ORIGINAL RESEARCH ARTICLE

Human papillomavirus in the oral cavity of children and mode of delivery: a retrospective study

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Summary: Our study aimed to examine the relationship between the presence of human papillomavirus (HPV) in the oral cavity of children and their mode of delivery. We investigated the presence of HPV infection in oral biopsies from 190 children (mean age: 7 years, range: 2–14 years) using the polymerase chain reaction (PCR) technique. Sixteen of 190 children (8.4%) were HPV-positive, with no significant difference between those delivered vaginally and by Caesarean section (C-section). The majority of the HPV-positive children were infected with type 16, whereas in the younger age group HPV type 11 was detected more frequently in children delivered by normal vaginal delivery (NVD) than by C-section. Our findings demonstrate the presence of HPV in the oral cavity of children delivered by both C-section as well as NVD. Further research on the possible modes of transmission of oral HPV infection will enable us to understand the natural history of HPV infection in childhood.

Keywords: human papillomavirus, HPV, children, oral cavity, Caesarean section, vaginal delivery

INTRODUCTION

Oral cavity mucosa seems to be a unique reservoir of mucosal human papillomavirus (HPV) infection in childhood. The presence of HPV DNA in oral swabs or washings from healthy asymptomatic children has been demonstrated in several studies.[1–7] HPV, including 'high-risk' mucosal HPV types 16 and 18, have also been detected in tonsillar and adenoid samples from children with normal mucosa, tonsillar hyperplasia, chronic tonsillitis or adenoid hyperplasia.[8–12] Recently, we evaluated the presence of HPV in adenoid and tonsillar tissues of 109 Greek children using polymerase chain reaction (PCR).[13] The presence of HPV in the oral cavity of both girls and boys has raised the possibility of early exposure to HPV.

Several researchers have proposed that HPV-positive mothers transmit HPV to their children vertically.[14–16] The highest detection rate of HPV DNA in the oral mucosa of children has been found in oral swabs of newborn babies, varying from 4% to 87%.[1,16] HPV infection appears to be acquired at birth; however, the possible modes of perinatal HPV transmission remain to be elucidated. Our study aimed to assess the potential vertical mode of HPV transmission in the oral cavity of children by investigating the maternal, antenatal and birth history of children with HPV-positive oral mucosa.

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METHODS

During the period 1996–2006, 190 biopsy specimens of oral mucosa were obtained from 190 children treated in the Aglaia Kyriakou Children's Hospital, Athens, Greece.

The present study was approved by the Ethics Committee of the University of Crete and parents of all children participating in the study gave written informed consent. Genomic and viral DNA was extracted from paraffin-embedded tissues and stored at −20°C. DNA purity was assessed by an ultraviolet (UV)-visible spectrophotometer estimating the A_{260}/A_{280} ratio and titrated to 200 μg/mL. HPV DNA detection was performed by a PCR-based technique using the general primers GP5+ and GP6+, as previously described.[13] The extracted DNA (1 μL) of each sample was amplified in a total volume of 30 μL containing 5 μmol/L of 10× PCR reaction buffer (200 mmol/L Tris-HCl, pH 8.4, 500 mmol/L KCl), 1.5 mmol/L $MgCl_2$, 200 μmol/L of each dNTP, 0.5 μmol/L of each primer and 0.6 U of recombinant Taq DNA polymerase (Invitrogen Ltd, Paisley, UK). The presence of amplifiable DNA was verified by performing PCR using primers specific for β2-microglobulin.[13]

For distinguishing HPV types we used separate specific pairs of primers for HPV types 16, 18, 33 and 11, giving a different length of amplified DNA, as previously described.[17] The extracted DNA (1 μL) of each sample was amplified in a total volume of 20 μL containing 10× PCR reaction buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L of each dNTP, 0.5 μmol/L of each primer (sense and antisense) and 0.8 U of recombinant Taq DNA polymerase (Invitrogen). PCR products were analysed on 2% agarose gel and photographed on a UV light transilluminator. PCR assay was carried out in a PTC-200

programmable thermal controller (MJ Research Inc, San Francisco, CA, USA). All PCR reactions included appropriate negative controls of DNA extracted from HPV-negative cervical samples collected from women who performed the HPV test at the Department of Virology of the University of Crete. DNA extracted from HeLa cells and the plasmid DNA of HPV types 16, 18, 33 and 11 were used as positive controls.

