Detection of Human Papillomavirus in Bronchoalveolar Lavage Samples in Immunocompetent Children

Ioannis N. Mammas, MD, PhD, Apostolos Zaravinos, PhD, George Sourvinos, PhD, and Demetrios A. Spandidos, PhD

Background: Human papillomavirus (HPV) has been detected in lungs of adults and has been proposed to play a role in lung carcinogenesis. However, no data are currently available on the incidence of HPV in the lower respiratory tract of children.

Purpose: To determine the prevalence of HPV deoxyribonucleic acid (DNA) in bronchoalveolar lavage (BAL) samples were obtained from asymptomatic immunocompetent children.

Methods: A total of 71 children between 2 and 12 years of age were prospectively enrolled. Detection of HPV DNA and HPV typing were performed using polymerase chain reaction-based techniques.

Results: Of the 71 BAL samples, HPV DNA was detected in 6 children. Coinfection with HPV 16, 18, and 31 was detected in 2 children, while 4 children were positive for non-"high-risk" HPVs.

Conclusions: This preliminary case-control study indicates the presence of HPV DNA in BAL samples in children. The possible presence of HPV in the lower respiratory tract of children requires further investigation to elucidate the actual epidemiologic condition, the potential modes of its transmission, and its possible causative relationship in lung carcinogenesis in adulthood.

Key Words: HPV, bronchoalveolar lavage, PCR, children

(*Pediatr Infect Dis J* 2011;30: 000–000)

Human papillomaviruses (HPVs) are double-stranded deoxyribonucleic acid (DNA) viruses that comprise a family of more than 130 different types of HPVs.[1] HPVs are common human pathogens associated with a wide range of cutaneous and mucosal infections in adults as well as in children.[2] Different HPV types can cause common warts, anogenital warts, "low-grade," and "high-grade" squamous intraepithelial cervical lesions as well as cervical cancer. Recurrent respiratory papillomatosis has also been associated with HPV infection in a number of studies.[2,3]

"High-risk" HPV types play a critical role in the development of cervical neoplasia in adulthood and its progression to cervical cancer.[1,4] Mucosal HPV types 16 and 18 are the most common high-risk types detected in the female anogenital system and in more than 70% of women with cervical cancer.[4] The recently introduced vaccination program against HPV is expected to prevent HPV 16- and 18-related cervical cancer in women. Recently, further evidence for the presence of HPV in nongenital cancers was noted by a number of researchers proposing the potential role of HPV in nongenital carcinogenesis.[5] Notably, HPV detection rates in lung cancer samples were found to range from 0% to 24.5%.[6,7] Additionally, it is considered as the second most common risk factor for lung cancer if the cause-effect link is indeed valid.

Because the question regarding the mode of HPV transmission in human lungs has yet to be determined, we conducted a prospective study to determine the presence of HPV in bronchoalveolar lavage (BAL) from asymptomatic immunocompetent children, using polymerase chain reaction (PCR) techniques. At present, the epidemiology of HPV infection in the lungs of children remains unknown; no data on the epidemiology of HPV in BAL or in lung tissues in children are available in the literature.

MATERIALS AND METHODS

BAL samples were obtained after approval from immunocompetent children who underwent bronchoscopy at the "Penteli" Children's Hospital in Athens, Greece. The indications for bronchoscopy included suspicion of foreign body, chronic wet cough, persistent atelectasis, and recurrent pneumonias. The children's parents were informed about the survey, and 71 samples were obtained following informed consent between 2009 and 2010. The histologic analysis of the material was performed at the Department of Pathology of the "Sismanoglio" General Hospital in Athens, Greece.

Genomic and viral DNA was extracted from all of the collected samples as previously described[8] and stored at −20°C. DNA concentration was calculated using the NanoDrop 1000 Spectrophotometer. Specimens were examined for the presence of amplifiable DNA using a set of primers for the *β2-microglobulin* gene. A total of 20 ng of genomic DNA was applied to each PCR reaction, and all samples were run in duplicate. The samples were initially examined for the presence of nontype-specific HPV DNA using the general HPV primers, GP5+/6+.[9] Appropriate negative and positive controls were included in each PCR reaction to exclude contamination events and to establish the specificity of primer-directed amplification. Recombinant plasmid-carrying HPV type-generic type sequence served as a positive control for GP-HPV genome detection. For the general screening of HPV DNA, HeLa cells transfected with conserved L1 sequences among the HPV strains were used as a positive control.

