Detection of Human Papillomavirus 16 and 18 DNA in Epithelial Lesions of the Lower Genital Tract by In Situ Hybridization and Polymerase Chain Reaction: Cervical Scrapes Are Not Substitutes for Biopsies

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Human papillomavirus (HPV) types 16 and 18 in 66 women with histologically documented lesions of the genital tract and 64 control cohorts were investigated. The efficacies of in situ hybridization and polymerase chain reaction (PCR) in detecting HPV 16 and 18 DNA were analyzed. In order to assess the usefulness of replacing biopsies with cervical scrapes, the two samples were compared by PCR. The prevalence rates of HPV infection by PCR were 59.1 and 10.9% in patients and controls, respectively. PCR was three times more sensitive than in situ hybridization (52.6 versus 17.8%). However, the need to improve PCR sensitivity by subsequent dot blot hybridization reduced one of the main advantages of PCR, i.e., expeditious diagnosis. Cervical scrapes were less sensitive than biopsies (15.6 versus 53%), although with four (6.1%) patients with intraepithelial neoplasias, HPV DNA was identified only by means of cervical scraping. We conclude that obtaining biopsy specimens and cervical scraping are complementary sampling procedures.

Human papillomaviruses (HPVs) are members of the Papovaviridae family and contain circular double-stranded DNA approximately 8 kb in length (2). The application of DNA hybridization techniques led to the characterization of distinct HPV types (15, 19). More than 66 different genotypes have been identified (45), and approximately 18 of them have been associated with neoplastic lesions of the anogenital mucosa and skin (26). The association of particular types with genital cancer is now well documented (18, 54). On this basis, HPVs have been categorized into low-risk (e.g., HPV 6 and 11) and high-risk (e.g., HPV 16, 18, 31, 33, and 35) virus types reflecting their oncogenic potential. HPV 6 and 11 frequently coexist with cervical condylomata and minor dysplasias (4), while HPV 16 and 18 have been found in high-grade premalignancies and invasive cancers (26, 41).

Important differences in the physical states of HPV DNA have been demonstrated with benign and malignant lesions. HPVs are maintained as extrachromosomal circular DNA episomes in benign cervical lesions, whereas the viral DNA appears to be integrated within the host genome in most cervical carcinomas (17). The integration of HPV DNA sequences can be an early event in the progression of genital tumors, but this is not in itself sufficient to transform the cell (39). Current data suggest that other initiating events, such as herpes simplex virus infections (53), cigarette smoking (21), and prolonged use of oral contraceptives (7), could interact synergistically with HPVs in the development of malignancies of the genital tract (11).

Growth of HPV in cell cultures has not yet been obtained. Methods of detection of HPV infection include colposcopic examination, cytology-histology, electron microscopy, and immunocytochemistry (6, 14, 36). However, the viral antigens which can be detected immunocytochemically do not specify the HPV type. Nucleic acid hybridization techniques with radiolabeled or nonspecific isotope probes are the only methods available for typing HPVs present in infected tissues (5, 36). The presence of HPV DNA extracted from tissue samples can be determined by Southern blot or dot blot hybridization. These procedures, however, do not permit hybridization data and histology of the tissue to be correlated. In situ hybridization (ISH) is a less sensitive technique than Southern blot analysis, but it allows exact correlation between histology and individual viral genomes. The use of polymerase chain reaction (PCR), an in vitro DNA enzymatic amplification procedure (37), enhances the sensitivity of DNA hybridization probes and has contributed to an increase in the detection of HPV DNA in scrapes and biopsies from women with carcinomas and precancerous lesions of the cervix (29, 46). Prevalence studies using different hybridization techniques to examine HPV-related lesions or normal Papanicolaou smears have provided conflicting results (13, 20, 29, 42, 51). Interlaboratory variations due to the use of differing protocols or deviations in test performance as well as geographic differences in the distribution of the various HPV types may account for the varying prevalence rates of HPV genotypes (16, 24).

