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Detection of Human Papillomavirus(HPV) DNA in Children's Genital and Respiratory Tract Papilloma and in Birth Canals of Their Mothers†

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= Abstract = **In order to study the epidemiologic relationship between children's papillomaviral disease and papillomaviral infection of mothers, five anogenital warts and seven laryngeal papilloma in children were analyzed by polymerase chain reaction to detect HPV DNA. HPV type 6 was found in 8 cases and HPV type 11 in 7 cases. Both types were found in 3 cases. From these results, anogenital warts and laryngeal papilloma in children are found to be pure viral diseases caused by HPV type 6 and 11. Nine cases of DNA extracted from cervical swabs from mothers of children with condyloma or laryngeal papilloma, were examined to identify possible latent infection of HPV. Among 9 cases, HPV DNA was found in two cases. These results suggest that inapparent infection of HPV type 6, 11 in the birth canal may contribute to the development of these viral diseases in their offspring aside from sexual abuse.**

Key Words: *Human Papillomavirus, Polymerase chain reaction, Condyloma acuminatum, Laryngeal papilloma*

INTRODUCTION

Condyloma acuminatum(Fig. 1) occurs in the genital area by infection of human papillomavirus(HPV), mainly type 6, 11 and occasionally type 16, 18(Oriel 1987; Gross *et al.* 1985). Laryngeal papilloma is the most common benign tumor in the larynx. But its cause had not been established until Gissman *et al.* (1983) demonstrated HPV from a laryngeal papilloma. Since then a few investigators have shown HPV from laryngeal papilloma lesions.

The mode of infection of laryngeal papil-

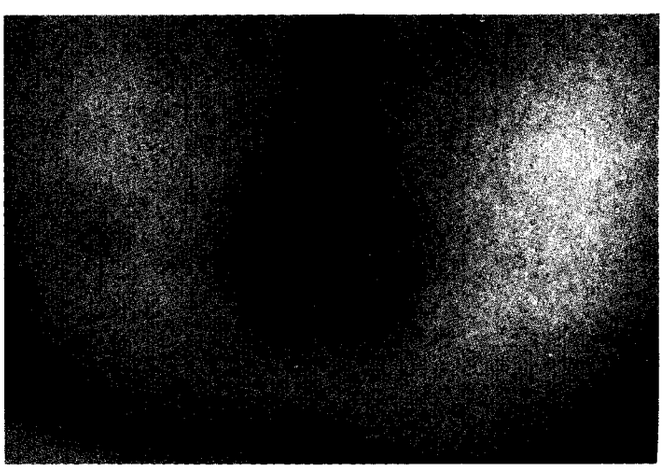

Fig. 1. Perianal condyloma acuminatum

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loma is not yet clear, in contrast to condyloma which is sexually transmitted. It is reported that there are close relationship between laryngeal papilloma of children and genital wart of their mothers(Hallen and Majmudar, 1986). Therefore, we attempted to investigate the etiologic types of HPV of these tumors and the possible epidemiologic relationship between children's papilloma viral disease and papillomaviral infection of their mothers.

MATERIALS AND METHODS

Samples

The patients were all children from the dermatologic and otolaryngologic outpatient clinics of Seoul National University Hospital. Five condyloma acuminatum and seven laryngeal papilloma biopsy samples were stored at -70℃. Nine cases of cervical swabs from mothers of children with condyloma or laryngeal papilloma, were collected with cotton ball from cervix. The collected material was suspended in 2ml phosphate-buffered saline and centrifuged at 4℃ for 10min at 2000rpm. The pellet was stored at -70℃.

Extraction of cellular DNA

The tissues and cervical swabs collected and stored seperately, were digested with proteinase K and RNase. DNA was isolated by phenol and chloroform extraction, and an ethanol precipitation.

