Detection of Human Papillomavirus DNA in Patients Referred to a Family Practice Colposcopy Clinic

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Background: **Human papillomavirus (HPV) is strongly implicated in the pathogenesis of cervical neoplasia. The ability of a commercially available kit (Virapap/Viratype) to detect evidence of HPV is compared with cervical cytology, colposcopy, and directed biopsies.**

Methods: **During a period of 16 months, cervical samples from 241 consecutive new patients referred for a colposcopy examination were obtained for HPV-DNA hybridization typing according to the kit instructions. Samples were sent to a reference laboratory for testing. The results were compared with results of the colposcopy examination, cervical cytology, and directed cervical biopsy samples processed and evaluated by our hospital laboratory.**

Results: **HPV DNA was detected in 27 of 107 patients who had abnormal colposcopy findings for a sensitivity of 25 ± 7.5 percent at the 90 percent confidence interval. One of 134 patients with normal findings was positive for a specificity of 99 ± 5 percent at the 95 percent confidence interval. Based on a 75 percent probability of HPV in the population, the positive predictive value was 99 percent and the negative predictive value 30 percent.**

Conclusions: **With the low negative predictive value and sensitivity, HPV-DNA testing by this commercial kit is not an adequate tool for screening HPV in this population. (J Am Board Fam Pract 1996;9:162-6.)**

Human papillomavirus (HPV) infection of the lower genital tract is increasing in incidence worldwide and might be the most common viral sexually transmitted disease today.[1] Molecular hybridization and epidemiologic studies have associated HPV in the pathogenesis of cervical squamous intraepithelial lesions and invasive cervical cancer.[2-4] With nearly 450,000 cases each year worldwide, cervical cancer is the second most common malignancy.[5] In the United States the American Cancer Society estimates there will be 15,700 new cases of cervical cancer and 4900 deaths in 1996. New human papillomaviruses are still being identified, and there were about 60 distinct types detected in 1989.[6]

Histologic confirmation of HPV infection by the finding of koilocytosis has been the reference standard for detection.[7,8] In the late 1970s and 1980s, methods for detecting HPV infection using nucleic acid hybridization techniques were developed with reported detection rates of 77 to 95 percent.[9-11] Nucleic acid amplification techniques, such as the polymerase chain reaction, have also become important.[12] With these new techniques certain HPV types classified as high-risk and low-risk for invasive cervical carcinoma were identified.[13,14] Recently kits have become commercially available to screen for and type HPV DNA that could be present in cervical samples.

Studies to date have described various methods to collect samples, including tissue from cervical biopsies[3,11,13-18] and exfoliated cells collected by a cytologic sampling brush,[19] spatula,[20] swab,[14,21,22] or cervicovaginal lavage.[15,23-26] The detection rate for HPV was best for the studies using specimens from cervical biopsies. None of the collection methods (other than cervical tissue from biopsy) consistently outperformed the others with respect to HPV detection. Different methods of analysis for HPV DNA have been used, such as Southern blot,[3,14,18,25,26] dot blot,[20] in-situ hybridization,[11,13,15,17,19,22] immunoperoxidase staining,[16] and polymerase chain reaction DNA amplification.[21,23,24] In 12 of 15 studies reviewed, a university hospital referral-based population

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has been used. The other three studies used a community-based population. All of the analyses for HPV DNA were done by the group who collected the samples or by the laboratory in their institution. None was sent to a separate laboratory for evaluation.

Family physicians provide a substantial amount of women's health care involving screening, diagnosis, treatment, and follow-up of cervical neoplasia. For every 1000 cervical smears done, approximately 40 to 50 of these will require further evaluation by colposcopy and directed biopsies.[27] The natural history of low- and high-grade lesions is different regarding progression to more advanced lesions. Approximately two thirds to three fourths of low-grade lesions will regress or remain unchanged without treatment, whereas up to 85 percent of high-grade lesions progress to more advanced lesions.[28-30] With the identification of low- and high-risk HPV types, screening patients for HPV DNA could select those at higher risk for development of cervical neoplasia.

While the death rate from cervical cancer has dropped remarkably since the 1930s, estimates of the number of deaths from this disease in 1996 are nearly 5000.[31] Because the typical course of cervical carcinoma is one of slow progression from low-grade to high-grade to invasive neoplasia during the years, detection and monitoring are possible. The Papanicolaou cervical smear is an effective screening tool. Coupled with colposcopy and directed cervical biopsy for diagnosis, women with cervical neoplasia can have it detected and treated appropriately before it progresses to invasive disease. Identification and typing of HPV could be a useful adjunct to the current methods of screening allowing more accurate determination of patients at low- and high-risk for development of cervical carcinoma.

The purpose of this study was to determine whether a commercially available kit was useful for screening patients for HPV who were referred to a community-based colposcopy clinic. The study was designed to simulate as closely as possible how samples would be collected and analyzed by the typical clinician.