The aims of this prospective study were (i) to compare the usefulness of ISH with that of PCR for detecting HPV 16 and 18 in tissue samples from women with squamous-cell abnormalities of the lower genital tract, (ii) to analyze the efficacies of PCR for detecting HPV 16 in biopsies and cervical scrapes, and (iii) to determine the incidence of HPV 16 in cervical scrapes from a control cohort with normal cervical smears.

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MATERIALS AND METHODS

Clinical specimens. The study population consisted of 66 women aged 20 to 67 years (mean, 34 years) who had been consecutively referred to our institution with either abnormal Papanicolaou smears or abnormal biopsies. For all, second samples of cervicovaginal smears for routine cytologic examination as well as cervical scrapes for PCR analysis were obtained. In addition, a total of 95 biopsies, including cervical, vaginal, and vulvar samples, were collected for histopathologic and HPV DNA assays. The control cohort consisted of 64 women aged 16 to 40 years (mean, 29 years) with normal Papanicolaou tests. The smear, PCR amplification and routine cytologic examination were taken by cervicovaginal scraping.

Histologic diagnosis. Biopsy specimens were fixed in 10% formalin and embedded in paraffin wax. Sections for histologic examination were stained with hematoxylin and cosin; 5- to 10-μm-thick sections for HPV DNA assays were left unstained. Cervical or vulvar intraepithelial neoplasia (CIN or VIN) grading was in accordance with World Health Organization criteria. The histologic diagnoses distinguished between normal epithelium, CIN I (mild dysplasia), CIN II (moderate dysplasia), CIN III (severe dysplasia and carcinoma in situ), and invasive carcinoma. Biopsy specimens were also examined for the histologic features of koilocytosis. All microscopic slides were reviewed by three independent observers.

ISH. Tissue sections were mounted on slides coated with an aqueous solution of poly-D-lysine (Sigma Chemical Co., St. Louis, Mo.). The sections were dewaxed, rehydrated, washed sequentially in phosphate-buffered saline–0.5% Triton X-100, and air dried. Afterwards, the slides were treated with proteinase K–3% hydrogen peroxide, rinsed with a stream of wash buffer, dehydrated, and air dried. The hybridization was performed with DNA biotinylated probes to HPV 16 and/or 18 (Enzo Diagnostics Pathogene assay; Enzo Diagnostics, Inc., New York, N.Y.). After being incubated, sections were treated with buffered sodium chloride and formamide. The reaction was detected with a streptavidin-horseradish peroxidase complex, and the sections were counterstained with hematoxylin. Appropriate controls were used in each assay; CaSkI cell lines (cervical carcinoma cells containing 500 integrated copies of HPV 16 DNA per cell) included in the kit were used as positive controls, and PCR-negative sections from cases of chronic cervicitis were used as negative controls.

Preparation of samples for PCR. Three 5- to 10-μm-thick sections from paraffin-embedded tissue blocks were cut and put into an Eppendorf tube under sterile conditions. The sections were treated by the procedure described by Shibata et al. (42) and modified by Wright and Manos (50). Briefly, each section was dewaxed twice in xylene and rinsed twice in 100% ethanol. Tissues were pelleted between each extraction. Then, the samples were dried in a heat block at 55°C and resuspended in 100 to 200 μl of digestion buffer (200 μg of proteinase K per ml in 50 mM Tris-HCl [pH 8.5]–1 mM EDTA–0.5% Tween 20). Samples were incubated overnight at 37°C. Proteinase K was inactivated at 95°C for 8 min.

