RECENT ADVANCES IN THE DIAGNOSIS OF GENITAL HPV INFECTIONS

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ABSTRACT

Genomic typing is considered the method of choice for detection of genital infections with human papillomaviruses. Most assays are based on determination of DNA:RNA-hybrids by autoradiographic or enzymatic staining. According to our results, the sensitivity of a conventional dot-blot method was limited by the amount of cellular substrate gained for testing. In situ-hybridization applied to cytologic swabs was not useful, while applied to histological samples it allows direct assessment of the epithelial affection. Polymerase chain reaction dramatically increases the sensitivity and specificity of subsequent hybridization tests. It can be used in selected cases. Southern blot assays and other highly specific tests are exclusively performed to answer scientific questions. In contrast to that, a relatively new chemoluminescence assay can be recommended for routine use. It allows the differentiation between two viral groups of different malignant risk as well as the detection of single viral strains. Compared to other routine methods, the chemoluminescence assay combines the advantage of easy handling with high sensitivity in the high and low risk spectrum of viral strains.

KEY WORDS

human papillomavirus, hybridization, bio-molecular assays, chemoluminescence

INTRODUCTION

Soon after infection of genital epithelia with human papillomaviruses (HPV) the viral genome is inserted into the genome of host cells. (4,6,16) Therefore genomic typing remains the only way to verify a viral infection (1). Hybridization of viral sequences with RNA probes has turned out to be the method of choice (8).

HPV infections are widely spread within the population; therefore there is an epidemiologic interest for diagnostic assessment. (8,14,17). Furthermore, high-risk strains (HRS) potentially can cause the onset of malignant tumors, especially in the susceptible region of the cervix uteri (4,8,9). Consequently routine screening in risk groups must be followed by specific tests for HPV infections in case of certain patients and their sexual partners (7,12).

Due to technical and financial aspects, among several methods available the most proper one is to be chosen (5). The present study gives a survey over these methods, for the most part based on own results.

METHODS

Dot-blot hybridization

Cells are gained e.g. from cervical swabs; after breaking open their membranes by proteases DNA can be extracted and denatured. On a paper carrier single stranded DNA is hybridized with complementary RNA and detected either by autoradiography or by enzymatic staining. Using DNA:RNA hybrids allows to minimize background signals by a ribonuclease step.

Dot-blot hybridization (DBH) provides nonspecific viral detection (e.g. VIRA-PAP™) as well as the possibility of targeting viral subgroups of various malignancy risk (e.g. Vira-Type™).

In situ-hybridization (ISH) is a modification of the test. The integrity of cells and histological samples is maintained due to careful and limited exposition to protease activity. Detection is done by enzymatic staining. There exist non-specific (e.g. Biohit In situ Screening-Test™) as well as specifically targeted assays (e.g. Biohit In situ Typing Test™).

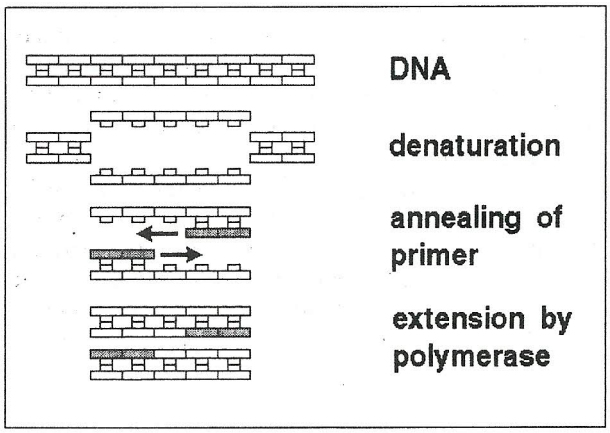

Fig. 1. Polymerase chain reaction

Polymerase chain reaction

Polymerase chain reaction (PCR) was introduced into laboratory medicine several years ago. It helps to overcome the problem of low levels of viral DNA (1). In an enzymatic cycle doublestranded host cell DNA is denatured and split into single strands (Fig. 1). Short primer molecules find their complementary sequences within the viral genome and build the starting point for further extension of complementary DNA by a heat-stable polymerase. The resulting doublestranded DNA copy serves as template for the next reading cycle. Every step within the cycle is controlled by changes of temperature; the cycle may be repeated up to 30 times. Thus the original amount of viral DNA can be amplified millionfold. This procedure is followed by a conventional hybridization assay (11).