Polymerase Chain Reaction

PCRs were performed on each sample. one for the detection of HPV6, the other for HPV11 using the oligonucleotide primers by Melchers *et al.* (1989)(Table 1.). Extracted DNA(0.25-0.5ug) was mixed with PCR buffer(Tris HCl(pH 8.3), KCl 50 mM, 1.5 mM MgCl2, 200ug gelatin/ml), 1 uM upstream primer, 1 uM downstream primer, 200 uM dNTP and 2.5 unit of Taq DNA polymerase(Perkin-Elmer Cetus, CT, USA). The amplification was performed through 30 cycles (denaturation, 94℃ ; annealing, 50℃ ; extension, 72℃) on a programmable heat block(Hybaid thermal reactor: Hybaid Ltd, U. K). After amplification, amplified reaction mixture was then separated by electrophoresis in a 2% agarose gel or 8% polyacrylamide gel. To avoid false positive PCR results, one negative control was processed in each experiment.

RESULTS

After 30 cycles of amplification, amplification products were analyzed by gel electrophoresis. Figure 1 and 2 shows that the sizes of the amplified products were as

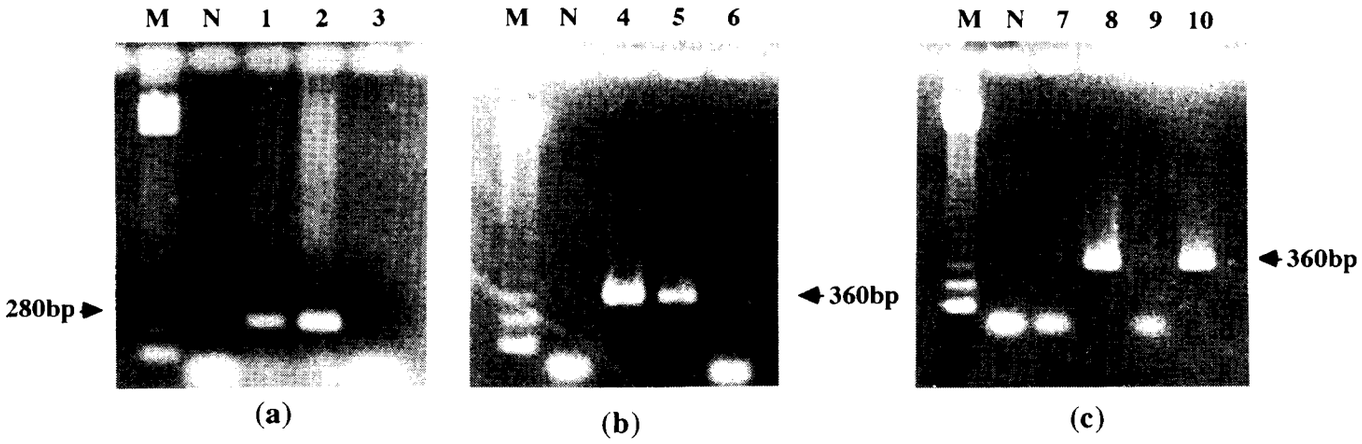

Fig. 2. DNA isolated from condyloma acuminatum and laryngeal papilloma was subjected to 30 cycles of amplification with primers specific for HPV6(a) and HPV11(b). (c): Agarose gel analysis of HPV11 specific PCR amplification of DNA extracted from cervicovaginal cells from mothers of children with condyloma acuminatum and laryngeal papilloma.(M; marker, N; negative control; lane 1, 2: HPV 6; lane 4, 5, 7, 9 : HPV 11)

Table 1. Oligonucleotide primers of HPV 6 and 11

primers	Sequence	Size of product (bp)
HPV 6/E5	: TAGTGGGCCTATGGCTCGTC(20mer)	280
	TCCATTAGCCTCCACGGGTG(20mer)	
HPV 11/L1	: GGAATACATGCGCCATGTGG(20mer)	360
	CGAGCAGACGTCCGTCCTCG(20mer)	

Table 2. Types of HPV in children's condyloma acuminata and juvenile laryngeal papilloma and in birth canals of their mothers

