The clinical utility of HPV DNA testing in cervical cancer screening strategies

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Cervical cancer continues to be the commonest cause of death among women in developing countries, largely due to the failure to sustain effective cytology-based screening programs. While this burden may come down following implementation of the human papillomavirus (HPV) vaccine, screening will still be required. HPV DNA testing is a promising new technology for cervical cancer prevention and is the most reproducible of all cervical cancer screening tests. Presently, the two assays most widely used for the detection of genital types are the polymerase chain reaction (PCR) and Hybrid Capture 2 assays (hc2). Rapid, affordable tests are expected to be available soon. HPV DNA testing can be used in a variety of clinical scenarios that include primary screening in women older than 30 yr; as an adjunctive test to cytology; in the triage of women with an equivocal cytologic report, e.g., ASC-US; or for follow-up post-treatment for cervical intraepithelial neoplasia (CIN). HPV DNA testing can also be performed on self-collected samples, which allows screening in remote areas and also in women who refuse gynecologic examination.

Key words Cervical cancer - cervical intraepithelial neoplasia (CIN) - HPV - screening

Introduction

Cervical cancer remains a leading cause of cancer death among women living in developing countries. The benefits of cytologic screening have been known for the last three decades, yet there are an estimated 132,000 new cases and 74,000 die of this preventable disease in India each year. The necessary resources, infrastructure and technological expertise, together with the need for repeated screening at regular intervals make cytologic screening difficult to implement in low-resource countries. The human papillomavirus (HPV) prophylactic vaccine against types 16 and 18 has the potential to eliminate up to 70 per cent of cervical cancers, depending on the coverage and acceptance of the vaccine. However, screening will have to continue to deal with cancers caused by non-vaccine types as well as for women already infected prior to vaccination.

Two advances in recent years have been the development of affordable and effective alternative screening tests based on visual inspection techniques and the attempt at reorganization of programs. However, visual inspection techniques have their own limitations, mainly because of poor specificity. The development of a rapid and affordable test for HPV DNA makes this a viable alternative to cytologic screening. This is particularly important for countries that have not begun to invest resources and effort in establishing cytology-based screening programs.
HPV testing

Cervical cancer has been recognized as a rare outcome of a common, sexually transmitted infection. Persistent infection with high-risk oncogenic HPV types is a necessary cause of cervical cancer\textsuperscript{4,5}. With optimal testing systems, HPV DNA can be identified in nearly all specimens of invasive cervical cancer and in the vast majority (>95\%) of the immediate cervical cancer precursors, namely high-grade squamous intraepithelial lesions (HSILs) - also known as cervical intraepithelial neoplasia 3 (CIN 3) or carcinoma in situ\textsuperscript{6-8}.

Techniques

HPV cannot be grown in culture and detection of the virus relies on a variety of techniques used in immunology, serology, and molecular biology. Presently, the two assays most widely used for the detection of genital types are PCR with generic primers and the Hybrid Capture 2 assay (Digene Corp, Gaithersburg, MD, USA). The PCR-based assay is based on target amplification and care must be taken to avoid contamination. Its advantage is that it allows identification of different types of HPV and can discriminate between multiple infections. However, it is expensive, time-consuming and laborious and essentially a research tool, not suited to be applied as a mass screening test.

The Hybrid Capture assay (hc2) is a batch test based on hybridization in a solution of long synthetic RNA probes. Probe B is complementary to the genomic sequence of 13 high-risk types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68) while probe A measures 5 low-risk (6, 11, 42, 43, 44) HPV types. However, HPV testing is currently more expensive than other screening tests and requires sophisticated laboratory infrastructure that includes testing equipment, storage facilities for samples, and trained technicians.

The new rapid test developed by Digene Corp is a modification of the hc2 test that will measure 14 high-risk types, with the addition of HPV-66. It requires only basic laboratory tools, can be set up easily in the field and can be easily taught to health workers. This test has shown good concordance with the previous test and has the maximum potential to be applied as a population screening tool\textsuperscript{9}.

