Review Article

Screening for human papillomavirus: Is urine useful?

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Abstract

Persistent infection with high-risk Human papillomavirus (hr-HPV 16, 18, 31, 33, and 45) is the main risk factor for developing malignant genital lesions. Screening methods and follow-up schedules for cervical cancer are well known. A golden standard to screen and monitor men does not exist yet, because HPV-related, life threatening malignancies in men are rare. The importance of male HPV screening lies mainly in HPV vaccination. Young females are the target group for HPV, but men are considered to be the reservoir for HPV and to have a role in the perpetuation of the infection in the general population. We looked at the usefulness of urine as a tool for HPV screening. Pubmed was searched with the words "HPV", "Urine," and "HPV-DNA". The chance of finding HPV-DNA in urine is higher in men with lesions in the urethra than outside the urethra, and in women with abnormal cervical cytology. In general, the results of testing urine for HPV-DNA are better for women than for men, probably because of the anatomical position of the urethra to the vagina, vulva, and cervix. In both genders, urine HPV prevalence is higher in HIV^{pos} patients and in high-risk populations. Urine, to screen asymptomatic low-risk-profile (wo)men seems less useful because their urine samples are often inadequate. If urine proves to be the best medium to screen, a low-risk population remains controversial.

Key words: Human papillomavirus, screening, urine

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Introduction

Human papillomavirus (HPV) infection is a sexually transmitted infection (STI). Low-risk HPV (lr-HPV) 6 and 11 are responsible for genital warts. Persistent infection with high-risk HPV (hr-HPV 16, 18, 31, 33, and 45) is the main risk factor for developing genital (pre) malignant lesions.^[1] Co-risk factors are sex at an early age, promiscuity, alcohol and drug abuse, and lack of condom use.^[2] In women HPV-DNA-PCR on cervical scrapes is used to identify the HPV-type responsible for the detected lesions. The golden standard to screen men is still under research.

HPV Screening

Persistent infection with hr-HPV is the main risk factor for developing cervical, penile, and anal cancer. Cervical cancer is the second most common cancer in women worldwide and a significant cause of death. Cervical scrapes for HPV-DNA-PCR are more sensitive than cytology and have become the golden standard for primary screening and follow up of women.

Standardized screening of men has not been fully elaborated yet, because HPV-related, life-threatening malignancies in men are rare. However, literature about the best way to diagnose HPV in asymptomatic men is growing. Of late a combination of samples of the penile shaft and the glans penis/coronal sulcus, combined with a scrotal, perianal or anal sample was proposed.^[3]

This review looks at the usefulness of urine as a screening tool for HPV in both sexes. Urine would be an ideal sample for screening large populations and children, as well as for monitoring adolescents and adults. A urine test may increase participation and compliance, since physical scrapes, sometimes unpopular because of the dislike of physical examination or because of religious reasons, are avoided. Reliable screening kits using PCR-testing on the first voided urine (FVU) are available for other STTs, for example, Chlamydia trachomatis.^[4] HPV-DNA in FVU represents infected cells shed from the epithelium of the urethra/urethral meatus and from the cervix and vagina in women. The β -Globin concentration in a sample is the internal test for the amount of shed cells and for