Downstream processing was performed for the general HPV-positive samples with the high-risk HPV typing Real-TM kit (Sacace Biotechnologies, Commo, Italy) to qualitatively detect and genotype HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. The primers used targeted the region E7 of the HPV genome. After an initial denaturation at 95°C for 15 minutes, the samples were subjected to 45 cycles of incubation at 95°C for 20 seconds and at 60°C for 60 seconds. Fluorescence was measured at 60°C on the channels FAM, HEX, ROX, and Cy5. *β-globin* was used as an internal control. Threshold fluorescence was set at 100 for channel CY5, 200 for channel ROX, 50 for channel HEX, and 300 for channel FAM.

Accepted for publication October 26, 2010.
From the Department of Clinical Virology, School of Medicine, University of Crete, Heraklion, Crete, Greece.
Address for correspondence: George Sourvinos, PhD, Department of Clinical Virology, School of Medicine, University of Crete, Heraklion 71110, Crete, Greece. E-mail: sourvino@med.uoc.gr.

ISSN: 0891-3668/11/3005-0001
DOI: 10.1097/INF.0b013e3182039a4b

RESULTS

BAL samples from 71 children, 33 girls and 38 boys were studied. The mean age of our study population was 7 years (Standard deviation, ±2.6 years), ranging between 2 and 12 years. In 8 children, the indication for bronchoscopy was the suspicion of a foreign body, in 42 children chronic wet cough, in 4 children persistent atelectasis, and 17 children were investigated because of recurrent pneumonias. HPV DNA was detected in 6 children; 3 girls and 3 boys. Notably, coinfection with high-risk HPVs 16, 18, and 31 was detected in 2 children; 1 boy and 1 girl. Non-high-risk nontyped HPVs were detected in 4 children. Among the HPV-positive samples, in 4 children the indication for bronchoscopy was chronic wet cough, in 1 child persistent atelectasis, and 1 child was investigated because of recurrent pneumonias. In all samples, histologic analysis did not reveal any specific characteristic for HPV infection.

DISCUSSION

This is the first study to report the presence of HPVs in the lower respiratory tract of children. Our findings suggest that lower respiratory tract mucosa can be a unique reservoir of both high-risk and non-high risk mucosal HPV infection in childhood. At present, several researchers have demonstrated the presence of HPV DNA in the upper respiratory tract of children by studying oral swabs or washings from healthy asymptomatic children.[10–12] HPVs, including high-risk mucosal HPVs 16 and 18, have also been detected in tonsils or adenoid samples from children with normal mucosa, tonsillar hyperplasia, chronic tonsillitis, or adenoid hyperplasia.[13,14]

The effect of the presence of these oncogenic HPV types in lungs of children has yet to be clarified. Almost 20 years after initial reports[15–17] about the association of HPV and lung carcinogenesis, the presence of HPVs in lung carcinoma samples has only been recently highlighted again. In a review by Klein et al,[6] the overall incidence of HPV in lung cancer samples was 24.5%. This observation indicates the potential role of HPV in lung carcinogenesis and denotes HPV as the second most common risk factor for lung cancer, if the cause-effect link is indeed valid. Notably, there is geographic variation in the HPV detection frequency in lung cancer samples, with higher mean incidence rates reported in Asia (35.7%) compared with Europe (17%) and America (15%). This variation can be attributed to the epidemiology of the HPV itself, although information on HPV prevalence worldwide is absent.[6]

The question regarding the route of HPV transmission to lung tissue has yet to be determined. It is known that HPV normally invades healthy tissue by direct mucosal contact,[18] and it is postulated that it reaches the lung site via blood circulation,[19] while the possibility of transmission from the cervix to the oral cavity and then to the larynx and lung is also plausible.[7] Once HPV presents to host cells, it is believed to attach to cells expressing heparin sulfates, which act as primary receptors for HPV and it is internalized to interfere with p53 and Rb proteins.[20] Importantly, our findings indicate lung tissue to be permissive to multiple HPV infection.

