JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2008, p. 369–370 Vol. 46, No. 1
0095-1137/08/$08.00+0 doi:10.1128/JCM.01819-07

Detection of Respiratory Viruses and Atypical Bacteria in Children's Tonsils and Adenoids▽

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Received 13 September 2007/Returned for modification 18 October 2007/Accepted 26 October 2007

The tonsils and adenoids of 44 children were analyzed for the detection of respiratory syncytial virus, influenza virus, parainfluenza virus, adenovirus, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. Viruses were detected in 47.7% of the children and 37.3% of the specimens, with adenovirus and parainfluenza viruses being the most frequently detected microorganisms.

Even though tonsillectomy and adenoidectomy are among the most frequently performed interventions in the pediatric population, with indications varying from recurrent or chronic disease to hypertrophy and obstructive sleep disorders, a consensus as to the main causative organisms seems to be lacking (4).

Although the incidence of viruses in respiratory tract infections has been evaluated by different authors, their real role in infections still remains underestimated, and it remains largely unknown whether they have to be considered "bystander" microorganisms.

This study evaluated the presence of respiratory syncytial viruses (RSVs) A and B; influenza virus A (IVA); influenza virus B (IVB); human parainfluenzaviruses 1, 2, and 3 (HPIVs); human adenoviruses (HAds); and atypical bacteria (*Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*) in the tonsils and adenoids from 44 patients (33 males; mean age, 5.78 ± 3.05 years) undergoing tonsillectomy and/or adenoidectomy for the first time during the period from 1 May to 31 July 2006.

The inclusion criteria, according to the Italian National Program for Clinical Guidelines (3), were obstructive sleep apnea syndrome (OSAS; $n = 17$), severe recurrent adenoiditis (SRA) and/or severe recurrent tonsillitis (SRT; at least five episodes in 1 year) (SRA/SRT; $n = 17$), and persistent otitis media with effusion (OME; 9 months of bilateral effusion with hearing loss) and/or recurrent acute otitis media (rAOM; at least six episodes in 1 year) (rAOM/OME; $n = 10$). The exclusion criteria were an acute respiratory infection in the previous 4 weeks and at the time of surgery, antibiotic treatment in the previous 4 weeks, craniofacial abnormalities, immunological disorders, and chronic medical diseases.

Cold tonsillectomy with extracapsular dissection and adenoidectomy under endoscopic control were performed for all the patients while they were under general anesthesia, after preoperative antibiotic prophylaxis with expanded-spectrum cephalosporins or penicillin. The local ethics committee approved the study protocol, and written informed consent was obtained from the parents of all study participants and from children >7 years old. Information regarding the medical history of each child, including the frequencies of upper and lower airway infections, otitis media, and tonsillitis and antibiotic treatments, was also recorded.

Both adenoid and tonsillar specimens were obtained from 23 children, while only adenoid specimens or tonsillar specimens were collected from 19 and 2 children, respectively. Reactive hyperplasia of the tonsillar and/or adenoid tissue with no evidence of malignancy was detected in all specimens.

Immediately after removal, the tissues were frozen at −80°C until nucleic acid extraction, when frozen aliquots of about 20 to 30 mg were homogenized for 20 to 40 s with an Ultra-Turrax T8 homogenizer (Janke & Kunkel GmbH & Co., Staufen, Germany) in the lysis buffers provided with the nucleic acid extraction kits, as described below.

The detection of RSV, IVs, HPIVs, and HAds was carried out by a real-time reverse transcription-PCR (RT-PCR) on a Rotor-Gene 3000 instrument (Corbett Research, Cambridge, United Kingdom) and by using commercially available RT-PCR assays (Fast set; Arrows Diagnostics S.r.l., Genoa, Italy).

Nucleic acids were extracted by using an RNeasy mini kit (Qiagen), according to the protocol for the isolation of nucleic acids from animal tissues. After tissue homogenization in 600 μl buffer, each sample was eluted in 100 μl RNase-free water.

Primers were designed from conserved regions of genes encoding the matrix protein and the nucleoprotein of IVs, the fusion protein of RSV, hemagglutinin neuroaminidase of HPIVs, and the hexon antigen of HAds (8).