Cervical scrapes were collected with a wooden Ayre spatula and placed in 3 ml of TEN buffer (10 mM Tris-HCl [pH 7.5], 50 mM EDTA, 150 mM NaCl, 0.1% sodium azide). The material was vigorously vortexed for 1 min, and the spatula was discarded. The suspension was centrifuged at 3,000 × g for 10 min. The pelleted cells were resuspended in digestion buffer (50 mM Tris-HCl [pH 8], 10 mM EDTA, 0.5% sodium dodecyl sulfate (SDS) containing 200 μg of proteinase K per ml and incubated at 37°C for 16 h. Samples contaminated with erythrocytes were treated with lysis buffer (20 mM Tris-HCl [pH 8], 10 mM EDTA, 5 mM MgCl2) before being incubated with the digestion buffer. DNA was purified by serial phenol-chloroform and chloroform-isomyl alcohol extractions. Subsequently, DNA was precipitated with 0.1 volumes of 3 M sodium acetate (pH 5.2) and 3 volumes of absolute ethanol at −20°C overnight. Then DNA was pelleted at 14,000 × g for 15 min, rinsed once in 70% ethanol, and precipitated again. DNA was resuspended in 50 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]), and the concentration was determined spectrophotometrically.

PCR. Samples were amplified separately for HPV 16 and 18 with a thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.) and primers specific for each viral type derived from previously published sequences (12, 40). The sequence of primers for HPV 16 DNA were D, 5'TTTTGGTGTTACA CATTATTACAG3' (residues 7684 to 7885), and R, 5'TGTC TGTTTTTATATAACCCG3' (residues 57 to 78), which generated a 119-bp product from the URR region. The sequence of primers for HPV 18 DNA were D, 5'GACACCTTAATG AAAAACGACAG3' (residues 406 to 482), and R, 5'CCTCG TTGGAGTGTCTTCTG3' (residues 543 to 562), which amplified a 103-bp fragment from open reading frame E6. Oligonucleotide sequences were synthesized with a DNA synthesizer (Applied Biosystems 380 A) by the beta-cyanoethyl phosphoramidite method.

For the amplification reaction, 1 to 3 μg of cervical scrape DNA or 17 μl of tissue extract was added to 100 μl of the reaction mixture containing 10 mM Tris-HCl (pH 8.4), 1.5 mM MgCl2, 50 mM KCl, 0.01% (wt/wt) gelatin, and 200 μM (each) dATP, dGTP, dCTP, and dTTP. For the specific reaction, 65 pmol of each primer and 2 μl of thermostable Taq DNA polymerase (Perkin-Elmer Cetus) were used. DNA from tissue samples was subjected to 45 cycles of amplification. Each cycle involved heating to 94°C for 45 s (DNA denaturation) followed by cooling to 54°C (for HPV 16) or to 58°C (for HPV 18) for 1 min (primer annealing) and heating again to 74°C for 30 s (chain elongation). DNA purified from scrapes was amplified through 38 cycles. Each cycle consisted of denaturation for 30 s at 95°C, annealing under the same conditions as those for biopsies, and primer extension at 74°C for 1 min. After the last cycle, 25 μl of the reaction mixture was analyzed by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. One hundred nineteen- and 103-bp specific bands for HPV 16 and 18, respectively, were identified. Precautions to minimize contamination during sampling and subsequent processing included frequent changes of gloves and careful handling of samples in cabinet hoods. Special precautions to minimize sample-to-sample contamination and amplicon carryover to avoid false-positive results consisted of spatially separated pre- and post-PCR areas, aliquoting of the reagents used in each assay, and the use of positive-displacement pipettes and different sets of laboratory materials for pre- and post-PCR areas.