In the upper respiratory tract, the highest detection rates of HPV infection have been observed in oral swabs of newborn babies, varying from 4% to 87%.[21–23] HPV infection in oral mucosa of infants appears to be acquired at birth.[21] It has been proposed that newborn babies are exposed to the cervical HPV infection of their mothers, and oral HPV infection persists for at least 6 months of age, with a rate of decrease during the first 3 years of life.[21] It is still unclear how frequently perinatal infection progresses to clinical lesions, whether genital, laryngeal, or oral. The concordance of HPV types detected in newborn babies and their mothers ranges from 57% to 69%, and it has been proposed that infants may also acquire the HPV infection postnatally.[22,23] This hypothesis is supported by various researchers, who found a bimodal age distribution of HPVs, with the highest HPV prevalence in children aged less than 1 year and adolescents and young adults aged 13 to 20 years.[10,12] These findings are crucial because they demonstrate that HPV oral infection is acquired at birth as well as gradually in childhood. In the present study, the presence of HPV infection in the lungs of the studied immunocompetent children indicates that HPV infection of the lungs can be acquired before adolescence.

Future research from various ethnic populations and a larger case series are required to investigate the presence as well as the clinical significance of HPV in human lungs during childhood.

ACKNOWLEDGMENTS

The authors thank all parents and their children who participated in the survey and the staff of the Pediatric Department and the Department of Clinical Microbiology of the "Penteli" Children's Hospital and Dr D. Mermiri, Dr C. Priftis, Dr A. Makri, and Dr A. Voyatzi for their assistance with sample collection.

REFERENCES

1. zur Hausen. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer.* 2002;2:342–350.
2. Mammas I, Sourvinos G, Spandidos DA. Human papilloma virus (HPV) infection in children and adolescents. *Eur J Pediatr*. 2009;168:267–273.
3. Mammas I, Sourvinos G, Vakonaki E, et al. Novel human papilloma virus (HPV) genotypes in children with recurrent respiratory papillomatosis. *Eur J Pediatr*. 2010;169:1017–1021.
4. Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer*. 2002; 88:63–73.
5. Mammas IN, Sourvinos G, Zaravinos A, et al. Vaccination against human papilloma virus (HPV): epidemiological evidence of HPV in non-genital cancers. *Pathol Oncol Res.* 2010 Jul 18. [Epub ahead of print].
6. Klein F, Amin Kotb WF, Petersen I. Incidence of human papilloma virus in lung cancer. *Lung Cancer*. 2009;65:13–18.
7. Chen YC, Chen JH, Richard K, et al. Lung adenocarcinoma and human papillomavirus infection. *Cancer*. 2004;101:1428–1436.
8. Zaravinos A, Kanellou P, Spandidos DA. Viral DNA detection and RAS mutations in actinic keratosis and nonmelanoma skin cancers. *Br J Dermatol*. 2010;162:325–331.
9. de Roda Husman AM, Walboomers JM, van der Brule AJ, et al. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol*. 1995;76:1057–1062.
10. Summergill 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.
11. Cason J, Mant C. High-risk mucosal human papillomavirus infections during infancy & childhood. *J Clin Virol*. 2005;32:S52–S58.
12. 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;26:836–840.
13. Syrjanen S. HPV infections and tonsillar carcinoma. *J Clin Pathol*. 2004; 57:449–455.
14. 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;43:1408–1410.
15. Syrjänen K, Syrjänen S, Kellokoski J, et al. Human papillomavirus (HPV) type 6 and 16 DNA sequences in bronchial squamous cell carcinomas demonstrated by in situ DNA hybridization. *Lung*. 1989;167:33–42.
16. Kulski JK, Demeter T, Mutavdzic S, et al. Survey of histologic specimens of human cancer for human papillomavirus types 6/11/16/18 by filter in situ hybridization. *Am J Clin Pathol*. 1990;94:566–570.
17. Béjui-Thivolet F, Liagre N, Chignol MC, et al. Detection of human papillomavirus DNA in squamous bronchial metaplasia and squamous cell

carcinomas of the lung by in situ hybridization using biotinylated probes in paraffin-embedded specimens. *Hum Pathol*. 1990;21:111–116.

18. Stanley MA, Pett MR, Coleman N. HPV: from infection to cancer. *Biochem Soc Trans*. 2007;35:1456–1460.
19. Tseng CJ, Pao CC, Lin JD, et al. Detection of human papillomavirus types 16 and 18 mRNA in peripheral blood of advanced cervical cancer patients and its association with prognosis. *J Clin Oncol*. 1999;17:1391–1396.
20. Selinka HC, Florin L, Patel HD, et al. Inhibition of transfer to secondary receptors by heparan sulfate-binding drug or antibody induces noninfectious uptake of human papillomavirus. *J Virol*. 2007;81:10970–10980.
21. Rintana M, Grenman S, Jarvenkyla 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;41:1728–1733.
22. Syrjanen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. *Crit Rev Oral Biol Med*. 2002;11:259–274.
23. 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;71:593–598.