		Patients sample		Mother's cervix	
	Age/sex	HPV6	HPV11	HPV6	HPV11
Condyloma acuminatum	F/3	+	+	NT	
	F/2	+	−	−	−
	F/4	−	+	−	+
	M/2	+	−	−	−
	M/3	−	+	−	+
	M/5	+	+	−	−
	F/1	−	+	−	−
Laryngeal papilloma	M/5	−	+	NT	
	M/7	+	−	−	−
	F/5	+	−	−	−
	M/6	+	−	−	−
	F/14	+	+		NT

NT ; not tested

predicted from design of the primers 280 base pairs for HPV type 6 and 360 base pairs for HPV type 11. In a previous experiment, we demonstrated the specificity of the resulting product by oligomer hybridization(Park *et al.* 1991).

Among the five condyloma acuminata three cases had HPV type 6 and three had HPV type 11; one of these had both types. Among the seven cases of laryngeal papilloma five cases had HPV type 6 and four had HPV type 11; two of these had both types. Of the nine cervicovaginal swab specimens from mothers who had children with condyloma acuminatum or laryngeal papilloma, HPV DNA was found in two cases. The details of the results are summarized in table 2.

DISCUSSION

Polymerase chain reaction is an ingenious new tool for molecular biology. It is so sensitive that a single DNA molecule has been amplified, and single-copy genes are routinely extracted out of complex mixtures of genomic sequences and visualized as distinct bands on agarose gel(Guyer and Koshland 1989). Practical applications of the PCR method in dermatology include the identification of viruses, bacteria and parasites in tissue specimens, the identification of autoimmune-linked human leukocyte antigen[HLA] alleles in patients with cutaneous disease and the identification of specific viruses in certain malignancies(Kurban and Mihm 1992; Kwok and Sninsky 1989; Ou *et al.* 1988).

It has been reported that condyloma acuminatum and laryngeal papilloma appear to be caused by HPV6 or 11 infections(Gissmann *et al.* 1983). Hallden and Majmudar(1986) had shown that in 50% of the child cases of laryngeal papilloma their mothers had a history of condyloma acuminatum in the delivery or pregnancy periods. And they had insisted the close relationship between the two diseases. In the present study, all cases of laryngeal papilloma were found to have HPV DNA, and relative positivity for HPV 6 and 11 were similar with condyloma acuminata as reported by Park *et al.* (1989) with Southern blot hybridization method. Therefore it suggests that the two diseases might be caused by the same virus and be closely associated in the epidemiological respect.

HPV type 16, 18 known as a major cause of cervical neoplasia has been isolated from

normal cervices as well as from cervical dysplasia. This has led to speculation that HPV 16, 18 is able to persist in tissues in an asymptomatic or latent state. But it has not been found that the presence of inapparent infection of HPV 6, 11 is associated with condyloma acuminatum and laryngeal papilloma. Recently Pao *et al.* (1990) has reported that HPV type 6 and 11 was found in cervicovaginal cells in about 30% of normal individuals but with low levels of HPV, and that it appeared transiently. They said that the reasons for the changing appearance of HPV were not clear and the importance of HPV in cervicovaginal cells demanded a more in-depth investigation. In our study, HPV DNA was found in 2 cases among 9 cases of mothers of children with condyloma acuminatum or laryngeal papilloma. This suggests the presence of inapparent infection of HPV. But it is not clear that the infection was transmitted through either inapparent viral infection of the birth canal or by other routes. The clinical importance of this inapparent infection is unclear, but if immunity of the host is compromised, clinical apparent disease may occur by HPV as recurrence of Herpes virus infection.

Therefore, this suggests that inapparent infection of HPV 6, 11 in the birth canal may contribute to development of these viral disease in their offspring aside from sexual abuse. Latency of HPV in vaginal canal need to be studied to determine the importance of this virus in the pathogenesis of genital wart and laryngeal papilloma in these children.

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