Current Infectious Disease Reports (2019) 21: 13
https://doi.org/10.1007/s11908-019-0674-9

FEMALE GENITAL TRACT INFECTIONS (J SOBEL, SECTION EDITOR)

Self-Collection and Molecular Diagnosis for Detection of Human Papillomavirus: Why Incorporate It?

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Published online: 19 March 2019

Abstract

Purpose of Review Cervical cancer, the third cause of death by cancer among Brazil's women, is associated with human papillomavirus (HPV) infection. In some countries of South America, North America, Europe, and Oceania, initial screening for HPV DNA and subsequent follow-up with HPV-positive patients using colposcopy and cytological testing are used as preventative measures.

Recent Findings For HPV DNA detection, it is necessary to obtain cervical cells by conventional clinical collection method or self-collection of the cells that flake off from the uterine cervix and vaginal canal. Self-collection has been shown to be a viable option for obtaining samples and is a less invasive method that is more accepted by women. Thus, it can potentially decrease the limitations of the conventional clinical collection methods.

Summary The efficiency of the self-collection method aligned with the implementation of HPV molecular testing, if adopted by public and private health care systems, may extend the reach of current cervical cancer prevention efforts. In addition, considering all phases from triage to treatment, this method may reduce health care costs and the time spent by patients and health care teams to conduct examinations and collect samples.

Keywords Cervical cancer · HPV · Prevention · Diagnosis, self-collection method · Molecular testing

This article is part of the Topical Collection on *Female Genital Tract Infections*

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Introduction

Cervical cancer is the fourth most common type of cancer among women worldwide. A study carried out in 2015 revealed the occurrence of 528,000 new cases with an incidence rate of 14 per 100,000 women, where most patients (70%) were living in areas with poor human development [1]. Incidence rates vary between regions and countries. The African continent is a high-risk region, with incidence varying from 30.6 to 42.7/100,000. Regions that exhibit the lowest incidences are New Zealand (5.5/100,000) and Western Asia (4.4/100,000). Among the Americas, the Caribbean and Latin American regions combined have the highest incidence rate at 23.4/100,000 inhabitants, while the lowest incidence rate is in North America with 7.1/100,000 inhabitants. In Brazil, the incidence is 15.85/100,000 inhabitants. As for deaths, 5727 women in Brazil died of cervical cancer in 2015, and it is estimated that worldwide, 265,000 deaths occur each year [2••].

Human papillomavirus (HPV) is the main etiological agent causing neoplastic changes in the uterine cervix. HPV infection can be potentiated by viral persistence and load, patient's immunological condition, and the presence of other microbial infections, increasing the risk of progression to cervical cancer. Pathogens such as *Chlamydia trachomatis*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, and other microorganisms that are sexually transmitted or those even common to the cervical microenvironment can facilitate or aggravate HPV infection [3, 4].

In developing countries, gynecological follow-up of women occurs annually or every 6 months, during which cytology tests are used to identify neoplastic changes and other infections. To this end, during the medical consultation, health professionals collect uterine cervix samples using a vaginal speculum. However, the required infrastructure, the low reproducibility of the screening program, and the need for repeated and regular examinations are limitations of this prevention scheme. In programs that collect a high number of samples daily, substantial errors may occur [5, 6]. With regard to cytology tests, results indicate an HPV infection only when cellular changes associated with the condition are observed. However, the absence of cellular changes does not exclude the existence of an ongoing HPV infection. Alternatively, other methods may be used, such as HPV DNA screening.

Some developed countries adopt HPV DNA detection as an initial screening method, with HPV-positive patients referred for colposcopy and cytological testing [7•] while HPV-negative patients are re-examined for HPV DNA in intervals of up to 3 years. For HPV DNA detection, cells must be obtained from the uterine cervix, which can be performed by conventional clinical collection or self-collection of the cells that flake off from the uterine cervix and vaginal canal. The inclusion of self-collection as an HPV detection method is an option for addressing the limitations of conventional clinical collection, especially as self-collection is considered a less invasive method and is well accepted among women [6, 8]. In addition, the introduction of molecular tests for HPV detection can be a promising strategy to increase the coverage and efficiency of cervical cancer prevention efforts.