Dot blot analysis. The amplified product was confirmed by dot blot and hybridization of 32P-labeled oligonucleotide probes to an internal area of the amplified sequence on each genotype (internal probe for HPV 16, 5' TAAATCTAATAACTA CAATAATTCATG3'; internal probe for HPV 18, 5' CATAG CTGGGGCAACTATAGG3'). Oligonucleotide sequences were synthesized by the same method used for the primers. They were end labeled with [32P]ATP (3,000 Ci/mmol; Amer-
TABLE 1. Distribution of positive rates for HPV DNA 16 and 18 according to histopathologic diagnosis with 95 biopsies of the female lower genital tract studied by ISH and PCR

<table>
<thead>
<tr>
<th>Histopathologic diagnosis (no. of biopsies)</th>
<th>Result* by ISH</th>
<th>Result* by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 and/or 18</td>
<td>16</td>
</tr>
<tr>
<td>Chronic inflammation (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vulvar condyloma (9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical condyloma (7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intraepithelial neoplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN I (31)</td>
<td>11</td>
<td>35.4</td>
</tr>
<tr>
<td>VIN I (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIN II (13)</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>CIN III (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VIN III (3)</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Invasive squamous-cell carcinoma (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total 95</td>
<td>17</td>
<td>17.8</td>
</tr>
</tbody>
</table>

* Except as indicated, results are numbers of biopsies positive for the indicated virus type(s).

sham Iberica S. A., Madrid, Spain) by standard procedures (27). Amplification products were denatured by heating to 98°C in 0.4 M OHNa-25 mM EDTA for 2 min. After being cooled on ice, 90 μl of the mixtures was spotted twice onto GeneScreen Plus nylon membranes (Biotechnology Systems NEN Research Products, Dupont, Boston, Mass.) (once for each genotype) with a dot apparatus (Hybirdot manifold; Bethesda Research Laboratories). Filters were baked at 80°C for 2 h and stored at room temperature until hybridization.

Prehybridization was performed at 65°C for 2 h in a solution (1 ml/12 cm² of nylon membrane) containing 5 x SSC (1x SSC is 15 mM sodium citrate and 50 mM sodium chloride), 5 x Denhardt solution (1x Denhardt solution is 0.02% bovine serum albumin, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone), 0.5% SDS, 50 mM sodium phosphate buffer, 1% inorganic PP, 0.2% sodium heparin, and 0.5 mg of denatured, sonicated herring sperm DNA per ml. Nylon membranes with amplified sequences for HPV 16 and 18 DNA were hybridized to their specific probes at 55 and 57°C, respectively, for 1 h. Hybridization was performed with volumes and solutions identical to those used for prehybridization but with 0.2% dextran sulfate and 0.5 × 10⁶ cpm of 32P-labeled probes per ml for each type added. The blots were washed three times for 5 min at room temperature in 1x SSC with 0.1% SDS and twice at 60°C in 1x SSC with 0.1% SDS. Autoradiography was performed for 16 to 96 h with Agfa Curix RP2 film with intensifying screens (Agfa) at −80°C.

RESULTS

Histology. Histologic examination of the 95 cervical biopsy specimens revealed a spectrum of lesions. Seventeen cases (17.8%) were classified as chronic inflammation (2 localized in the vagina and 15 in the cervix), 16 (16.8%) were classified as condylomata (9 from the vulva and 7 from the cervix), 59 (62.1%) showed various grades of intraepithelial neoplasias (1 VIN I, 31 CIN I, 13 CIN II, 3 VIN III, and 1 CIN III), and 3 (3.1%) were invasive squamous-cell carcinomas. Koilocytosis appeared in 73 cases (76.8%) (3 cases of chronic inflammation, 16 of condylomata, 51 of intraepithelial neoplasias, and 3 of invasive carcinomas).

Detection of HPV DNA by ISH. HPV 16 and/or 18 DNA could be detected by ISH in 17.8% of lesions (17 of 95) (Table 1; Fig. 1). None of the chronic inflammation or condylomata cases were HPV positive. In contrast, 17 (29%) of the 59 cases of intraepithelial neoplasia were positive for HPV by ISH. The frequency of positive results decreased with the severity of the lesions, from 35% with CIN I to 0% with CIN III. The three VIN III cases were HPV positive by ISH. One invasive squamous-cell carcinoma was HPV negative.

