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The Application of Human Papillomavirus Testing to Cervical Cancer Screening

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Although cytologic screening has considerably reduced the incidence of cervical cancer, there are some problems which remain to be solved, such as the low sensitivity of this procedure. HPV testing is fundamentally different from conventional cytologic testing, because it evaluates the HPV infection itself, the most important causative factor for cervical cancer. In this study, the roles and clinical applications of HPV testing in cervical cancer screening are examined from 3 standpoints: in primary screening, in the management of women with low-grade cytologic abnormalities, and in the follow-up after treatment of pre-invasive or early invasive lesions.

Key Words: Cervical cancer, HPV testing, screening

INTRODUCTION

Invasive cervical cancer is one of the most common malignant tumors of the female genital tract and a leading cause of cancer mortality in women in developing countries. However, in developed countries, the incidence of cervical cancer has been substantially reduced, mainly due to the introduction of well-organized cytology-based screening programs for cervical cancer. It is suggested that potential reductions in cervical cancer incidence of 60-90% are possible in the first three years following the implementation of a screening program.[1,2] However, a recent study found that 47% of fully invasive cancers in women under the age of 70 occurred in spite of an apparently adequate screening history, thus showing the limits of the cytologic screening test.[2] Although cytologic screening for cervical cancer has the merit of being convenient, reasonably specific and inexpensive, it also has certain disadvantages such as processing problems (inadequacy of specimen collection and fixation), difficulties in the follow-up and management of abnormal tests, and trouble in interpreting the results (false negative results). Walker et al. reported that out of 93 women who had been diagnosed as having cervical cancer, only 26 (28%) women had undergone a cervical smear at any time before the diagnosis of invasive cancer. Among these women, 15 (60%) had had a smear taken which was reported as being negative within the previous 5 years, and six (6%) within the previous year. When eleven of these 15 slides were subsequently reviewed, three (20%) were regarded as positive (false negative) and three (20%) were re-classed as too scanty for conclusive assessment.[3] False negative rates have been reported as being in the range of 5-50%, depending on the medical center involved.[4-6]

Therefore, it is the limit of the cytologic screening method itself which constitutes the problem needing to be resolved in cervical cancer screening. Moreover, cytologic screening is not effective in the diagnosis of human Papillomavirus (HPV) infection, the most important causative factor for cervical cancer, and cannot predict the natural outcome of lesions, in terms of whether they are likely to regress spontaneously or to progress. This is due to the fact that cytologic screening alone cannot detect the HPV

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infection when it is subclinical or latent and cannot discriminate between high risk and low risk infections.

HPV infection: natural history and cervical carcinogenesis

HPV infection is a sexually transmitted disease. HPV is a DNA virus that proliferates in the nucleus and is found in cytoplasm in an episomal form. HPV can cause various benign proliferations, such as warts, genital squamous intraepithelial lesions and laryngeal papillomas. Moreover, certain types of HPV have been linked to cervical cancer.[7]

Among women with normal cytology, HPV infections peak first in those who are less than 25 years old, decline up to about age 40 - 45 years, and then peak again at age 55 years or older, with non-cancer-associated types of HPV and uncharacterized HPV types being predominant.[8] Most infections are transient with a median duration of 12 months, and have no risk of cervical neoplasia. But 10 - 20% of the HPV infections become persistent and these type-specific persistent HPV infections, particularly those with a high viral load, give rise to chronic cervical dysplasia.[9,10]

It has been established by both epidemiological and molecular biological studies that HPV infection is the principal etiologic factors for cervical cancer. Liaw et al reported the results of 5 years' follow-up of 17,654 women with initially normal cytologic screening results. In comparison with initially HPV-negative women, women who tested positive for HPV DNA at enrollment were 3.8 times more likely to have low-grade SIL diagnosed during follow-up and 12.7 times more likely to develop high-grade SIL.[11] HPV can be detected in about 90% of cervical cancers. HPV 16 and HPV 18, described as high-risk types, have frequently been detected in cervical intraepithelial lesions and invasive carcinomas. Other HPV subtypes including HPV 31 and HPV 33 are also found in cervical cancers.