Self-Collection of Gynecological Samples

Self-collection is a method for obtaining cervical samples and is carried out by the patient herself without the need of a professional's assistance [9] or without having to travel to a health unit. Women can follow the guidelines of printed material and the health assistant may provide verbal instructions, but the patients collect samples in privacy [10].

Self-collection is carried out using a cervico-vaginal swab, cervical brush, or cervico-vaginal washing buffer. A swab is a device that consists of a shaft with a small piece of soft-tipped hygroscopic material. The swab is provided in a dry form in a plastic tube or in a wet form in a tube containing 1 mL of liquid transport medium (ESwab®, Copan, Brescia, Italy). Both dry and wet swabs show good concordance (85.7%) in their sample results and ability to maintain specimen integrity [11]. However, there are some limitations of using swabs as a self-collection tool. There is a greater chance of microscopic sample contamination with blood, which can alter the results of molecular analyses. Moreover, the samples would need to be maintained under refrigeration until processing [6].

The endocervical brush is the most used device for self-collection. Similar to the swab, the brush mainly collects cells that easily detach from the cervix and vaginal walls. This device is flexible, easy to use, and it can be maintained and transported under dry conditions [5, 9]. The quantity of cells collected is at least three times lower than that obtained through self-collection with swab, but is sufficient for molecular tests. The limitation of this device is that the samples contain mainly vaginal cells; this could potentially influence molecular test results, as the cervix and vaginal canal are distinct sites though both are subject to HPV infection [6].

Cervico-vaginal wash buffer is a solution that is introduced into the vagina. Cervico-vaginal wash has the advantage of covering a greater surface area and collecting a high volume of sample, which can be fractionated for various tests. The collection device uses an irrigation syringe, a disposable female urine catheter and a container with 15 mL of sterile phosphate-buffered saline solution [12]. The limitation of using the cervico-vaginal wash is the potential concern a patient may have with the opaque appearance of the irrigation fluid. Moreover, the samples obtained from a cervico-vaginal wash need to be diluted prior to processing, which reduces experimental sensitivity. Another main disadvantage is that this type of sample is not easily transported to the laboratory [6].

Considering the advantages and disadvantages described thus far [5, 6, 9], the use of the cervico-vaginal brush would be the most suitable for self-collection, mainly in communities difficult to access, such as Indian villages, and agricultural and riparian communities. The advantages for use in these communities include easier transportation of the samples to the laboratory for processing, lower costs, and the convenience of the collection method.

Studies demonstrate that self-collection with the endocervical brush has shown good concordance with conventional collection regarding high acceptability and cost-benefits [5, 13]. There have been many questions regarding whether HPV DNA detection in self-collected samples is efficient as the primary method for the prevention of cervical cancer. In meta-analysis carried out in 2007, the accuracy of clinical collection and self-collection in identifying women with HPV genital infection was verified, and both methods performed equally [14]. In a study carried out by Campos et al. [15], samples obtained by self-collection were found to

be viable for HPV DNA detection with high concordance of viral genotypes.

The acceptability of the self-collection method has been extensively evaluated in developing countries. Women participants in the study by Igdibashian et al. [8] stated that self-collection was easier to carry out and that it was not uncomfortable or painful [6]. Self-collection seems to be the most attractive option for obtaining samples for HPV DNA detection because it dispenses the use of the speculum, dispenses the presence of a health care professional, or a visit to the doctor's office for material collection. In addition, it is adequate for residents of distant regions, residents of localities where health units are difficult to access or medical care is provided by mobile units and for patients in general who will have the convenience of not seeking assistance at a health unit until they are diagnosed.

It is understood that self-collection can extend screening coverage because women who feel inhibited, are afraid of clinical collection, have cultural restrictions or do not understand the relevance of screening could benefit from its practicality [16]. In developing countries and remote regions, it is more difficult to visit the clinics, limiting the adherence to prevention programs that include the cervical cancer screening process. As an alternative strategy, the use of self-collection to obtain cervical samples for viral DNA detection would widen the reach of cervical cancer screening.