Detection of HPV DNA by PCR. HPV 16 and/or 18 DNA could be identified by PCR in 52.6% of the lesions (50 of 95) (Table 1). HPV 16 DNA was detected in 40 cases (80%), HPV 18 DNA was detected in 5 cases (10%), and both viral genotypes were detected in the remaining 5 cases (10%). Seven (41%) of the 17 chronic inflammation cases and 11 (68.7%) of the 16 condylomata cases were HPV positive. Five of the 11 PCR-positive condylomata cases corre-
sponded to four women who also had CIN and/or VIN lesions, all but one of which were positive by PCR (identical HPV types). None of the seven PCR-positive chronic inflammation cases presented CIN, VIN, or invasive carcinoma simultaneously. In cases of intraepithelial neoplasia, the detection rate (54.2%, 32 of 59) increased with the severity of the lesions, from 48.3% with CIN I to 63.6% with CIN III. All cases of VIN were HPV positive by PCR. The three biopsies of the single case of squamous-cell carcinoma were all HPV negative.

Detection of HPV infection by ISH and PCR in dysplastic tissue. Of the 59 sections showing various grades of intraepithelial neoplasias, 36 (61%) were positive for HPV 16 and/or 18. HPV infection was detected by both ISH and PCR in 36.1% of the cases (13 of 36), by ISH only in 11.1% of the cases (4 of 36), and by PCR only in 52.7% of the cases (19 of 36).

Detection of HPV DNA in biopsy specimens and cervical scrapes. Table 2 compares the detection rates for HPV infections in 66 patients from whom biopsies and cervical scrapes were taken (when a patient had more than one cervical biopsy, only a PCR-positive specimen was considered). Overall, the detection rate for HPV infection was higher in biopsy specimens (ISH, 18.1% [12 of 66]; PCR, 53% [25 of 66]) than in cervical scrapes (PCR, 13.6% [9 of 66]). HPV DNA was identified only by cervical scraping with 4 (6.1%) of the 66 women (2 CIN I, 1 CIN II, and 1 CIN III). In the subgroup of 46 patients with CIN, the detection rate for HPV infection was also higher with biopsy specimens (ISH, 26% [12 of 46]; PCR, 54.3% [25 of 46]) than with cervical scrapes (PCR, 17.3% [8 of 46]) (Table 2). In the control cohort, the prevalence of HPV infection identified by PCR was 11% (7 of 64).

Electrophoresis on agarose gels and ethidium bromide staining were less sensitive than PCR and subsequent dot blot hybridization for the detection of HPV amplification products (Fig. 2). Fourteen (25%) of the 55 biopsy cases which were positive by PCR and dot blot hybridization were positive by electrophoresis. With cervical scrapes, the corresponding figures were 5 of 10 (50%). Altogether, HPV 16 and/or 18 DNA was detected by PCR with 39 (59%) of the 66 patients for whom both cervical biopsies and scrapes were analyzed (35 patients with PCR-positive biopsies and 4 patients for which PCR was only positive with cervical scrapes). In contrast, HPV 16 and/or 18 DNA was detected by ISH in 12 (18.1%) of the cases.

**DISCUSSION**

In the present study, HPV 16 DNA was detected by PCR in a significant proportion (35%) of chronic inflammation cases; all cases were negative by ISH. In a study carried out in China (43), HPV 16 DNA was detected by PCR in 20% of chronic cervicitis cases, 50% of dysplasias, and 60% of invasive carcinomas. The episomal form of HPV DNA was present in cervical benign lesions, whereas integration was associated with malignancy. None of the 16 cases of condylomata was ISH positive. This finding agrees with the fact that condylomata are mainly related to replicative HPV 6 and 11 genotypes (36, 54). However, 69 and 20% of the cases of condylomata were PCR positive with biopsy specimens and cervical scrapes, respectively. The coexistence of replicative HPV 6 and 11 with minimal amounts of HPV 16 and 18 may account for positive results by the PCR technique. The presence of HPV 16 DNA in condylomata has been previously reported (36).