We investigated in detail the maternal, antenatal and birth history of the children included in the study by reviewing their case-notes retrospectively. A questionnaire was created to assess the maternal history at childbirth, at the time of sampling and five years after sample collection. The mothers were contacted and asked to complete the questionnaire. Clinico-pathological data (age, gender, ethnic origin, residence and medical history) were available for the patients included in the study. Statistical analyses were performed using SPSS software (version 11.5 SPSS Inc., Chicago, IL, USA). Pearson's chi-square test was performed in order to compare the rates of HPV detection in different groups of samples according to the mode of delivery. The *t*-test was used to compare the mean age between HPV-positive and HPV-negative children included in our study. Statistical significance was set at $P < 0.05$.

Table 1 **Demographic characteristics and detection of oral HPV infection in children delivered vaginally ($n = 62$) and by Caesarean section ($n = 128$)**

Children's group	Children delivered vaginally	Children delivered by Caesarean section
	62 (100%)	128 (100%)
Gender		
Boys	28 (45.2%)	60 (46.9%)
Girls	34 (54.8%)	68 (53.1%)
Age group (years)		
Less than five	40 (64.5%)	77 (60.2%)
Five or older	22 (35.5%)	51 (39.8%)
Ethnicity		
Hellenic	34 (54.8%)	89 (60.5%)
Albanian	28 (45.2%)	39 (30.5%)
Residence		
Urban	49 (79.0%)	108 (84.4%)
Rural	13 (21.0%)	20 (15.6%)
Age (years)		
Mean age (SD)	6.8 (3.5)	7.0 (4.1)
Age range	2–13	2–14
HPV		
HPV-positive	6 (9.7%)	10 (7.8%)
HPV-negative	56 (90.3%)	118 (92.2%)

SD = standard deviation; HPV = human papillomavirus

RESULTS

The clinical and epidemiological characteristics of the children included in our study are presented in Table 1. Sixty-two children, 28 boys and 34 girls, were delivered vaginally, while 128 children, 60 boys and 68 girls, by Caesarean section (C-section). The mean age of children delivered vaginally was 6.8 years (standard deviation [SD] 3.5), ranging between two and 13 years. The mean age of children delivered by C-section was 7.0 years (SD 4.1), ranging between two and 14 years. There was no statistically significant difference in the mode of delivery according to maternal age, ethnicity or place of residence ($P < 0.05$).

The histological morphology of the samples showed normal oral mucosa, with no features of HPV infection (koilocytes, dyskeratotic cells or parakeratosis). None of the children had any wart lesions in his or her oral cavity. There was no clinical evidence of child sexual abuse in any child. HPV DNA was detected in 16 (8.4%) of the 190 collected specimens. The most frequently detected type was HPV16 (10/16), while HPV11 was detected in four (4/16) children and HPV33 in one (1/16) child. HPV18 and six were not detected at all, while in one sample the HPV type remained undetermined. No multiple HPV infections were detected in any specimen. Children aged less than five years old showed a higher prevalence of HPV DNA than children aged five years or more ($P < 0.05$).

The mean maternal age during delivery was 28 years (SD 4.8) in the HPV-positive children and 32 years (SD 8.2) in the HPV-negative children ($P < 0.05$). Ten out of 16 HPV-positive children were delivered by C-section and six by normal vaginal delivery (NVD) (Table 1). Interestingly, among children aged less than five years HPV11 was detected more frequently in children delivered by NVD than by C-section ($P < 0.05$). No statistical correlation was observed between the mode of delivery and the presence of HPV DNA infection.

The participation rate of mothers who completed the questionnaire was 175/190 (92.1%). There was no benign or malignant cervical lesion reported by the mothers when the samples were collected (Table 2). Two mothers were diagnosed with high-grade squamous intraepithelial lesions two and five years after the sample was obtained from the children, respectively (Table 2). Interestingly, oral HPV16 was detected in the child of the first mother; this child was delivered by NVD. The child of the second mother was also delivered by NVD, but was HPV-negative.

DISCUSSION

We demonstrate the presence of HPV DNA in the oral cavity of infants delivered by C-section as well as NVD. This finding is of great importance since C-section does not appear to be protective against HPV oral transmission.

Acquisition of HPV infection in the oral mucosa by infants at birth has been proposed by several researchers.[1–7] Neonatal HPV infections may occur transplacentally via amniotic fluid during gestation and delivery and through direct exposure to cervical and genital lesions during birth.[14–16] They are predominantly caused by types 16 and 18 and persist for at least six months in both the genital area and oral cavity.[7] The concordance of HPV types detected in newborn babies and their mothers is in the range of 57–69%.[16] In the study of Cason *et al.*,[7] the transmission rate of HPV from HPV-positive mothers to their infants at 24 hours after delivery was approximately 73% while, at six months of age, persistent HPV16 DNA was detected in 83.3% of cases. HPV18 DNA persistence at this time was only 20%. In a study of oral scrapings from 324 infants during their first three years of life, HPV infection was still detectable in 10% of infants, indicating a decreasing rate of carriage of HPV DNA during the first three years of life.[1] It is still unclear how frequently perinatal HPV infection progresses to clinical lesions, whether genital, laryngeal or oral.