Southern blot

After extraction and denaturation cellular DNA is partitioned by endonuclease enzymes into defined base pair segments. These fragments are separated by double-step electrophoresis and detected by autoradiography. The method is time-consuming and requires a well-equipped laboratory.

Chemoluminescence assay

This new system (Digene Hybrid Capture™ System) has been used at our department for more than a year. Hybrids containing viral DNA and complementary RNA are captured by a solid phase-bound antibody and then detected by a second antibody, which is conjugated with alkaline phosphatase. The enzyme turns a luminogen precursor molecule into a lumi-

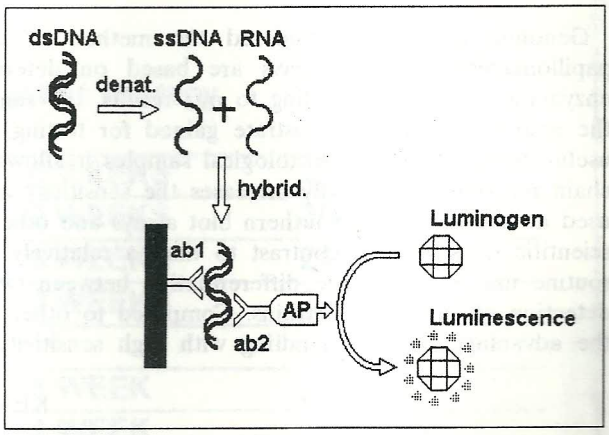

Fig. 2. Chemoluminescence assay

nescent agent (Fig. 2). On the one hand the method provides a diagnostic screening by determination of two viral groups; one of them covering low risk HPV types (6,11,42,43,44), the other one high and intermediate risk types (16,18,31,33,35,45,51,52,56). In the same way particular viral strains can be determined. As a result, the assay even gives some quantitative information about the extent of viral infection.

Statistics

X^2-test comparing two binomial distributions.

RESULTS

Dot-blot hybridization

120 samples of patients (males and females) attending our STD outpatient clinic were examined. All of these patients clinically were suspected of genital HPV infections. In 43 samples (35.8%) HPV could be found; amongst them low risk viral types (6/11) in 37.2% of HPV positives, high risk types (16/18) in 44.2% and intermediate risk types (31, 33, 35) in only 9.3%. Taking into account cases with multiple strain infections these percentage values increased to 40% (low risk types) and 62.8% (intermediate and high risk types).

In situ-hybridization

In situ-hybridization of cervical cell scrapes was insensitive because of technical and handling problems. Histologic samples were not included in our studies.

Polymerase chain reaction (PCR)

In cooperation with the II. clinic for gynecology and obstetrics of the Vienna University cervical swabs of female patients belonging to various risk groups were examined by PCR (3).

Prevalence of infections with HPV 11,16 and 18 was significantly higher in cases of cervical cancer and dysplastic Papanicolaou stain (PAP IIID + IV). In contrast, only 16% of those, who had been operated from cervical carcinoma and 20% of patients belonging to an unselected reference group were HPV-positive (unpublished data). Hybridization without preceding PCR revealed markedly lower results.

Chemoluminescence test

The assay was applied to a routine screening distinguishing infections with low risk strains from those with high risk strains. We examined 382 samples of patients attending our STD outpatient clinic because of clinical signs pointing to a genital HPV infection. All examinations were done for the first time; from some patients we got two samples of different genital regions. Women turned out to be clinically affected nearly three times as often (74%) than were male patients (26%). Nevertheless, regardless of gender the rate of HPV-positive samples was approximately equal in males (58%) and females (61%). Among males we predominantly found single strain infections with low risk-types (m:79%; f:39%); among females significantly more often high risk types (m: 9%; f: 44%; p=0.001).