The role of HPV in the carcinogenesis of infected lesions is generally considered to be associated with the integration of the viral genome into the host cell's chromosomes. The integration of viral DNA into the host genome occurs in a way that results in the loss of viral E1 and E2 gene expression, which regulates viral transcription (disruption of open reading frames), whereas the E6 and E7 open reading frames frequently remain intact and are actively transcribed. E6 protein binds to p53 tumour-suppressor protein through its interaction with the E6-associated protein ligase, leading to the ubiquitin-dependent degradation of p53.[12] HPV can overcome the growth-arrest and apoptosis-inducing functions of p53 by preventing the accumulation of P53 in cells.[13] E6 can also inhibit Bak-induced apoptosis through an interaction between the proteins, resulting in degradation of Bak protein, probably as a result of ubiquitination by the E6-associated protein.[14]

It has been reported that E7 can induce phosphorylation and enhanced degradation of Rb protein, a critical regulator of entry into the cell cycle, via the ubiquitin-proteasome pathway. pRb negatively regulates the activity of several transcription factors, including members of the E2F family via direct association. The phosphorylation of pRb leads to the disruption of PRb/E2F complexes with the subsequent release of active E2F transcription factors, which in turn activate the transcription of genes essential for S-phase progression.[15]

Method of HPV detection

Although a range of methods have been developed to detect HPV, two consensus primer PCR systems, the MY09/11 and the GP5+/6+, and the second-generation Hybrid Capture system (HC-II) with high risk probes seems to be the methods of choice.[16-18]

The Hybrid Capture II test (HC II), approved by the U.S. Food and Drug Administration, is based on the formation of RNA-DNA hybrids between HPV DNA that may be present in clinical specimens and complementary unlabeled HPV RNA probes. The RNA-DNA hybrids are captured and immobilized by antihybrid antibodies. Immobilized hybrids are reacted with a monoclonal antibody that is conjugated to alkaline phosphatase, and the complexes are detected via a chemiluminescent substrate reaction.[17]

Detection and typing of HPV infection by

HPVDNAchip™ (Biomedlab Co., Seoul, Korea) is now under investigation. In our preliminary results, high-risk HPV was detected in 83.3% (70/84) of cervical cancer. It would be useful to be able to provide information not only about the HPV infection status but also concerning the multiple infections and genotypes of HPV.

Clinical application of the HPV testing

The HPV testing can be applicable to the clinical setting in 3 ways.

1. In primary screening.
2. In the management of women with low-grade cytologic abnormalities.
3. In the follow-up after treatment of preinvasive or early invasive lesions.

In primary screening

The role of HPV testing as a screening tool needs to be evaluated by comparing two different groups of women at risk - communities with excellent access to health care (developed countries) and communities where access is sub-optimal (developing countries). Age is another important consideration in HPV testing, because HPV infections peak in women below 25 years old, leading to the low specificity of the tests performed in the younger age group.

In a French study of the efficiency of HPV detection, the ability of the Hybrid Capture II test to detect histologically confirmed high-grade lesions was compared with that of conventional cytology. All smears evocative of high-grade lesions were positive for high-risk HPV, and high-risk HPV was detected in all 34 cases presenting a histologically proven high-grade lesion, and in 68 (97.1%) of the 70 cone biopsy samples showing a high-grade lesion or an invasive carcinoma. The sensitivity of the Hybrid Capture II test was found to be superior to that of cytology (85.3%). The authors recommended HPV testing for the screening of high-grade lesions on a large scale, in association with classic cytology.[19]

In many developing countries, cytologic screening programs are not feasible, due to lack of financial resources to establish the needed infrastructures.[20] HPV testing on patient-collected samples is under evaluation in these numerous areas where resources are inadequate and there are limited numbers of clinicians trained in performing speculum examinations. Wright et al. showed that HPV testing of self-collected vaginal swabs is less specific than, but as sensitive as, Papanicolaou smears for detecting high-grade cervical disease in South African black women aged 35 years or older, and HPV testing offers an important new way to increase screening in settings where cytology cannot readily be performed.[21] In this study, of women with histologically confirmed high-grade disease, high risk of HPV was detected in 66.1% of the women, according to the self-collected vaginal samples, while 67.9% of them had an abnormal Pap smear. Schiffman et al. also showed that HPV testing with HC II was more sensitive but less specific than conventional cytologic screening in the Guanacaste province in Costa Rica, where the incidence of cervical cancer is high. HPV testing with a cut-off value of 1.0 pg/mL detected 88.4% of 138 high-grade lesions and cancers (all 12 cancers were HPV-positive), with colposcopic referral of 12.3% of women. Papanicolaou testing, using atypical squamous cells of undetermined significance as a cut off point for referral, resulted in 77.7% sensitivity and 94.2% specificity, with 6.9% of the women being referred.[22] The problem of low specificity leading to higher rate of referral to colposcopy than conventional cytologic screening remains to be solved.