Comparison with routine cytology

A meta-analysis of studies from the United Kingdom (HART, Hammersmith), France (Reims), Germany (Hannover and Tuebingen, Jena), the Netherlands (Amsterdam) and Canada found that the sensitivity for detection of CIN 2+ disease by HPV testing was 96 per cent and by cytology was 53 per cent, while the specificity was 92 and 97 per cent respectively\textsuperscript{10}. The performance of HPV testing was found to be similar in different areas of Europe and North America. However, the sensitivity of cytology was highly variable in different countries.

In addition, testing for high-risk types of HPV DNA (hrHPV) has a very high negative predictive value (NPV), with most studies reporting values greater than 99 per cent and some reporting 100 per cent\textsuperscript{11}. The high NPV for hrHPV has important implications for screening programs. First, screening intervals may be significantly increased in women older than 30 yr who have tested negative hrHPV as the risk of these women developing cervical cancer over a 5- to 8-year period is negligible. Secondly, when combining HPV DNA testing with cytologic testing, women who test negative with both methods may receive a very high level of reassurance that they will not be at risk for cervical cancer for a long time, perhaps the next 10 years.

The advantages of HPV DNA testing as a screening test compared with cytologic evaluation or visual inspection of the cervix are as follows: (i) Its higher sensitivity is particularly important in settings where women will be screened only once or twice in their lifetimes. This has been seen with the new, rapid HPV test as well\textsuperscript{12}. (ii) HPV DNA testing not only identifies women with cervical disease but also those at risk for developing CIN within the next 3 to 10 years. This is particularly important for developing countries that might not have sufficient resources to screen all women at 5- to 10-year intervals, but might have the resources to screen a small subset of high-risk HPV DNA-positive women at more frequent intervals. (iii) The interpretation of the test is objective and does not have the inherent subjectivity of visual screening methods or cervical cytology.

Clinical utility of HPV testing

HPV DNA testing can be incorporated into screening programs in different ways, as follows:

1. HPV DNA testing as a primary screening test

In some studies, up to 70 per cent of college-going women are found to be HPV DNA positive that is usually transient\textsuperscript{13,14}. The rate of HPV positivity after the
age of 30 years declines considerably as the infection is cleared spontaneously in about 90 per cent of women. In developing countries, rates of HPV DNA positivity in women older than 30 yr vary from 6 to 18 per cent. Therefore HPV screening should be initiated after the age of 30 yr in order to exclude transient positive cases, which increases the cost-effectiveness and also saves the woman from unnecessary anxiety. HPV testing in women older than 30 yr has an average sensitivity and specificity of 89 and 90 per cent with NPV greater than 97 per cent. Women detected to be positive on HPV testing can then undergo visual inspection with acetic acid (VIA) or colposcopy, with directed biopsy, for confirmation of disease and appropriate management.

A comprehensive assessment of the cost-effectiveness of cervical cancer screening strategies was conducted in 5 countries with differing epidemiological profiles but where conventional cytology screening programs had not been sustainable. In all 5 countries the lifetime cancer risk was reduced by approximately 25 per cent with a single lifetime screening of women aged between 35 and 40 yr and consisting of either a 1-visit VIA or a 2-visit HPV testing; and it was found to be reduced by nearly 50 per cent with strategies targeting women 3 times per lifetime with spacing of about 5 years.

II. Combined screening with HPV DNA plus cytology

When HPV DNA testing and cytologic testing are done in parallel, the high negative predictive value means that women who test negative with both methods receive a very high level of reassurance that they will not be at risk for cervical cancer for a long time. The algorithm for management of cytology plus HPV DNA testing is shown in the Fig.

III. HPV DNA testing in the triage of equivocal (ASC-US) or low-grade (LSIL) cytologic findings

The ASCUS/LSIL Triage Study (ALTS) trial is a large, multicentric, randomized trial specifically designed to evaluate three methods of managing women with cytologic findings of ASC-US (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesion) designed to evaluate three methods of managing women with cytologic findings of ASC-US (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesion). (i) immediate colposcopic examination for all women; (ii) HPV testing and referral for colposcopy if the HPV test result was positive; and (iii) repeated cytologic assessment with referral for colposcopy if the smear showed the presence of HSIL (high-grade squamous intraepithelial lesion). Approximately 80 per cent of the women who had a cytologic diagnosis of LSIL were found to harbor HPV DNA. The high rate of HPV positivity among women found to have LSIL on cytologic evaluation significantly undermined the ability of HPV testing to discriminate between clinically non-significant cytologic abnormalities and abnormalities representing true cervical cancer precursors. The study concluded that HPV testing was not of value in the management of women found to have LSIL on cytologic evaluation. The American Society for Colposcopy and Cervical Pathology (ASCCP) recommends that these women undergo colposcopy instead of HPV testing.