the DNA quality. However, 6% of male samples were found to be positive for HPV-DNA, although negative for β -Globin.^[5] Efforts have been made to detect the presence of HPV-DNA in urine in the most reliable way, using liquid hybridization, conventional PCR, and real-time, PCR-based methods. Optimization of the technique was described in 2004.^[6] Different factors with a possible influence on the results were examined. The authors concluded that the presence of proteins in urine ameliorates amplification, while nitrites decrease amplification. Adding a dilution step and a concentration step before applying the Qiagen protocol increased the amplification of β -Globin (from 50 to 63%) and of the HPV L1 gene (from 13 to 33%). Refrigerating the specimens at four degrees Celsius overnight produced better amplification, versus immediate processing or versus freezing the specimen for 24 hours. Another two groups published good results with PCR and real-time-PCR (RT-PCR). Daponte examined 100 consecutive women referred to the colposcopy clinic with abnormal cervical cytology and normal urine parameters.^[7] Paired urine and cervical specimens were submitted for an in-house PCR method (HPV type 16 and 18) and a multiplex PCR method (HPV type 6, 11, 16, 18 and 33). He found a urine/cervix HPV detection sensitivity of 76.5% in cases with high-grade lesions and of 45.5% in cases with low-grade lesions. In all concordant cases the same HPV type was detected in both samples. The sensitivity enhanced when there were two or more epithelial cells per field, in urine microscopy. Two years later he confirmed his results by submitting the urine of another 100 cervical HPV-16-positive women to RT-PCR.^[8] Urine sensitivities for cancer, high grade lesions, and low grade lesions were, 93.3, 83.3, and 38.8%, respectively, with RT-PCR vs. 86.7, 72.2, and 32.7% with conventional PCR. The mean viral load in urine was significantly lower than in the cervical swab. Payan had similar results using RT-PCR on cervical (n = 333) and urine (n = 177) samples of women referred for gynaecological examination and PAP-smear.^[9] Respectively, 45 and 37% of the samples were HPV positive: the latter with a significant 50-fold-lower mean viral load.

When using urine for HPV-screening in asymptomatic women, the results vary depending on the chosen population. In a population of sexually unexposed college girls only six out of 100 urine samples tested positive.^[10] In the USA 3262 sexually active women, aged 18 to 25 years, were included in the WAVE III Study (National Longitudinal Study of Adolescent Health), and each produced a urine sample.^[2] Overall, HPV prevalence was 26.9%, and 14.3% in women with one lifetime partner. High-risk types were detected

in 20%, of which 10% were infected with types covered by the current vaccines. Jacobson found any HPV in 90% of cervical scrapes and in 75% of urine samples in a group of 80 sexually active Afro-American adolescents.^[11] These last two studies show that urine may be a good tool to screen a mass population.

Overall, the results of detecting HPV-DNA in FVU are better in women than in men, probably because of the anatomical position of the urethra to the vagina, vulva, and cervix. Sellors described that the concordance between cervical specimens and the vaginal, vulvar, and urine specimens for the presence of HPV was 0.76, 0.55, and 0.41, respectively.^[12]

Human papillomavirus prevalence increases when abnormal cervical cytology and/or HIV-positivity is involved. Forslund found that 65% of the urine samples of women with HPV^{pos} cervical scrapes were HPV-positive, while 85% of the patients with HPV-DNA^{pos} urine also had HPV-DNA in their cervical scrapes.^[13] Vossler found additional value in urine testing in women with cervical dysplasia.^[14] The same good results were observed in Korea when urine and cervical swabs of women with cervical lesions were matched: HPV-DNA was found in 70 / 100 cervical samples and in 47 / 90 urine samples.^[15] Type-specific agreements were good for HPV 16, 18, 52, and 58. Similar results were seen in Greece,^[7] in patients with HPV-16-infection, and in Zimbabwe,^[16] in women who had invasive cervical cancer. Fambrini successfully used urine for the follow up of women after conization of their cervical lesions; this resulted in 96.6% concordance between cervical scrapes and urine.^[17] Powell found that young girls (children) with Lichen Sclerosus (LS) and non-LS-related vulvar pathology had a higher chance of intermediate and high-risk-HPV than girls with no known vulvar disease.^[18] Recent studies showed HPV prevalence in HIV^{pos}-women's urine and cervical smear samples, of 48-81.5 and 51.9-58%, respectively.^[19,20] Concordance between the samples was 71% in both studies.