For the detection of atypical bacteria, after DNA extraction (High Pure PCR template preparation kit; Roche Applied Science, Indianapolis, IN) nested PCRs were performed as described previously (1, 2). Primers for MP-1 and MP-2 and for the major outer membrane protein were chosen for *M. pneumoniae*- and *C. pneumoniae*-specific amplification, respectively (1, 2, 7). All samples were analyzed by PCR for the

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▽ Published ahead of print on 7 November 2007.

TABLE 1. Microbiological findings according to clinical disease requiring surgery

Pathogen(s)	No. of indicated tissue samples with pathogen from patients with[a]:							
	OSAS (n = 17)		SRA/SRT (n = 17)		rAOM/OME (n = 10)		Total (n = 44)	
	Tonsils (n = 5)	Adenoids (n = 17)	Tonsils (n = 17)	Adenoids (n = 15)	Tonsils (n = 3)	Adenoids (n = 10)	Tonsils (n = 25)	Adenoids (n = 42)
HAds	2	5	2	4	0	3	4	12
HPIVs	0	0	2	0	2	0	4	0
IVs	1	0	0	0	0	0	1	0
HAds + HPIVs	0	0	1	0	1	0	2	0
HAds + *C. pneumoniae*	0	0	0	0	0	1	0	1
C. pneumoniae	0	0	0	0	0	1	0	1
Total positive samples	3	5	5	4	3	5	11	14

[a] Among the 21 patients (47.7% of the total) in whom microbes were detected, 6 had OSAS (35.3% of the total in the OSAS group), 8 had SRA/SRT (47.1% of the total in the SRA/SRT group), and 7 had rAOM/OME (70.0% of the total in the rAOM/OME group).

presence of β-actin DNA in order to confirm the presence of DNA in the samples.

Tonsillar and/or adenoid specimens from 21 of 44 patients (47.7%) were found to be positive for at least one of the microorganisms for which tests were conducted (Table 1). In two patients, one undergoing tonsillectomy for SRT and the other undergoing adenotonsillectomy for rAOM/OME and SRT, HAds and HPIVs were simultaneously found in the tonsils. HAds and *C. pneumoniae* were simultaneously detected in the adenoids from one patient with ear disease. In the only patient positive for IVB in the tonsils, HAds were detected in the adenoids. HAds were detected in both the adenoids and the tonsils of two children.

Among the tonsillar tissue samples, 11/25 (44.0%) samples were found to be positive, with HAds and HPIVs being the most frequently detected viruses (6/25; 24.0%), followed by IVs (1/25; 4.0%). HAd was the only virus detected in the adenoid tissues (13/42; 30.9%), while *C. pneumoniae* was detected in two specimens (4.8%). RSV and *M. pneumoniae* were never detected.

To the best of our knowledge, this is the first study to have simultaneously evaluated tissue specimens from children undergoing elective surgery in the absence of an acute upper respiratory infection for the presence of viruses and atypical bacteria. The persistence of some microorganisms, such as atypical bacteria or viruses that have not necessarily been cleared, in specific cells or tissues of infected individuals may involve stages of both silent and productive infection without rapid killing or even the production of excessive damage to the host cells. The reactivation or persistence of microbes may trigger various stimuli, including changes in cell physiology and physical stress or trauma, in which case only surgical removal can sometimes resolve the infection. The significance of the presence of HAd in adenoids and/or tonsils remains unclear. The virus could contribute to the pathogenesis of clinical conditions requiring surgery. A defective or suboptimal immune response to these microorganisms might cause chronic antigen stimulation, sustaining a low grade of inflammation and favoring either adenoidal or tonsillar hypertrophy or recurrent infection. This hypothesis seems to be supported by the finding of samples positive for HAd in patients with either OSAS or recurrent tonsillar and ear infections at the same frequency.

The low rate of detection of *C. pneumoniae* seems to be in contrast to the data reported in a similar study by Normann and colleagues, who found by a immunohistochemical procedure a positivity rate of 98.5% for children undergoing adenoidectomy (5). This difference might be due to the different detection method used, since immunohistochemical assays generally show higher sensitivities than PCRs with tissue specimens (5).

The correlation between persistent infections or simply the presence of some microorganisms, such as HAds, HPIVs, and IVs, in our samples, involving both modulation of the virus and cellular gene expression and modification of the host immune response, is far from being able to be postulated. For this purpose, further studies with a large population are needed to fully evaluate the relationship between virus infection and hyperplasia.

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