In developed countries, HPV testing on self-collected samples may also play a role in primary screening. Harper et al showed that self- collected samples were equivalent to clinician- collected samples for the detection of high-risk HPV infection in his study in the USA. Although the false-positive rates were higher in the group involving HPV testing on self-collected samples than in the group where the Pap smear was employed, HPV testing of self-collected vaginal samples provides a new, interesting opportunity for extending cervical cancer screening coverage to many unscreened women, especially those regions of the world where resources are limited.[23]

In the management of women with low-grade cytologic abnormalities

How to manage optimally the patient with low

grade cytologic abnormalities (i.e. ASCUS, LGSIL) is still the issue under thourough investigation. This is another limit of cytologic screening, and has led to a search for a superior method, although none has yet been formally approved. Some recommend immediate colposcopic examination for these abnormalities because of possibility of underlying HGSIL (7-20%) and poor compliance with follow-up.[24] Kinney et al. also reported that of the total number of cases of histologically confirmed high-grade cervical neoplasia present in the population, the largest proportion (38.8%) was in women with smears showing ASCUS.[25] However, it is unclear whether the immediate colposcopic referral of patients, on the basis of low-grade cytologic abnormalities, represents a satisfactory solution, in view of its lack of cost-effectiveness. Manos et al. evaluated the efficiency of HPV testing, using material taken from liquid-based Pap tests for colposcopy testing, as the triage of women with ASCUS Pap results, in comparison with repeat Pap testing. In their study, 6.7% were found to have histologic HSIL or cancer. For women with histologic HSIL+, the HPV test was positive in 89.2% with the specificity being 64.1%, while the repeat Pap smear result was abnormal in 76.2%. Triage based on HPV testing alone or on repeat Pap testing alone resulted in similar proportions being referred (approximately 39%) as compared to colposcopy. The sensitivity of HPV DNA testing for HSIL was equivalent to the repeat Pap test. They concluded that for women with ASCUS Pap tests, HPV DNA testing of residual specimens collected for routine cervical cytology can help identify those who have underlying HSIL.[26]

The 2001 Bethesda System subdivides atypical squamous cells (ASC) into 2 categories: "of undetermined significance" (ASC-US) and "cannot exclude HSIL" (ASC-H).[27] According to the American Society for Colposcopy and Cervical Pathology (ASCCP) 2001 consensus guidelines, the management of women with atypical squamous cells (ASC) depends on whether the Papanicolaou test is subcategorized as ASC-US or ASC-H. They postulated that women with ASC- US should be managed using a program of 2 repeat cytology tests, immediate colposcopy, or DNA testing for high-risk types of human papillomavirus (HPV), and that testing for HPV DNA is the preferred approach when liquid-based cytology is used for screening. However, in most instances, they postulated that women with ASC- H should be referred for immediate colposcopic evaluation.[21]

Evaluation of HPV testing as a triage strategy for low-grade squamous intraepithelial lesions (LSILs) was also performed by the ALTS (The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study) group. Among the 642 women referred with LSIL, HPV DNA was detected in cervical samples from 532 (82.9%) women. Because this very high percentage of women, with an LSIL diagnosis from Pap smears, is positive for HPV DNA by HC II testing, they concluded that there is limited potential for this assay to direct decisions about the clinical management of women with LSIL.[28] So the role of HPV testing in the triage of LSIL has yet to be defined. Moreover, the ASCCP 2001 consensus guidelines recommended that low-grade squamous intraepithelial lesion should be referred for immediate colposcopic evaluation.[21]

The cost-effectiveness of HPV testing has been a major block to its clinical application so the use of HPV testing has not been formally approved. However, the use of liquid based cytology, which eliminates an additional office visit to perform HPV sampling and a repeated smear test, may decrease the cost of screening, especially for ASC-US results.[29]

In the follow-up after treatment of pre-invasive or early invasive lesions

The role of HPV testing in post-treatment surveillance of high-grade CIN and early invasive cancer is under evaluation.

It has been reported that a minority of the patients with normal cytology after treatment of cervical dysplasia had detectable HPV DNA, while a high prevalence of HPV DNA was found in cervical smears of patients with abnormal cytology following treatment for cervical dysplasia.[30] It was demonstrated that after treatment, none of the patients with abnormal cytology, but having HPV DNA negative smears, had a recurrence of cervical intraepithelial neoplasia. This suggests the value of supplementary HPV DNA

testing during the follow-up of patients treated for cervical dysplasia.

In a recent study, 184 women, who were treated for CIN 2 or 3, were prospectively monitored by cervical cytology and high-risk HPV testing after treatment.[31] The authors showed that a positive high-risk HPV test 6 months after treatment was more predictive for the post-treatment CIN 2/3 than abnormal cervical cytology (sensitivity 90% and 62% respectively, with similar specificity). Although there relatively few studies have been done, it is suggested that it may be possible to return women to routine cervical cancer screening if they become HPV-negative 1 or 2 year after treatment.[18,31]

Conclusions

HPV testing is fundamentally different from conventional cytologic testing, because it evaluates the HPV infection itself, the most important causative factor for cervical cancer. It can be introduced into the cervical cancer screening program as a method of triage for the cytologic diagnosis of ASC-US and could play a role in primary screening, especially in those areas where financial and manpower resources are limited. The development of technologies for improving both its sensitivity and specificity would allow the clinical application of HPV testing to be further extended.

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