Methods

During a 16-month period, 241 consecutive new patients referred to the Puget Sound Family Medicine Residency for colposcopy had cervical samples taken for HPV DNA hybridization typing. The patients were referred by their primary provider or clinic for the examination. The majority were from the Women's Health Clinic (58 percent) or the Family Practice Clinic (31 percent). The Ambulatory Care Clinic (4 percent), civilian health care providers (4 percent), and the county health department (3 percent) also contributed patients. The majority of referrals in this study were because of dysplastic (37 percent) or atypical (31 percent) smears. Other reasons included external condyloma (8 percent), abnormal appearance of the cervix (7 percent), history of dysplasia with inadequate follow-up (7 percent), and repeated inflammatory findings on Papanicolaou smears (6 percent). All patients were active-duty personnel or otherwise eligible for care in the military system. The age range was 13 to 67 years with a mean age of 29 years and a median of 27 years. The majority of patients were white (82 percent). Asians and Pacific Islanders (12 percent), African-Americans (3 percent) and Hispanic patients (3 percent) made up the rest of the study population. Only patients referred for their initial colposcopic examination were included in the study.

Complete colposcopic examinations (repeat cervical smears, acetic acid washings, an endocervical curettage, and directed cervical biopsies of abnormal areas) and cervical HPV sampling were performed by the author and other credentialed staff family physicians. Second- and third-year family medicine residents who were undergoing colposcopy training as part of their curriculum also conducted the examinations. All residents were directly supervised by a credentialed faculty member during the sample collection until the resident showed adequate ability to collect the specimen properly. Endocervical curettage and cervical biopsies were not done in pregnant patients. All histologic and cytologic samples were labeled and taken to the Bremerton Naval Hospital laboratory for processing and evaluation.

Cervical samples were also obtained from each patient to be used with the Virapap/Viratype kit (developed by Life Technologies and manufactured by Digene Diagnostics, Inc, Beltville, Md). The samples were obtained according to the instructions included with the kit. After insertion of the unlubricated speculum and before any other manipulation, the transformation zone and endocervical canal were rubbed with the supplied

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swab, and the sample was transferred to the transport medium. Samples were labeled for patient identification and delivered to a reference laboratory for evaluation.

The results from the Virapap/Viratype were compared with the results of cervical cytology, endocervical curettage, directed biopsies, and colposcopic impression. The HPV results were reported positive or negative, with further typing for strains 6, 11, 16, 18, 31, 33, and 35 done on the positive samples. Cytologic samples, endocervical biopsies, and directed cervical biopsies were considered positive for HPV if they showed evidence of koilocytosis or squamous dysplasia (low- or high-grade squamous intraepithelial lesions). Atypia not associated with koilocytosis was considered negative. Our laboratory had not yet converted to the Bethesda system of reporting for cervical cytology and histology, so the data are reported in the Richter classification (dysplasia).

Sensitivity and specificity were determined along with confidence intervals (CIs). The positive and negative predictive values were calculated based on a probability of HPV of 75 percent in new colposcopy referrals.

Results

Of 241 patients, 107 had abnormal findings on colposcopic examinations, cervical cytology, or histologic examination. Twenty-seven of these 107 patients had positive screening test results for HPV DNA by the in-situ hybridization method done at our reference laboratory. The sensitivity was 25 ± 7.5 percent (CI 90 percent). One hundred thirty-four patients had normal findings on examination and normal results reported by the cytology or histology laboratory. One of these patients screened positive for HPV DNA. The specificity was 99 ± 5 percent (CI 95 percent). The positive predictive value of the test was 99 percent, and the negative predictive value was 30 percent.

Nontypable HPV was detected in 9 patients. High-risk types 16 and 18 were detected in 8 patients. The intermediate-risk types 31, 33, and 35 were found in 7 patients. Low-risk types 6 and 11 were found in 4 patients. The distribution of HPV types compared with cervical cytology or histology findings showed that types 6 and 11 were found only in the low-grade lesions (mild dysplasia or atypia), but the other types appeared in no definite pattern. Types 31, 33, and 35 were found in 4 low-grade lesions and 3 high-grade lesions (moderate or severe dysplasia). Types 16 and 18 were found in 5 low-grade and 3 high-grade lesions. Nontypable HPV was detected in 3 low-grade and 6 high-grade lesions. The results of HPV screening with the Virapap/Viratype kit are compared with the results obtained by colposcopic examination and cytologic or histologic examination in Table 1.

Table 1. Colposcopic Examination and Human Papillomavirus Screening Kit Results.

