



Respiratory viruses are continuously detected in children with chronic tonsillitis throughout the year



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ABSTRACT

Objective: To evaluate the oscillations on the viral detection in adenotonsillar tissues from patients with chronic adenotonsillar diseases as an indicia of the presence of persistent viral infections or acute subclinical infections.

Study design: Cross-sectional prospective study.

Setting: Tertiary hospital.

Methods: The fluctuations of respiratory virus detection were compared to the major climatic variables during a two-year period using adenoids and palatine tonsils from 172 children with adenotonsillar hypertrophy and clinical evidence of obstructive sleep apnoea syndrome or recurrent adenotonsillitis, without symptoms of acute respiratory infection (ARI), by TaqMan real-time PCR.

Results: The rate of detection of at least one respiratory virus in adenotonsillar tissue was 87%. The most frequently detected viruses were human adenovirus in 52.8%, human enterovirus in 47.2%, human rhinovirus in 33.8%, human bocavirus in 31.1%, human metapneumovirus in 18.3% and human respiratory syncytial virus in 17.2%. Although increased detection of human enterovirus occurred in summer/autumn months, and there were summer nadirs of human respiratory syncytial virus in both years of the study, there was no obvious viral seasonality in contrast to reports with ARI patients in many regions of the world.

Conclusion: Respiratory viruses are continuously highly detected during whole year, and without any clinical symptomatology, indicating that viral genome of some virus can persist in lymphoepithelial tissues of the upper respiratory tract.

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1 Introduction

Acute and chronic respiratory diseases are highly frequent and associated with excessive morbidity and mortality, especially in children, thus having great impact on public health [1,2]. Respiratory viruses play major roles as etiologic agents of acute

respiratory infections (ARI) in children, but their association with chronic respiratory diseases, especially with adenotonsillar hypertrophy, has only recently become the focus of investigation [3–5].

Adenotonsillar hypertrophy is the most common cause of sleep apnea in children, resulting in craniofacial growth changes and, in severe cases, leading to right ventricular dysfunction and cor pulmonale [6–8]. As a consequence, adenotonsillectomy is the most frequent surgical procedure performed by otorhinolaryngologists [9]. The aetiologies of chronic hypertrophic adenotonsillar diseases have not been properly established, but are believed to be multifactorial, including allergies, bacterial colonisation and viral infections [3,10,11].

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We have previously reported high rates of detection of respiratory virus genomes in tonsils and adenoids from patients with chronic adenotonsillar diseases, suggesting a significant association of viruses, particularly picornaviruses, with severe tonsillar hypertrophy [3]. However, no conclusive evidence of productive – acute or persisting – viral infection, as opposed to virus latency, has been established.

In general, peaks of respiratory virus detection in children with ARI occur with marked seasonal variations in temperate and subtropical regions [12,13]. In tropical areas, the seasonal pattern of viral detection is more difficult to be analysed, due to the heterogeneity of data in several parts of the world. However, respiratory viruses have been mainly observed during the rainy seasons [14]. In southeast Brazil, a region of transition between tropical and subtropical climates, peaks of viral ARI tend to occur during cooler months [15–17]. The present paper reports analyses of over time variations in rates of detection of respiratory viruses in tissues and secretions removed from children undergoing tonsillectomy while in the absence of ARI symptoms. The rationale was that if detection of respiratory viruses in hypertrophic tonsillar tissues oscillated with variations in temperature and rainfall, in a way similar to what occurs among ARI patients, this would suggest an association with acute subclinical respiratory viral infections, rather than prolonged asymptomatic harbouring of viral nucleic acids in the tissues.

2 Patients and methods

2.1 Patients and sampling

Respiratory virus genomes were searched in adenoids (AD), palatine tonsils (PT) and nasopharyngeal secretions (NPS) obtained from all 172 children (91 males) aged 1–13 years (mean 5.8 years) who underwent adenotonsillectomy to treat adenotonsillar hypertrophy with clinical evidence of obstructive sleep apnoea syndrome [6] or recurrent adenotonsillitis according to Paradise criteria [18]. Patients were treated at the division of Otorhinolaryngology of the School of Medicine of Ribeirão Preto, University of São Paulo, between May 2010 and June 2012. Patients with signs/symptoms of acute respiratory infections within the last four weeks prior to surgery and patients with immunodeficiencies were excluded from the study. Indeed, the exclusion of these patients was a safety criterion for surgery. All clinical samples obtained in this study were maintained in a preservative solution (RNA later – Invitrogen, Carlsbad, CA, USA) at -70°C until nucleic acid extraction.

