

The Association of Human Papillomaviruses with Anal Carcinoma

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Background Information

Papillomaviruses are ubiquitous in nature and have evolved to be associated with a range of human disease, each showing a high degree of tissue specificity. Recent advances in molecular biology demonstrate that human papillomaviruses (HPV) play a significant role in the development of benign and malignant neoplasms of the skin, anogenital and oro-respiratory tract. The epidemiology of HPV infections reveals them to be a serious problem, where in North America alone, as many as 100,000,000 people are afflicted. The most clinically significant of HPV-associated diseases is cervical cancer, but the virus is also known to cause precancerous and malignant lesions in the anal skin and rectal mucosa. Much like the strategy to use PAP smears as an effective screening test to diagnose early changes in cervical epithelium, the PAP smear is also an excellent test for the evaluation of cells from the anal canal and rectal verge. With the marked increase in the prevalence of HIV infection, the importance of evaluating these sites, in both men and women, is pronounced as HPV-associated malignancy is greatly increased when concurrent with HIV. The following are guidelines for the effective use of PAP smear techniques for HPV testing as a means to diagnose premalignant and malignant lesions of the anus and rectum. In general, the details of the types of specimens, the manner of transport and the expected results of such tests are identical to those of monitoring disease of the cervix.

Anal intraepithelial neoplasia: Classification for premalignant disease of the anal tissues

The anus is anatomically derived from the same tissues as the skin and portions of the external genital tract. As such, the covering cell layers are similar to these sites, and are primarily comprised of keratinocytes. Human papillomaviruses commonly infect epithelial cells that produce keratin, which are among the cells infected by HPV. Not all HPV types infect the anal tissues, but many of the types that commonly infect the female genital skin, the penile skin in males and the epithelium that covers the larynx are found. Specifically, HPV 6 and 11 are very common. Infections associated with these viruses

cause outward growing lesions known as condyloma acuminatum, commonly referred to as anogenital warts. These lesions can occur as single well-defined lesions, or can grow in multiple sites in what is referred to as a "field effect". In some examples, a solitary lesion can be disfiguring and grow very large, causing symptoms such as obstruction of the anal canal, the vagina or urethra. Once removed, these lesions typically recur at the same site and may also spread locally to involve other body sites covered by skin or a tissue of a similar character.

Important in the monitoring of anogenital condylomas is the fact that patients with altered immune systems are predisposed to progression toward dysplastic and malignant change within these lesions. This problem is common in patients with immunosuppression due to organ transplantation, and in selected inherited immune diseases. HIV infection is the most common setting for the malignant transformation of HPV associated lesions. In a recent study of HIV and non-HIV infected males, HPV lesions in the anus recurred more frequently and had a nearly 9 fold increased risk of malignant conversion in HIV positive men. This risk was apparent even when the condylomas involved the so-called non-oncogenic type viruses like HPV 6 and 11. When malignancy was diagnosed, invasive squamous cell carcinoma was universal, and most cases demonstrated a biologically aggressive course. The incidence of conversion from condyloma to cancer was also strongly inversely correlated with CD4 counts.

Simple methods for detecting HPV in the anus and anogenital skin

As is true for HPV in general, approximately 1% of infections are clinically detected. HPV related changes to infected cells are often silent. As a result, direct visualization by physical examination or anoscopy are largely insensitive as a means to diagnosing most HPV associated disease. For this reason, microscopic evaluation in concert with molecular genetic tests for HPV DNA are necessary for purposes of finding the infection and monitoring the course of disease. The polymerase chain reaction (PCR) is a technique used to amplify specific segments of viral DNA so that it can then be detected by selected analytic techniques. PCR is exquisitely sen-

sitive and therefore can employ samples which are simple and noninvasive to collect. Utilizing techniques similar to that used to obtain a PAP smear, such devices as a wooden spatula, a cytobrush or even a cotton tipped swab are effective at collecting the skin cells from the anus. Using one hand, brush the collection device against the affected tissue 3-4 strokes, turning the device between one's fingers to ensure covering the entire surface. Once collected, place the device into a small vial of sterile saline or culture media, again turning the device to remove the cells. Although no material will appear to come off the device, there is likely to be enough suitable sample released, that testing on extracted DNA can be performed. Transport to the molecular genetics laboratory within 1-2 days will be sufficient to ensure that DNA can be extracted and the testing successfully performed. Alternatively, if a PAP smear is to be used, collect the sample and smear the cells onto a series of 1-3 additional slides. Do not fix these slides with any preservative, but rather simply allow them to air dry. Ship the labeled glass slides to the molecular genetics laboratory, where the technologists can wash the cells from the glass and proceed to extract DNA. A third and preferred technique is to employ the commercial liquid PAP collection kits. In this case, the collection device is a plastic brush that

is placed into a collection media. This media can be used to prepare a portion for microscopic examination and the remainder for HPV DNA analysis.

Expected Results

DNA based testing is fast and highly sensitive. The methods of PCR are preferred when looking for disease without clinically evident lesions. PCR can also be used to characterize the specific types of HPV. This is significant because an individual might harbor multiple HPV types due to repeated infections, and some HPV types are known to be highly associated with the development of cancer. Some consideration to the amount of HPV virus and the corresponding risk of cancer has been studied. In the case of HIV, greater viral load of HPV is typical, however the number of viral molecules does not directly correspond with the probability of cancer. The genotype identification is more important in risk assessment. Once a sample is received in the laboratory, the test is completed within 3-4 days. Results are reported as either the presence or absence of HPV DNA and when positive, the type or types are listed. Additionally, each report will provide a comment of the associated risk of the specific HPV types with cancer.

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