

Detection of Genital Human Papilloma Viruses Using PCR

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Fifteen cases of genital warts were examined for the presence of human papillomavirus (HPV) using polymerase chain reaction (PCR). HPV6/11 DNA were found in all cases of genital warts. The PCR-based methods described here provide a sensitive, accurate means of detecting genital HPVs. (Ann Dermatol 3:(1) 37-39, 1991)

Key Words: Human papillomavirus, Polymerase chain reaction

The genital human papillomaviruses (HPVs) are a group of over 20 distinct virus types that are associated with a number of disease and cancer.¹ For instance, types 16 and 18 are often found in cervical dysplasia and carcinoma, while type 6 and 11 are associated with benign condylomas.^{2, 3, 4} The detection and typing of genital HPVs in normal and diseased tissue samples are important in studies concerned with defining the etiologic role these viruses play in cancers and benign disease. Although a sensitivity of 0.1 copy per cell and good specificity can be achieved with Southern blot hybridization,⁵ the recently developed polymerase chain reaction (PCR), a primer-directed method for the enzymatic amplification of specific DNA sequences, is a more sensitive and technically less difficult method of virus detection.¹

In this study, DNA extracted from 15 patients of anogenital warts were tested for the presence

of HPV6/11 using PCR.

MATERIALS AND METHODS

Patients

The patients comprises fifteen patients with anal and anogenital warts, four children and eleven adults including one case of giant condyloma acuminatum.

Collection of samples

Samples taken from patients were immediately frozen and stored in liquid nitrogen or deep freezer until DNA preparation. DNA was extracted according to a modified procedure previously described.⁶ Samples were treated separately to prevent contamination of viruses.

Polymerase chain reaction

The target sequence for amplification was 280bp in the E5 open reading frame of HPV6 and 360bp in the L1 open reading frame of HPV11. HPV type 6, 11 primers, designated in Table 1 were synthesized on a DNA synthesizer (model 380: Applied biosystems.) Primer sequences were the same as those by Melchers et al.⁷ Two polymerase chain reaction were performed on each sample, one for the detection of HPV6, one for the detec-

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Table 1. Oligonucleotide primers and probes of HPV6, 11

Primers	Size of product (bp)
HPV6/E5 : TAGTGGGCCTATGGCTCGTC (20mer)	280
TCCATTAGCCTCCACGGGTG (20mer)	
HPV11/L1 : GGAATACATGCGCCATGTGG (20mer)	360
CGAGCAGACGTCCGTCCTCG (20mer)	
Probes	
HPV6 : CATTAACGCAGGGGCGCCTGAAATTGTGCC (30mer)	
HPV11 : CGCCTCCACCAAATGGTACACTGGAGGATA (30mer)	

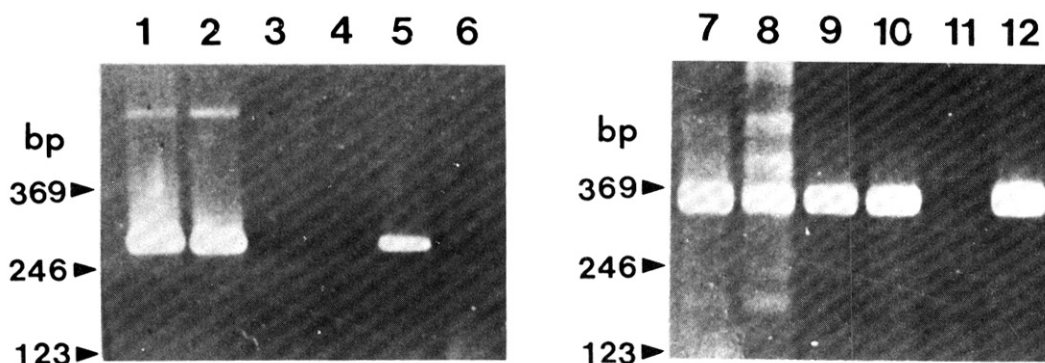


Fig. 1. DNA (0.25 μ g) isolated from genital warts was subjected 30 cycles of amplification with primers specific for HPV6 and 11. Lane 1-6 represent amplified HPV6 fragment from cases of anogenital warts. Lane 7-12 represent the same series of samples amplified for HPV11.

tion of HPV11. PCR was performed according to a modification of the procedure described by Innis and Gelfand.⁸ Amplification of the HPV target sequences was carried out in 100 μ l of a reaction mixture containing 0.25-0.5 μ g of the extracted DNA, 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% gelatin, 200 μ M of each dNTPs, 100 pmol of each primer needed for the specific reaction and one unit of thermostable Taq DNA polymerase (BRL). The samples were overlaid with mineral oil (~100 μ l) to prevent condensation and subject to 30 cycles of amplification using programmable heat block (Hybaid thermal reactor, Hybaid Ltd, U.K.). Each cycle involved heating to 94°C for 30 sec (DNA denaturation), followed by cooling to 50°C for 30 sec (Annealing), and again heating to 70°C for 2 min (chain elongation).

After the last cycle, 10 μ l of the reaction mixture was analyzed for amplification products by gel electrophoresis and ethidium bromide staining.

The final products were transferred on nylon filter and detected with a ³²P end-labeled oligomer probe. The filter was washed in succession with 2x SSC at RT for 15 min, 1x SSC at RT for 15 min and finally 5x SSC at 50°C. Autoradiography was done with intensifying screens.

RESULTS

Figure 1 illustrates the visualization of PCR products on 2% agarose gel electrophoresis. As expected, 280bp and 360bp length fragments were amplified by PCR performed for the detection of HPV6 and HPV 11 respectively.

HPV6 was detected in 10, and HPV11 in 11 out of 15 cases. HPV DNA was found in all cases of genital warts.

DISCUSSION

DNA hybridization procedures are useful for both the detection and typing of HPV. Until recently, Southern blot analysis of extracted tissue DNA was the most sensitive method for HPV detection.⁵ Using new in vitro enzymatic DNA replication methods, even a single 5-10 μ m paraffin embedded tissue section can be examined for the presence or absence of HPV.¹ Theoretically a 10^{13} amplification is achieved by a 40 cycle of in vitro amplification,⁹ allowing possible detection of a single HPV molecule in samples. In this study using 0.25-0.5 μ g of chromosomal DNA, HPV DNA was detected in all cases of condyloma acuminatum. Compared to previous studies PCR is found to be a more sensitive method than the other techniques such as Southern blot hybridization and in situ hybridization.^{10, 11}

Because the synthesized amplification product can be visualized on agarose gels and its specific length estimated, no radioactive labeling or hybridization is required. Compared to hybridization techniques, specific HPV typing is easy to perform by PCR, using primers synthesizing specific HPV fragments with different lengths. Even double infection, sometimes difficult to interpret by Southern blot hybridization, is easily differentiated by PCR. In this study, length of amplified product was designed to be 280bp for HPV6, 360bp for HPV11. Synthesized amplification products hybridized well with oligomers which corresponded the internal segments of amplified products (data not shown). Agarose gel electrophoretic visualization of amplified product was sufficient for the identification of specific HPV type. HPV6 was found in 10 out of 15 cases, and HPV11 in 11 out of 15 cases. There was amplification of both HPV types in 6 out of 15 cases. Although cross contamination can not be ruled out in this study, PCR has been reported to be an excellent technique for detecting double virus infection.

The PCR method is simple to perform, easy to

interpret, and should be included in HPV screening program to gain more insight into the epidemiology of HPV related disease.

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