We have to consider that many women do not know the anatomy of their own bodies and that broad guidance with simple vocabulary and figures illustrating the method will become necessary for ensuring adequate self-collection. If the patient does not comprehend how the collection must be performed, there may be submission of insufficient or even improper sample for laboratory analyses.

Human Papillomavirus Diagnosis

The morphological alteration of cervical epithelial cells is observed through histopathological testing. Applied since the 1950s in the USA, this examination is responsible for a noticeable global reduction in cervical cancer cases. Cervical cancer is still one of the most common cancers worldwide, due to the structuring of prevention programs and cytological testing limitations [17].

In Brazil, the cytology test is the standard method for cervical cancer screening. However, this technique is based on the observation of cytological changes that may be related to neoplasia and/or HPV infection. Unlike molecular tests, it does not detect viral genetic material. In Canada, Chile, Denmark and some European countries, initial screening is carried out by detection of HPV genetic material. If the patient tests positive for the presence of HPV DNA, she will be referred for cytology and colposcopy and monitored biannually in an outpatient facility with a physician that specializes in HPV infections [6, 7•].

This approach aims to monitor the evolution of infection, since approximately 50% of HPV-infected women are able to eliminate the virus via an effective immunological response. Patients testing negative for the presence of HPV DNA at the initial screening are directed to repeat the molecular test every 2–3 years depending on the local screening program [18•].

The implementation of molecular tests for HPV DNA diagnosis is directly associated with early intervention in the appearance of the lesion, contributing to the reduction of the cervical cancer diagnosis and mortality rates. The molecular test is the most objective HPV screening technique with the advantages of greater replicability, easy training, and quality assurance. Besides HPV detection, molecular tests also enable detection of other pathogens by using the same sample. Such pathogens, when present in the microenvironment, can propagate infection through microlesions generated in the epithelium, or change the immunological response profile to facilitate the evolution of HPV infection [3].

Conclusion

The implementation of molecular testing for HPV DNA screening in the public health service was provided in the 2011–2015 agenda of the Health Surveillance Secretariat from the Brazilian Ministry of Health and in determinations of the Brazilian Decree n° 7508/2011, but such implementation has not occurred nationwide. The opinions against implementing molecular tests as a screening method for Brazilian women are based on the high cost of the test and the lack of professionals trained for performing the analysis. With regard to costs, HPV molecular tests have been demonstrated to be more effective and at lower cost for the public system when compared to cytology tests, resulting in the reduction of the average lifetime costs per woman [19]. Furthermore, there is a considerable decrease in cost considering the evolution of molecular methods over the last few years along with the guidance to re-evaluate patients with negative test results every 2–3 years instead of annually investing in cytology tests for the entire female population.

As for the lack of skilled professionals, this is being addressed through the dissemination of the technique among researchers, professional training opportunities developed by the government, and increasing vacancies in the health courses in technical, higher education, and graduate programs [6, 20].

The efficiency of the self-collection method along with the implementation of HPV molecular testing, if adopted by public and private health systems in developing countries, may expand the current prevention coverage for cervical cancer, reduce time spent by patients, and health care teams for sample collection. Considering all phases of the process from

triage to treatment, costs may be reduced up to US$ 2,000,000 by year per life saved [19, 20]. Moreover, it may be possible to identify critical regions with high incidences of HPV infection, prioritize access to information on the prevention, and care against HPV infections in the populations of these areas, ultimately resulting in the decrease HPV global incidence through early detection.

Following the implementation and consolidation of molecular testing for HPV DNA screening in the public health service, it is expected that the Ministry of Health, by means of the Department of Surveillance, Prevention and Control of Sexually Transmitted Infections, makes available technical guidance documents as "Technical Manual for Diagnosis" or "Clinical Protocols and Treatment Guidelines" for diagnosing HPV infections.

Compliance with Ethical Standards

Conflict of Interest Larissa Zatorre Almeida-Lugo, Camila Mareti Bonin-Jacob, Vanessa Terezinha Gubert de Matos, and Inês Aparecida Tozetti declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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