In our study, the mean age of HPV-positive children was lower than that of HPV-negative children. This finding agrees with the results from other reports, including serological studies, showing that higher HPV positivity rates are related

Table 2 **HPV detection and maternal history when samples were obtained (A) and five years after sample collection (B) of children delivered vaginally and by Caesarean section**

	Oral HPV status						
	HPV-positive	HPV typing					
		16	18	11	33	Other	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
(A) HPV detection and maternal history when samples were obtained							
Positive maternal history	0 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Negative maternal history	175 (100%)	14 (8.0%)	11 (6.2%)	0 (0%)	1 (0.6%)	1 (0.6%)	1 (0.6%)
(B) HPV detection and maternal history five years after sample collection							
Positive maternal history	2* (100%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Negative maternal history	173 (100%)	13 (7.5%)	10 (5.7%)	0 (0%)	1 (0.6%)	1 (0.6%)	1 (0.6%)

HPV = human papillomavirus
*High-grade squamous intraepithelial lesion (SIL)

to younger age groups.[8,9,13,18] In the study by Smith *et al.*,[8] a bimodal age distribution was observed, with the highest HPV prevalence in the youngest group aged less than one year and in the oldest group aged 16–20 years. In the study by Chen *et al.*,[9] HPV DNA was detected in 11.5% of preschool children with a mean age of 4.6 years, while the frequency in school children aged 10.4 years was 6.5%. The age-specific prevalence rates of HPV in children and adolescents demonstrate that HPV infection is acquired at birth as well as gradually in childhood.

HPV typing analysis among HPV DNA-positive samples revealed a clear predominance of oral HPV16 infection. This finding is in accordance with the results from our recent analysis of 109 tonsillar and adenoid tissues from Greek children, where the HPV16 rate was 5.9%.[13] A similar predominance of HPV16 has been described in studies of oral swabs, scrapings, tonsils and adenoids from children;[3,4,9–12] HPV 11 was detected less frequently. HPV11 is a common cause of recurrent respiratory papillomatosis (RRP), a potentially life-threatening tumour in childhood.[19,20] Patients with RRP infected with HPV11 are prone to developing more aggressive papillomatosis and require more frequent surgical intervention.[21] HPV11 infection is also related to more frequent need for adjuvant therapies, tracheal and pulmonary disease and tracheostomy.[22]

Researchers have found that RRP rates are higher in children delivered vaginally than those delivered by C-section.[23] It is believed that HPV11 in RRP is transmitted vertically at birth.[22,23] Interestingly, in our study, among children less than five years old, HPV 11 was detected more frequently in children delivered vaginally compared with those delivered by C-section. However, other researchers have demonstrated no statistically significant association between the detection of HPV in the oral cavity and the method of delivery.[8,18] Modes of vertical HPV transmission to oral cavities of children remain controversial. Further research is required to elucidate the role of mode of delivery and RRP pathogenesis.

Although vertical transmission is a possible cause of HPV transmission, other pathways seem to be more likely, including sexual contact, autoinoculation and heteroinoculation and, possibly, indirect transmission via fomites.[16] Mant *et al.*[24] have suggested that HPV infection in the oral cavity of children is a transient event and is most probably acquired from their peers. In their study,[24] they re-assessed swabs from oral mucosa of 20 HPV16-positive and 19 HPV16-negative four- to eight-year-old children 30 months after the initial evaluation. This second visit showed that 40% of the HPV16-positive group had no detectable HPV16 DNA, while 63% of children who were originally HPV16-negative had acquired the virus. Our findings do not exclude the possibility of vertical transmission, but propose that other modes of HPV transmission later in childhood are more likely. Further research on the possible modes of transmission of HPV infection is required. This will enable us to understand the role and the natural history of HPV infection in childhood as well as its possible implications on the effectiveness of HPV vaccination.