These days highly effective vaccines for HPV type 6, 11, 16, and 18 are available, covering about 70% of the cervical cancers. By partial cross-protection against HPV 31 and 45, this could rise to 76%.^[21] The vaccines are most preventive when given before the first sexual encounter (age < 11 years). Although young females are the main target group for this vaccination, one could consider vaccinating men as well. A counter-argument is that men already profit from herd immunity when all young women are vaccinated. An argument pro male vaccination is aiming to protect all sexually active people. If an individual is infected with one HPV

type, vaccination will protect him against the other three types. The present vaccines protect men also from HPV types 6 and 11. We expect that the same will happen for HPV types 16 and 18, but this is still hypothetical. Furthermore, recent data indicate that males experience a longer duration of genital warts than women, which leads to greater treatment costs. Widespread use of HPV-vaccines can reduce the workload at STI clinics by approximately 10%, without a substantial impact on the diagnosis and treatment of other STI's.^[22] However, at present there is no treatment for asymptomatic high-risk men. A screening test capable of detecting subclinical HPV infections in males would be of value, should effective therapy be discovered.

In general, urine samples to detect HPV in asymptomatic men are less useful. Hillman conducted several studies on HPV-DNA in urine of men. He examined men with penile warts, genital gonorrhoea, and genital dermatoses: few positive samples were found. There was no relation between visual lesions and HPV positivity.^[23-25] Geddy tested urine sediments of 73 men attending the STI clinic; among them were 14 patients with genital warts that did not involve the urethral meatus.^[26] He did not find HPV-DNA in any of the specimens, while human β -Globin was identified in 40 out of the 73 samples. He concluded that urine is unhelpful for studying the prevalence of urethral HPV infection in men. Fife examined men visiting the STI polyclinic and found a low sensitivity for HPV in urine, mainly because of the inadequacy of the sample material.^[27] Melchers on the other hand detected HPV-DNA in 15 out of 17 male patients with condylomata accuminata in the urethral meatus.^[28] Iwasawa examined the urine of 29 patients with urethral condylomata, three patients with penile condylomata, and 15 control patients.^[29] HPV-DNA was found in 76, 0, and 0%, respectively. He found additional value in testing the urine for HPV screening of the male genital tract. Smits compared the urine of 114 HIV^{pos}-men and 115 HIV^{neg}-men.^[30] The prevalence of HPV in HIV^{pos} patients was 39.4%, being 81.4% high-risk type. In the HIV uninfected men 9.6% of the samples tested HPV positive.

Urine sampling for detection of HPV in women and their male partners is controversial. Nakazawa detected HPV-DNA in 2 / 8 urine samples of male partners whose wives were HPV positive.^[31] A few years later Astori collected urine and urethral swabs of 70 asymptomatic male partners of HPV-DNA positive women.^[32] He used the Dot blot Technique as well as PCR. PCR gave better results than the Dot blot; nevertheless 87% of the urethral swabs were inadequate and 21% of the urine was inadequate. In

Argentina, urine of men presenting with penile lesions and of men with HPV positive partners was examined with nested PCR.^[33] Urine tested positive in 79.5 and 68.8%, respectively. Eleven percent had a double infection. In India cervical scrapes and urine of 30 healthy women and of 30 women with cervical cancer were compared with their husbands' penile scrapes and urine.^[34] All groups showed a similar frequency of HPV infection both in urine and scrape samples, but there was a significant difference in the prevalence of high-risk type 16 in women with cervical cancer and their male partners. Our own study, where we tested 31 FVU of healthy females and 30 FVU of healthy men, showed 71 and 66.7% adequacy, respectively.^[35] HPV-DNA was found in 3.2% of female FVU. Our second study (unpublished observations), which included asymptomatic men, showed 20% adequacy, so we stopped urine testing - supported by the article of Giuliano.^[3]

Conclusion

Urine is an easy, self-obtainable sample, which could be ideal to screen for HPV, if reliable. Earlier articles mention a varying prevalence of HPV in urine, depending of the screening population. Recent articles find a high prevalence of HPV, but mainly in high-risk populations. Urine will probably become a diagnostic tool for follow up of women treated for (cervical intra-epithelial neoplasia) CIN / HSIL (high-grade squamous intra-epithelial lesions), for HIV^{pos} women, and for detecting the HPV-type in a high-risk (a)symptomatic male population; for example, partners of women with HR-HPV related lesions, male prostitutes, HIV^{pos} men, men repeatedly having unprotected sex with different (wo)men. Urine can be kept in mind for screening of mass population. Whether urine is the best medium for specific screening of asymptomatic low-risk men is questionable.

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