2.2 Ethics statement

The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the University Hospital Clinical Research Ethics Committee (file number 10466/2008). A written informed consent was obtained from all parents and guardians prior to enrolment.

2.3 Detection of respiratory viruses

Nucleic acids from AD, PT and NPS were obtained from 30 mg of tissue or 200 μL of secretion using the AllPrep DNA/RNA mini kit (Qiagen GmbH, Hilden, Germany) or QIAamp Min Elute Virus Spin Kit (Qiagen GmbH, Hilden, Germany), respectively. All samples were tested for the presence of genomes of human adenovirus (HAdV), human enterovirus (HEV), human rhinovirus (HRV), human bocavirus (HBoV), human respiratory syncytial virus (HRSV), metapneumovirus (HMPV), human influenza virus (FLU), human parainfluenza virus (HPIV) and human coronavirus (HCoV) by TaqMan real-time PCR.

The detailed description of each PCR, including the primers sequences, can be obtained in a previously published paper [3]. In the present study were included 51 patients, extending the observation period to two years, allowing that the seasonal pattern of viral circulation was determined in these patients. The analysis of the seasonality of respiratory viruses in adenotonsillar tissues was performed cross matching the virus presence to the temperature and rainfall.

2.4 Analysis of climate variations

Ribeirão Preto is a city in the state of São Paulo, southeast Brazil with a population of 619,746, located at $21^{\circ}10'40''\text{S}$ and $47^{\circ}48'36''\text{W}$, 500 m above sea level. The climate is a transition between tropical and subtropical conditions, with annual average temperature of 23°C , with dry mild winters and hot rainy summers. During this study, the mean monthly minimal and maximal daily temperatures were respectively 18.3°C (range, $13.5\text{--}20.5$) and 25.3°C (range, $23.5\text{--}26.8$). Rainfall is the major climate variable, with yearly rainy seasons between November and March and dry season from June to September. The mean monthly accumulated rainfall throughout the study was 108 mm, ranging from 0 to 533 mm. Accumulated rainfall and mean seasonal temperatures were obtained from the site of the Integrated Center of Agrometeorological Information of São Paulo State (<http://www.ciiagro.sp.gov.br>).

3 Results

Rates of detection of respiratory viruses in adenoids, tonsils and respiratory secretions were determined for 172 children. Genomes of at least one respiratory virus were detected in over 87% of the patients, without discernible seasonal variations (Fig. 1). Remarkably, high rates of virus detection were obtained from all three kinds of clinical samples throughout the study. The frequencies of virus detection ranged from 71.5% to 94.1% in adenoids, and from 37.5% to 86.6% in secretions and palatine tonsils. Of the three sample kinds, adenoid tissue yielded the highest frequencies of virus detection during almost the whole study, except for autumn months (March–June) of 2011, when nasal secretions yielded higher rates of positivity (Fig. 1).

The present analysis is based on results from a total of 172 patients, covering a 2-year period, and raises new issues made clear upon inclusion of additional cases to a study that was underway [3]. Overall, the most frequent viruses were human adenovirus (HAdV) detected in 52.8%, followed by human enterovirus (HEV) in 47.2%, human rhinovirus (HRV) in 33.8%, human bocavirus (HBoV) in 31.1%, human metapneumovirus (HMPV) in 18.3%, human respiratory syncytial virus (HRSV) in 17.2%, influenza virus (FLU) in 4.5%, human parainfluenzavirus (HPIV) in 4.5%, and human coronavirus (HCoV) in 2.7%. The frequencies of HAdV, HBoV and HRSV were higher in adenoids, whereas HRV was more frequently detected in nasal secretions and HEV in palatine tonsils. The rates of viral co-infections and the agreement between results from different tissues were high. In this 2-year study period, two or more viruses were detected in 62.2% of the patients, and 54% of them had the same virus detected in adenoids and palatine tonsils.

Overall, HAdV detection rates fluctuated from summer troughs of approximately 37.5% up to peaks of greater than 70% in spring-2011 and autumn-2012, without clearly seasonal periods (Fig. 2). HBoV detection rates were usually above 20% (12.5–46%) without discernible seasonality (Fig. 3). Detection frequencies of HAdV and HBoV were consistently higher in adenoids than in other samples. Rates of detection of the picornaviruses HRV and HEV were opposite in the summer, while the rate of HEV detection was at its

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