For cases of intraepithelial neoplasia, our detection rate of

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**TABLE 2. Positivity rates by ISH and PCR for 66 patients with cervical lesions**

<table>
<thead>
<tr>
<th>Histopathologic diagnosis (no. of biopsies)</th>
<th>Result* by ISH from biopsy</th>
<th>Result* by PCR from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 and/18</td>
<td>Frequency of positivity (%)</td>
</tr>
<tr>
<td>Chronic inflammation (14)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical condyloma (5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (24)</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>II (12)</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>III (10)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive squamous-cell carcinoma (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total 66</td>
<td>12</td>
<td>18.1</td>
</tr>
</tbody>
</table>

* Except as indicated, results are numbers of patients positive for the indicated virus type(s).

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**FIG. 2.** (A) Amplification products of HPV 16 DNA. PCR products were electrophoresed in 2% agarose gels and stained with ethidium bromide. PCR-positive samples from biopsy specimens (lane 1) and cervical scrapes (lanes 3 to 7) from patients with CIN are shown. The band of 119 bp corresponds to the DNA amplified. A PCR-negative sample from a cervical scraping from a control woman is also shown (lane 2). (B) Dot blot analysis of some biopsies whose PCR-positive samples are shown in panel A. Hybridization signals can be seen at positions A1, A3, C7, and D10.
29% by ISH is similar to that reported in other studies in which biotinylated probes were used (10). The frequency of HPV DNA detection is known to increase with the use of radiolabeled probes (38). The detection rate with biopsies of intraepithelial neoplasias by PCR was 54%. The four cases of VIN were positive for HPV 16 DNA. These lesions are predominantly associated with HPV 16 infection (32), as opposed to a more heterogeneous distribution of HPV DNA genotypes in cervical dysplasias (35). Our rate of HPV 16 infection in cases of intraepithelial neoplasia (45.7%) is lower than those reported by others (20, 46), which may be explained by geographic differences or interlaboratory variations in test performance, i.e., the use of a Southern blot to analyze the amplimers (16, 22, 23, 36). In addition, recent evidence of HPV 16 DNA genomic diversity in the viral transcription region (25) may also account for the varying prevalence rates of HPV types. Mixed infections represented 5% of the biopsy specimens analyzed. In studies including multiple genotypes, the occurrence of mixed infections varied between 18 and 21% (29, 31).

The detection rate for HPV 16 and/or 18 DNA infections decreased with the severity of the dysplastic lesion when ISH was used. In contrast, the detection rate for HPV 16 and/or 18 DNA increased with the severity of the lesion when biopsy specimens were analyzed by PCR. These HPV types have been associated with the progression of severe dysplasia to malignancy (9, 26). CIN I has been associated with several HPV genotypes, and when HPV 16 or 18 is present, it is mainly in a replicative form (18). However, high-grade dysplasias and carcinomas in situ are associated with a few viral types which are usually integrated within the host DNA (1, 17, 39); thus, PCR, which detects 1 DNA copy per 10^6 cells in 30 amplification cycles, is much more sensitive with severe dysplasias than ISH, which detects 5 to 800 copies per cell (3, 30, 38). The greatest differences in the detection rates were found with CIN III (ISH, 0%; PCR, 63%). Note that in four cases (3 CIN I and 1 CIN II), HPV 16 and 18 DNA was only detected by ISH. Sampling errors (23) or variations in the genomic sequence complementary to the oligonucleotide used may explain this discrepancy. This finding has also been reported when Southern blot was compared with PCR (30). The singular advantage of ISH is its ability to determine the location of the target DNA in the tissue sample.

