Detection of Human Papillomavirus DNA in Genital and Laryngeal Papilloma Using the Polymerase Chain Reaction

Hyang Joon Park, M.D., Kyung Chan Park, M.D.*

Department of Dermatology, Inje University College of Medicine, Pusan, Korea
*Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea**

Background: Compared to condyloma acuminatum, the cause of laryngeal papilloma has not been clearly established. Although mode of viral transmission is not known yet, laryngeal papilloma seems to be caused by same viruses as condyloma acuminatum.

Objective: This study was done to investigate the HPV types of condyloma acuminatum and laryngeal papilloma and the epidemiologic relationship between these two diseases.

Methods: The polymerase chain reaction (PCR) was done in fifteen cases of condyloma acuminatum and ten cases of laryngeal papilloma.

Results: Same types of HPV DNA (HPV6,11) were detected in all cases of condyloma acuminatum and laryngeal papilloma.

Conclusion: Laryngeal papilloma is considered a pure viral disease caused by HPV 6 or HPV11 and closely related to condyloma acuminatum caused by HPV 6, 11

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Key Words: Condyloma acuminatum, Laryngeal papilloma, Human papillomavirus

Condyloma acuminatum occurs in the genital area by infection of the human papillomavirus (HPV), mainly type 6, 11 and occasionally type 16, 18[1,2]. Laryngeal papilloma is the most common benign tumor in the larynx[3]. But its cause had not been established until Gissmann et al[4] demonstrated HPV from a laryngeal papilloma lesion. Since then a few investigators showed HPV types which were different from Gissmann's report[3,5,6]. Its mode of transmission is not clear yet, in contrast to condyloma which is sexually transmitted. Therefore, we attempted to investigate the etiologic types of HVP of these tumors and the epidemiological relationship between them.

MATERIALS AND METHODS

Samples: Subjects were 15 patients with genital warts and 10 patients with laryngeal papilloma. Samples taken from the patients were immediately frozen and stored in liquid nitrogen or a deep freezer until DNA preparation.

DNA extraction: Briefly, tissue samples were homogenized and treated with proteinase K and RNase. DNA was isolated by a series of phenol-chloroform-isoamyl alcohol extractions and finally an ethanol preparation. The DNA was collected by centrifiugal force and suspended in distilled water.

PCR: Two PCRs were performed on each sample, one for the detection of HPV 6, the other for HPV 11 using the same oligonucleotide primers as those by Melchers et al[7] (Table 1). Amplification of HPV target sequence was carried out in 100 μl of the reaction mixture containing 0.25-0.5μg of the extracted DNA, 50mM KCl 10mM Tris HCl (pH 8.3), 1.5mM $MgCl_2$, 0.01% gelatin, 200μM of each dNTPs, 100 pmol of each primer and 2.5 unit of thermostable Taq DNA polymerase (Perkin-Elmer Cetus, CT, USA). The samples were over laid with mineral oil (100μl) and subjected to 30 cycles of amplification using a programmable heat block (Hybaid thermal reac-

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Reprint request to: Hyang Joon Park, M.D., Department of Dermatology, Inje Unviversity College of Medicine, Pusan Korea

Table 1. PCR primers and oligonucleotide probes for HPV 6 and 11

Primers or probes	Sequences	Length of product (bp)
HPV 6 primer	+5'-TAGTGGGCCTATGGCTCGTC-3' −5'-TCCATTAGCCTCCACGGGTG-3'	280
HPV 6 probe	5'-CATTAACGCAGGGGCGCCTGAAATTGTGCC-3'	
HPV 11 primer	+5'-GGAATACATGCGCCATGTGG-3' −5'-CGAGCAGACGTCCGTCCTCG-3'	360
HPV 11 probe	5'-CGCCTCCACCAAATGGTACACTGGAGGATA-3'	

Table 2. Results of the polymerase chain reaction

	HPV 6	HPV 11	HPV 6/11
Condyloma acuminatum	11/15	10/15	6/15
(%)	(73)	(66)	(40)
Laryngeal papilloma	7/10	4/10	1/10
(%)	(70)	(40)	(10)
Total	18/25	14/25	7/25
(%)	(72)	(56)	28)