Mixed infections with low and high risk strains together existed about equally in males (12%) and females (17%). Among all HPV-positive patients, isolated low risk strains were found in 49%, high risk strains in 35%, and multiple strain infections in 16%. Adding the latter group, the rate of low risk type infections was 65%, of high and intermediate risk types together 55%.

DISCUSSION

Conventional dot-blot hybridization (DBH) methods mostly are based on autoradiographic detection which requires certain precautions. Their handling is not too complicated. They allow virologic screening as well as determination of viral subgroups of various malignancy risk. Sensitivity is limited by the amount of viral substrate, corresponding to the number of cells gained e.g. from cervical swabs. Our own experiences were in accordance with those of other authors. Law (1991) examined females with genital warts. In the same group of patients 85% of cervical biopsies were HPV-positive; which could be confirmed by only 40% of cervical swabs (8). The rate of HPV positive swabs or scrapes in our STD-group tested by DBH was slightly lower (35%), presumably due to inclusion of males. High risk strains predominated in our group, which underlines the importance of typing.

Past evaluation studies showed DBH to be more reliable than in situ-hybridization (ISH) (2). Corresponding to that, our experiences with ISH of cervical cell scrapes were disappointing and did not provide statistically relevant results (2,3). The ISH method is applicable more successfully to histologic samples to confirm the total surgical removal of an affected region.

PCR is able to overcome the problem of lacking enough detectable viral DNA. The million-fold amplification of genomic substrate markedly increases the sensitivity of following hybridization (15). This certainly is an advantage, but at the same time even traces of contamination can lead to false positive results. Specificity of the method depends on the quality of primer molecules (11). We would not recommend PCR for daily routine, but for application to selected cases.

Southern blot and other highly specified assays help to answer scientific questions in specially equipped

laboratories (2).

HPV-determination by hybridization and chemoluminescence detection (CLD) turned out to be easy to handle and proper for routine use. In contrast to radiographic methods no special precautions are required.

We used the system for differentiation of low risk types on the one side from intermediate and high risk types on the other side. Prevalence was comparable among males and females (mean value 60.2%) and markedly exceeded the corresponding value of DBH (35.8%). This in part seems to be influenced by the features of different test groups, which were not randomized (see above). Yet, CLD sensitivity was superior to DBH, obviously due to the expanded test spectrum in the low risk range, covering three additional viral types. No difference was found among detection rates of intermediate and high risk types. This at least confirms the success of conventional methods, the more so since less viral strains were included in DBH than in CLD. The extended number of intermediate and high risk types covered by CLD did not seem to influence the prevalence rate too much, obviously due to the predominance of types 16 and 18 (which were covered in both assays). Determination of particular strains by CLD will help to clear up this matter.

Samples for CLD analysis came from an unselected group of patients attending our STD ambulance. Among them females were affected about three times as often than were males. Furthermore, HPV-positive females in contrast to males had a threefold rate of infections with high risk strains.

These findings are in accordance with previous reports, confirming that predominantly women are at risk for HPV infections and their malignant sequelae. One reason could be the increased susceptibility of cervical epithelium (9,13). In young females Schiffman observed high rates of cervical infections, most of them clinically undetectable. As there can be expected from observations in elder groups, only a minority of these women years later would develop a neoplasia. Genetic or infectious cofactors are discussed (12).

Even without regard to gender, CLD results showed high risk viral strains in more than half of HPV infected persons. This proves the unrestricted importance of HPV diagnosis by means of highly specified methods.

Certainly, not every simple condyloma has to be subjected to bio-molecular analysis. Those are the criteria for typing:

Persisting or clinically undetermined lesions; unclear histologic findings; missing therapeutic effect; and all questions of control, like gynecological prevention, selected partner examination and post-surgical assessment.

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