Analysis of transforming gene regions of human papillomavirus type 16 in normal cervical smears

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Summary. Exfoliated cells from the uterine cervix of 102 Hungarian women with no cytological abnormality were screened using the polymerase chain reaction (PCR) for human papillomavirus (HPV) type 16 infection. Twenty-nine patients with histologically confirmed cervical intraepithelial neoplasia (CIN) served as reference cases. PCR was performed with 2 different HPV 16 specific oligonucleotide primer pairs flanking a 300 and a 200 base-pair fragment from the early 6 (E6) and early 7 (E7) genes, position 215–514 and 605–805. The specimens exhibited the same proportions of type 16 sequences specific for the tested regions. 8.8% (9/102) of normal samples showed amplification for HPV type 16 E6 and E7 regions, while 48.3% (14/29) of CIN biopsies were positive for the same gene sequences.

Key words: Cervical HPV 16 infection – PCR – Normal cervical smears – Cervical intraepithelial neoplasias

Introduction

Histologic studies have shown that genital lesions are frequently associated with dysplasia and progress to carcinoma in situ and invasive carcinoma. Studies with the techniques of deoxyribonucleic acid (DNA) hybridization have shown the dominant presence and expression of HPV 16 in pre-invasive cervical lesions and in cervical cancers (Munoz et al. 1988, Shirasawa et al. 1988, Czeglédy et al. 1989). This association has been extended and confirmed by using the polymerase chain reaction or PCR (Franquemont et al. 1989; Bevan et al. 1989). The biological significance of these reports has been challenged by Young and recently Johnson who showed that HPV 16 DNA was present in up to 90% of normal biopsies. In the Netherlands, however, HPV 16 was only detected in 2 to 4% of the normal specimens (Melchers et al. 1989; Van den Brule et al. 1989).
The genomes of the HPVs contain eight open reading frames (ORFs). The E6–E7 regions of the HPV 16 genome have been identified as retained, transforming genes and it appears that their transcripts are necessary for transformation (Tanaka et al. 1989; Munger et al. 1989). The purpose of this study was to determine the prevalence of HPV 16 DNA sequences in exfoliated cervical cells from healthy Hungarian women (without colposcopic and cytological abnormality) and in cervical biopsies taken from CIN patients as references cases. For this investigation we have designed two new primer pairs: 16ME21/16ME49 specific for the E6 region and 16ME60/16ME78 specific for the E7 ORF.

Patients and methods

Exfoliated cells from the ecto- and endocervix (3–5 × 10⁵ cells per swab) were obtained from women (mean age 37.0 years) with normal PAP (Papanicolaou test) smears and without signs of any genital infections. They were fitted with intrauterine contraceptive devices (IUD) and all of them were annually controlled at the Family Planning Centre of the Department of Obstetrics and Gynecology, Debrecen. The other group consisted of 29 women (mean age 38.6 years) with histologically confirmed CIN. For DNA extraction exfoliated cells were treated by nonionic detergents (Nonidet P40, Tween 20) and proteinase K (Higuchi 1989). DNA was prepared from biopsies by proteinase K digestion, phenol extraction (Fuchs et al. 1988).

PCR: 25 μl of the resuspended DNA was mixed with PCR buffer (16.6 mM (NH₄)₂SO₄, 67 mM Tris-HCl pH 8.8 at 25°C, 6.7 mM MgCl₂, 10 mM β-mercaptoethanol), d NTP-s at a final concentration of 0.08 μM of each of the oligonucleotide primers and 2 units of Taq DNA polymerase (Perkin-Elmer Cetus) to a 50 μl reaction volume. Before applying the HPV specific oligomers the presence of human DNA in the samples was controlled with an internal reaction control using α-globin primer pair (PC03/PC04) (Saiki et al. 1985). In the HPV specific PCR reaction each sample was subjected to 2 oligonucleotide primer pairs (16ME21/16ME49; 16ME60/16ME78) (Evander and Wadell 1990). The following temperature cycling scheme was applied in 35 cycles: melting 95°C, 30 s; annealing 55°C, 30 s; elongation 70°C 30 s in a thermal cycler (TECHNE, Duxford, Cambridge, U.K.). Positive (cloned HPV) and negative (without DNA) controls were amplified along with the gynecological specimens throughout the amplification reactions.

Analysis of amplified DNAs: Characteristics sizes of the PCR products were evaluated by electrophoresis of 20 μl aliquots from each reaction mixture in ethidium bromide stained 1.2% SeaKem ME (MC BioProducts, Rockland, Maine, USA) agarose gel. Bands were compared to the migration of a molecular weight standard 2 × 174/HaeIII. All the E7 amplified products were subjected to Southern blot hybridization as well. Blots (Hybond N nylon membrane; Amersham PLL, U.K.) were prehybridized for 1 h then 2 × 10⁶ dpm/ml P³² labeled entire genome of HPV 16 (Multiprime DNA labeling technique Amersham, England) was added into the same mixture for overnight at 42°C (50% formamide, 5 × SSC, 5 × Denhardt's solution, 1 mM EDTA, 0,1% SDS, 100 μg/ml salmon sperm DNA). After washing (high stringency) the filters were autoradiographed for 48 h at −70°C.

Results

All samples prepared contained suitable DNA according to the β-globin gene specific PCR. The results obtained with the oligonucleotide primer pairs 16ME21/16ME49 specific for the E6 and 16ME60/16ME78 specific for the E7 region of the HPV type 16 genome were concordant: these sequences could be