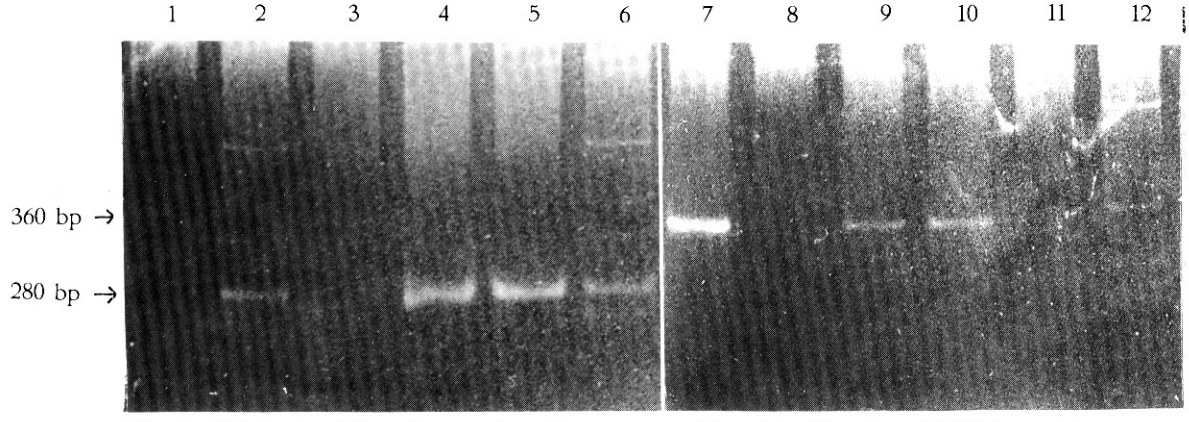

Fig. 1. DNA isolated from laryngeal papillomas was subjected to 30 cycles of PCR. Lane 1-6 represent amplified HPV 6 fragments (280 bp) and land 7-12 represent amplified HPV 11 fragments (360 bp).

tor, Hybaid Ltd, U.K.). Each cycle involved heating to 94°C for 30 sec (DNA denaturation), followed by cooling to 45°C for 30 sec (annealing), and again heating to 72°C for 2 min (chain elogation). After the last cycle, 10μl of the reaction mixture was analyzed by agarose gel electrophoresis. The final products were transferred on to nylon membrane and hybridized with radiolabeled oligonucleotide probes. After one or two days, autoradiography was done.

RESULTS

Figure 1 illustrates the visualization of PCR products on 2% agarose gel electrophoresis. As expected, 280 bp and 360 bp length fragments were amplified by PCR, performed for the detection of HPV 6 and HPV 11 respectively, confirmed by hybridization with virus type specific oligonucleotide probes (data not shown). As shown in Table 2, in all cases of condyloma acuminatum,

HPV 6 or HPV 11 or both could be detected (11 for HPV 6, 10 for HPV 11 and 6 for both). And also in laryngeal papilloma, HPV DNA was found in all patients (7 for HPV 6, 4 for HPV 11 and 1 for both).

DISCUSSION

Condyloma acuminatum and laryngeal papilloma are known to occur as a result of infection by HPV 6 and/or HPV 11,[3-6]. For years a virus, particularly HPV, has been implicated as the causative agent in laryngeal papilloma. However, this hypothesis was not proven until Mounts et al[6] isolated DNA from a laryngeal papilloma by Southern transfer. In 1982, Gissmann et al[4] also demonstrated HPV DNA from a laryngeal papilloma and they designated it HPV 11, in contrast to Mounts et al who characterized it as HPV 6. Hallden and Majmudar[3] reported that about 50% of laryngeal papilloma patients had a history of maternal condyloma during pregnancy or at the time of delivery and they suggested a close relationship between the two diseases. However, because the types of HPV detected are not consistent[4-6] and there are not many cases of laryngeal papilloma patients with maternal condyloma, their epidemiological relationship is not definitely established. In 15 cases of condyloma, we found 11 were positive for HPV 6 and 10 for HPV 11, which was similar to the results of Southern blot hybridization by Park et al[8]. Although there were no available data about laryngeal papilloma in Korea, we obtained similar positive rates to those of condyloma (7% for HPV 6, 40% for HPV 11). HPV 16/18, strongly suspected as a cause of cervical carcinomas, were found in cervical dysplasia and even in the normal cervix[9]. But, for HPV 6/11, it is unknown whether they may cause latent infection in addition to condyloma or laryngeal papilloma. Recently Pao et al[10] reported, using PCR, 40% of normal women had HPV 6/11 in their birth canals, and when repeated a few days later, half of them changed to show negative findings. Therefore, the clinical significance of HPV 6/11 in the birth canal remains to be elucidated and further studies are required to establish when they become pathogenic. The types and positive rates of HPV in both condyloma and laryngeal papilloma detected in our experiment provide strong evidence that the two diseases are caused by the same types of virus and are related to each other epidemiologically. For the next step, we are planning to study the presence of specific HPV In the birth canals of mothers of laryngeal papilloma patients and normal women.

The PCR used here is a recently developed technique for in vitro amplification of specific target DNA sequences to a millionfold[11]. It was used first in the diagnosis of sickle cell anemia[12] and has been valuable for genetic disease diagnosis, detection of pathogens, cancer diagnosis, and cancer research[7-13]. In terms of HPV study, PCR can detect the presence of a virus in a very small sample by amplifying target DNA sequences specific for each type and facilitate epidemiological investigation of the etiological role of HPV in various diseases[14].

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