The detection rates of HPV 16 DNA in patients with CIN are greater than those found with women with no evidence of histologic abnormalities, although a high prevalence of HPV 16 has also been detected in benign lesions of the cervix. Different physical states of HPV 16 DNA have been recognized in benign and malignant genital tumors (17), but it is an open question whether integration of HPV 16 DNA into the cell genome is a causative event for malignant transformation (11). The transforming properties of HPV 16 and 18 have been documented in studies using immortalized rodent cells, human foreskin, and cervical keratinocytes. The transforming genes have been mapped to E6 and E7 open reading frames (26). The integration of viral DNA in premalignant lesions results in the disruption of the E2 gene, which controls intraviral transcription. This implicates a disregulated expression of the viral oncoproteins. However, this fact has been revealed to be not enough for malignant transformation (39). Other cellular events, such as the level of expression of c-myc oncogenes when viral DNA is integrated in the vicinity (43) or the role of tumor suppressor genes found in chromosome 11 (55), have been examined.

The detection rate by PCR of HPV infection in cervical scrapes was lower than that in biopsy specimens. By cervical scraping, we were able to identify HPV 16 DNA in 13% of dysplastic lesions (figures in the literature range between 6.2 and 100% [20, 29, 48, 49, 51]) and HPV 18 DNA in 4.3% of the cases. Van den Brule et al. (49) identified HPV 18 DNA in 7% of the cases. Recently, Gjoen et al. (20) obtained similar prevalence rates of HPV infection with cervical smears and biopsies from 15 patients with condylomata and/or dysplasias. To our knowledge, this is the only study comparing the value of PCR with both types of samples. In our experience, HPV 16 DNA was detected in cervical scrapes from 50% of patients with PCR-positive biopsies and HPV 18 DNA was detected with only 25%. For four patients, however, HPV infection was detected only in cervical scrapes.

There are also a few studies comparing the efficacy of cervical scrapes or lavages with that of biopsy specimens when techniques other than PCR are used (8, 28, 33). Some authors have reported (8, 33) the efficacy of cervical scrapes to be higher than that of biopsies by nucleic acid sandwich hybridization or Southern blot and have emphasized the importance of adequate collecting methods for obtaining a sufficient number of cells. In most cases, results may vary according to the size of the lesions or the amount of tissue biopsied. McCance et al. (28), using filter ISH, obtained similar detection rates for HPV 16 DNA in cervical smears and biopsies. However, using dot blot hybridization, Hallam et al. (24) also failed to correlate HPV DNA positivity obtained with cervical scrapes and biopsies. These conflicting results between both samples may reflect the depth of location of HPV 16 and 18 within the cervical epithelium. Cervical scrapes are less representative than biopsies, probably because of their smaller cellular content and the fact that biopsies are colposcopically directed. These findings suggest that cervical scrapes do not constitute the most suitable sample for screening HPV infection, although with 6% of our patients the infection was detected only by scraping. Zhang et al. (52) have pointed out that both methods are complementary. Differences in sampling efficiency may be reduced by standardizing the amount of nucleic acid analyzed. In order to obtain valid results with cervical scrapes, a minimum number of 10^5 to 10^6 epithelial cells would be necessary (28, 33, 52).

The prevalence of HPV 16 DNA in cytologically normal smears varies between 2 and 84% (23, 29, 34, 44, 47, 49, 51). Using the PCR technique, we could detect HPV 16 associated with normal cytology in 11% of the controls.

In conclusion, PCR is a sensitive method for detecting HPV infection. It is not as expeditious as expected, because of the need to increase the sensitivity by dot blot, which at the same time confirms the specificity of the amplimers shown by electrophoresis on agarose gels (34, 49, 51). Biopsy specimens have been proven more suitable than cervical scrapes for HPV DNA detection, although a noninvasive sampling method such as cervicovaginal lavage also could be convenient. Conditions for the PCR technique, particularly the primers and the number of cycles, should be standardized in order to compare results from different laboratories and to draw conclusions about the role of HPV infection in genital carcinogenesis.

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