine sample. This is significant because this patient could have been diagnosed directly from a concentrated urine sample.

While our results are promising, further research is crucial to validate these findings in larger, more diverse populations and to refine the analytical methods to ensure optimal sensitivity and specificity. Longitudinal studies are needed to assess the long-term efficacy of urine-based HPV screening in preventing cervical cancer. Nevertheless, our data suggests that concentrated urine-based HPV testing offers a significant advancement in cervical cancer prevention by providing a noninvasive, easily accessible and cost-effective screening strategy with comparable accuracy with traditional methods. This strategy holds considerable potential for improving public health outcomes worldwide.

Conclusions

In conclusion, HPV testing in concentrated urine presents a promising alternative to traditional cervical smear screening methods. With advances in molecular diagnostics and public health strategies, concentrated urine-based HPV testing has the potential to enhance screening accessibility, improve patient compliance, and ultimately contribute to the reduction of HPV-related cancers. Continued research, education, and advocacy are essential to recognize the full potential of this innovative approach in different populations and healthcare settings, paving the way towards better management and prevention of cervical cancer.

Authors' Contributions

Neval Yurttutan Uyar organized the laboratory studies and analyzed the results, Harika Bodur was responsible of taking smear and urine samples from the volunteers, Merve Olcen Erdem and Irem Ayse Kanneci Altinisik provided support for PCR tests, Ece Aksoy and Tuba Polat did urine concentration and PCR tests, Figen Demir analyzed the results and did the statistical analysis, Osman Acar, Boran Aksakal, Gulnur Alizade, Narmin Nadirova and Alara Apa collected the patient data and did the coordination of the study between the hospital and the laboratory, Ozge Can and Tanil Kocagoz developed the urine concentration method, organized the research team and the study, analyzed the data and wrote the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Merve Olcen Erdem and Irem Ayse Kanneci Altinisik are the employers of Anatolia Geneworks. Tuba Polat and Ece Aksoy are the employers of Bio-T Biotechnology Solutions and Production. Ozge Can and Tanil Kocagoz are the founders and scientific advisors of Bio-T Biotechnology Solutions and Production. All the other authors declare no conflict of interest. HPV PCR kits were provided by Anatolia Geneworks and MyMagiCon® kits by Bio-T Biotechnology Solutions, free of charge. No other funding was available for the study.

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