REFERENCES

1 Rintala MA, Grénman SE, Järvenkylä ME, *et al.* High-risk types of human papillomavirus (HPV) DNA in oral and genital mucosa of infants during their first 3 years of life: experience from the Finnish HPV family study. *Clin Infect Dis* 2005;**41**:1728–33
2 Myhre AK, Dalen A, Berntzen K, Bratlid D. Anogenital human papillomavirus in non-abused preschool children. *Acta Paediatr* 2003;**92**:1445–52
3 Kojima A, Maeda H, Kurahashi N, *et al.* Human papillomaviruses in the normal oral cavity of children in Japan. *Oral Oncol* 2003;**39**:821–28
4 Rice PS, Mant C, Cason J, *et al.* High prevalence of human papillomavirus type 16 infection in children. *J Med Virol* 2000;**61**:70–75
5 Chatterjee R, Mukhopadhyay D, Murmu N, Mitra PK. Correlation between human papillomavirus DNA detection in maternal cervical smears and buccal swabs of infants. *Indian J Exp Biol* 1998;**36**:199–202
6 Armstrong DK, Handley JM. Anogenital warts in prepubertal children: pathogenesis, HPV typing and management. *Int J STD AIDS* 1997;**8**:78–81
7 Cason J, Kaye JN, Jewers RJ, *et al.* Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. *J Med Virol* 1995;**47**:209–18
8 Smith EM, Swarnavel S, Ritchie JM, *et al.* Prevalence of human papillomavirus in the oral cavity/oropharynx in a large population of children and adolescents. *Pediatr Infect Dis J* 2007;**26**:836–40
9 Chen R, Sehr P, Waterboer T, *et al.* Presence of DNA of human papillomavirus 16 but no other types in tumor-free tonsillar tissue. *J Clin Microbiol* 2005;**43**:1408–10
10 Strome SE, Savva A, Brissett AE, *et al.* Squamous cell carcinoma of the tonsils: a molecular analysis of HPV associations. *Clin Cancer Res* 2002;**8**:1093–100
11 Watanabe S, Ogura H, Fukushima K, Yabe Y. Comparison of Virapap filter hybridisation with polymerase chain reaction and Southern blot hybridisation methods for detection of human papillomavirus in tonsillar and pharyngeal cancers. *Eur Arch Otorhinolaryngol* 1993;**250**:115–19
12 Fukushima K, Ogura H, Watanabe S, *et al.* Human papillomavirus type 16 DNA detected by the polymerase chain reaction in non-cancer tissues of the head and neck. *Eur Arch Otorhinolaryngol* 1994;**251**:109–12
13 Mammas I, Sourvinos G, Michael C, Spandidos DA. Human papilloma virus in hyperplastic tonsillar and adenoid tissues in children. *Pediatr Infect Dis J* 2006;**25**:1158–62
14 Fredericks BD, Balkin A, Daniel HW, *et al.* Transmission of human papillomaviruses from mother to child. *Aust N Z J Obstet Gynaecol* 1993;**33**:30–32
15 Puranen M, Yliskoski M, Saarikoski S, *et al.* Vertical transmission of human papillomavirus from infected mothers to their newborn

babies and persistence of the virus in childhood. *Am J Obstet Gynecol* 1996;**174**:694–99
16 Syrjanen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. *Crit Rev Oral Biol Med* 2000;**11**:259–74
17 Mammas I, Zafiropoulos A, Koumantakis E, *et al.* Transcriptional activation of H- and N-ras oncogenes in human cervical cancer. *Gynecol Oncol* 2004;**92**:941–48
18 Summersgill KF, Smith EM, Levy BT, *et al.* Human papillomavirus in the oral cavities of children and adolescents. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;**91**:62–69
19 Hartley C, Hamilton J, Birzgalis AR, Farrington WT. Recurrent respiratory papillomatosis – the Manchester experience, 1974–1992. *J Laryngol Otol* 1994;**108**:226–29
20 Rimell FL, Shoemaker DL, Pou AM, *et al.* Pediatric respiratory papillomatosis: prognostic role of viral typing and cofactors. *Laryngoscope* 1997;**107**:915–18
21 Maloney EM, Unger ER, Tucker RA, *et al.* Longitudinal measures of human papillomavirus 6 and 11 viral loads and antibody response in children with recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2006;**132**:711–15
22 Wiatrak B. Overview of recurrent respiratory papillomatosis. *Curr Opin Otolaryngol Head Neck Surg* 2003;**11**:433–41
23 Gerein V, Schmandt S, Babkina N, *et al.* Human papilloma virus (HPV)-associated gynecological alteration in mothers of children with recurrent respiratory papillomatosis during long-term observation. *Cancer Detect Prev* 2007;**31**:276–81
24 Mant C, Kell B, Rice P, *et al.* Buccal exposure to human papillomavirus type 16 is a common yet transitory event of childhood. *J Med Virol* 2003;**71**:593–98

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