In the case of women found to have ASC-US on cytologic evaluation, the ALTS trial found that HPV testing detected 96.3 per cent of women with previously undiagnosed CIN 3 or cancer and resulted in the referral of only 56.1 per cent of women for colposcopy. This would significantly reduce referral to colposcopy, particularly if the management strategy is to perform colposcopy on all women found to have ASC-US on cytologic evaluation. ASCCP consensus management guidelines for the follow-up of women with ASC-US include repeated cytologic assessment, immediate colposcopy and HPV testing as options. However, if liquid-based cytology (LBC) was used for the initial cervical smear, then reflex HPV testing (that is, using the residual fluid in the LBC sample for HPV testing if the cytologic diagnosis is ASC-US) is the preferred option, as it makes a second clinic visit unnecessary.
IV. HPV DNA testing for follow-up post-treatment

Ablative or excisional techniques for the treatment of cervical cancer precursors are generally reported to be very effective, with cure rates up to 95 per cent reported. However, in approximately 5-15 per cent of cases, the precursor lesions may persist or recur. In addition, treated women remain at increased risk of cervical cancer for at least 8 years compared to the general female population. HPV testing has recently been investigated as an alternative to two diagnostic modalities (cytology and colposcopy) for the detection of persistent or recurrent disease. If HPV DNA is undetectable 6 to 8 months post-treatment, the likelihood of post-treatment persistence or recurrence of disease is negligible. After combining the results of 10 studies of post-treatment HPV testing, Lorincz estimated the sensitivity, specificity, and NPV of HPV testing for the post-treatment detection of CIN 2/3 to be 96.5, 77.3 and 98.8 per cent, respectively.

V. HPV DNA testing of self-collected vaginal samples

A number of methods have been used, including cervical scraping/brushing, cervicovaginal lavage and vaginal tampons. Evidence shows that HPV tests using self-collected samples are at least as sensitive for detection of preinvasive high-grade lesions and invasive cervical cancer as the Pap test. We have found a 94 per cent concordance between self- and physician-collected samples. The women collected these samples in an unsupervised manner or with help from a trained health worker. In contrast, self-collected cytology samples are not an effective substitute for conventional cytology screening, with a major limitation of decreased sensitivity for the detection of dysplastic lesions. These data suggest that, in settings where cytologic screening is not available or where women are reluctant to undergo a gynecologic examination, HPV DNA testing from self-collected samples may be useful for identifying women at risk for cervical disease.

<p>| Table. Comparison of self-collected HPV with clinician obtained HPV and conventional cytology |</p>
<table>
<thead>
<tr>
<th>% Self-collected HPV (sensitivity)</th>
<th>% Clinician-obtained HPV (sensitivity)</th>
<th>% Cytology (sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al, 2000</td>
<td>66</td>
<td>84</td>
</tr>
<tr>
<td>Belinson et al, 2003</td>
<td>87.5</td>
<td>96.8</td>
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</tbody>
</table>

Conclusions

The HPV vaccine can be an important tool for prevention of cervical cancer. Timely administration before sexual debut will allow control of at least half the cancers. The optimum method of screening for the remainder continues to pose a challenge in India. Conventional cytology has immense infrastructural and economic requirements, VIA and VILI lack specificity and quality control is a challenge. The rapid HPV test that will be provided at an affordable price to developing countries holds tremendous promise. It can be used as a primary screening method to select patients at risk of disease and allow limited resources to be targeted where they are most needed. A combination of HPV vaccination and HPV-based screening may help control cervical cancer in India.

References