HPV Screening Results*	Colposcopic Results		
	Normal	Abnormal	Total
Negative	133	80	213
Positive	1	27	28
Total	134	107	241

Sensitivity = true-positives / (true-positives + false-negatives) = 27/107 = 25%

Specificity = true-negatives / (false-positives + true-negatives) = 133/134 = 99%

Predictive value of a positive test = sensitivity × prior probability / (sensitivity × prior probability) + ([1 - specificity] × [1 - prior probability]) = 0.25 × 0.75/(0.25 × 0.75) + ([1 - 0.99] × [1- 0.75]) = 99%

Predictive value of a negative test = specificity × (1 - prior probability) / (specificity × [1 - prior probability]) + ([1 - sensitivity] × prior probability) = 0.99 × (1 - 0.75) / (0.99 × [1 - 0.75]) + ([1 - 0.25] × 0.75) = 30%

*Using the Virapap/Viratype kit, Digene Diagnostics, Beltville, Md.

Discussion

The sensitivity and specificity of the Virapap/Viratype kit were reported by Life Technologies (Gaithersburg, Md) as 94.5 percent and 95.5 percent, respectively, compared with Southern blot analysis (telephone conversation, Life Technologies representative, February 1995). Tissue from cervical biopsy specimens was used in the analysis performed by Life Technologies. We found a much lower sensitivity than that reported by the manufacturer. In our study the predictive value of a negative test was also low. The predictive value of a positive test was only somewhat above the estimated probability and thus contributed little added information.

The value of a diagnostic test not only depends on the sensitivity and specificity but also on the prevalence of the disease in the population. If a disease is rare, the incidence of false-positive results increases, and a screening test with a high specificity is then more clinically useful. Con-

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versely, if a disease is common, the rate of false-negative results increases, and a very sensitive test is most useful. The predictive values of a positive and negative result are good measures of overall clinical usefulness, because they take into account the sensitivity and specificity of a diagnostic test and the prevalence of a disease process. In this population, the Virapap/Viratype kit was not shown to be useful as a screening test for cervical HPV.

HPV types are currently divided into the low-risk (6, 11), intermediate-risk (31, 33, 35), and high-risk (16, 18) categories. For those patients whose screening test results are positive for HPV, the typing information can help the clinician in recommending treatment or follow-up intervals for patients with normal or low-grade findings. The distribution of the HPV types was even, and there were no obvious tendencies for higher-grade lesions to be associated with types 16 and 18.[4,14] The most frequently encountered category was nontypable. These HPV types are other than 6, 11, 16, 18, 31, 33, and 35 and have a variable risk.[14] In this study nontypable HPV types were associated with high-grade lesions more frequently than other HPV types. By using this kit, there is the potential that the clinician would not detect HPV in a number of women at higher risk to develop high-grade lesions or cervical carcinoma. More than one type of HPV has been shown to be present on the cervix.[3] For a patient with a histologically determined low-grade lesion, if only the low-risk HPV was detected, considerable morbidity or mortality could result, especially if a decision was made not to treat or to follow up with routine annual cervical smears. Further research could help decide whether HPV typing of tissue specimens in patients with low-grade lesions would be useful in recommending treatment or follow-up. The Virapap/Viratype kit has been revised by Life Technologies to improve detection of additional HPV types.

Because this study was done in a manner to simulate how a clinician would use the testing kit, there are several possible confounding variables. Although another study has reported a 70 to 75 percent probability of HPV evidence in new patients referred for colposcopy,[19] our patient population had the much lower rate at 40.6 percent, which could be related to the difference between a primary and tertiary care population base, fewer sexual partners, older age at initial intercourse, or different tissue specimen collection techniques. The lower rate of HPV would not have affected the statistical calculations for sensitivity and specificity, but the positive and negative predictive values would be changed. The generalizability of the population might be questioned, as the demographics show a majority of the patients were white and only those eligible for military care were enrolled. The population is typical of that encountered in many military family practice settings. Other possible confounders could include sampling bias, because different physicians collected the samples, laboratory handling error, and equipment malfunction. These potential problems, however, are encountered by the practicing clinician and need to be considered when evaluating a diagnostic test.

The cost of screening for cervical cancer was addressed in 1987 by Eddy.[32] Using Eddy's calculations for 100,000 women, Koss[27] estimated the cost of cervical cancer screening for 50 million adult women for 50 years (from age 20 to 70 years) to be $14.5 billion or $290 million per year. Our hospital is charged $50 for the Virapap/Viratype kit and the laboratory evaluation of the cervical samples for HPV. If all women had HPV screening annually with their cervical smears, the total cost of screening would be increased to $139 billion or $14.5 billion per year.

Screening for HPV has not evolved sufficiently to be a clinically useful tool for patients referred for colposcopy or to be a routine screening test for the general population.[5] Future investigation in this area could focus on nucleic acid amplification techniques, such as polymerase chain reaction to detect very small amounts of HPV DNA, screening for HPV DNA in cervical biopsy samples found to have histologic evidence of HPV, and the behavior of the nontypable HPV types not screened by commercial kits.

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