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Human bocavirus frequency in pre-school and school-aged hospitalized wheezing children and association to epidemiological risk factors of wheezing

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ABSTRACT

Recurrent wheezing in infancy is associated with acute respiratory infection (ARI) and atopy. The objectives of this work were to determine the frequency of respiratory human bocavirus (HBoV) in children hospitalized with acute wheezing and associated clinical-epidemiological variables. A cross-section observational study was performed. Nasal and pharyngeal swabs were obtained from pediatric patients hospitalized with acute wheezing and HBoV DNA was detected by PCR. Clinical and epidemiological data of patients were analyzed in relation with HBoV detection. Eight of 40 patients studied (20%) were pre-school age children (≤ 4 years old; average 3.5±0.5 yr.) and 32/40 (80%) were school-age children (5 to 14 years old; average 7.8±2.8 yr.). HBoV genome was detected in 22/40 patients (55%; IC95%: 40-69%), the majority of them school-aged children (19/22, 86.4%). No statistically significant differences were observed when comparing HBoV prevalence in groups of patients clustered by sex, age, antecedents of rhinitis, number of wheezing episodes in the previous year, and family history of atopy. Significant association was found between HBoV detection and (a) passive smoking: 17/23 (73.9%) patients with exposure to cigarette smoke were HBoV+ (p=0.006); (b) contact with cohabitant with ARI: 11/12 (91.7%) patients living with a cohabitant with ARI were HBoV+ (p=0.002). HBoV was frequent among school-age hospitalized

wheezing children. HBoV genome detection was associated with passive smoking and contact with cohabitant with ARI.

1. Introduction

Wheezing is a common symptom in children: up to 40% of children suffer from wheezing sometime during the first 6 years of life. Acute wheezing is one of the leading causes of admission to pediatric hospitals, representing a substantial burden for primary health care services. The etiology of recurrent wheeze in childhood is associated with respiratory tract infections and atopy (which are usually linked to asthma in older individuals). Smoking during pregnancy and exposure to cigarette smoke after birth are also significant risk factors (Chavasse et al 2002).

Among infections related to the development of wheeze, those of viral etiology are the most common and most significant. Respiratory syncytial virus, rhinovirus, human metapneumovirus and influenza are respiratory viruses associated with wheezing and development of asthma (Inoue and Shimojo, 2013); in addition, human bocavirus (HBoV) is also considered as a potential cause or risk factor (Jartti et al, 2012; Peltola et al, 2013).

HBoV and has been associated with acute respiratory infections (ARI) in children and adults (Ghietto et al, 2012a; Ghietto et al, 2012b; Guido et al, 2011). Although its role as an etiological agent of respiratory illness has been intensely discussed due to the high percentages of co-infections with other pathogenic viruses, there is growing evidence indicating that HBoV primary infection can cause acute respiratory disease in infants (Meriluoto et al, 2012; Zaghloul, 2011). In addition, some authors have detected HBoV in individuals without respiratory symptoms, but at significantly lower frequency or viral load compared to patients with acute respiratory illness (Jansen et al, 2011; Wiertsema et al, 2011). Thus, it is hypothesize that HBoV can establish persistent infections where high viral loads match with the production of disease, while low viral loads associate with excretion of virus in infected asymptomatic individuals (Schildgen, 2013). Alternatively, reinfections might be frequent especially among school age children (Meriluoto et al, 2012).

In order to provide data on the association and potential causality of HBoV in the development of wheezing, the aims of this work were to detect HBoV in respiratory specimens obtained from patients 3-14 years old hospitalized with acute wheezing and to analyze the frequency of clinical and epidemiological variables associated to HBoV detection.

2. Materials and methods

An observational, cross-sectional study was performed. The protocol was evaluated and approved by the Institutional Ethical Committee (CIEIS del Niño y el Adulto del Polo Hospitalario, Hospital de Niños de la Santísima Trinidad, Córdoba, Argentina).

2.1. Patients and clinical specimens

Inclusion criteria: children 3 to 14 years old presenting with acute wheezing (with or without previous antecedents of wheezing), requiring hospitalization according to the guidelines followed at the Emergency Department of the Children's Hospital Hospital de Niños de la Santísima Trinidad, from April 1st through June 1st, 2010. Parents or tutors of all potential participants were invited to participate in the study, and only those with signed informed consent and informed assent when appropriate (children ≥ 7 years old) were included. Exclusion criteria: previous chronic lung disease; associated systemic disease (diabetes, cystic fibrosis, kidney disease, heart disease, liver disease); septic clinical condition, toxic clinical condition; shock; primary or secondary immunodeficiency; and immunosuppressive therapy.

Fifty patients were admitted at the hospital with the inclusion criteria (and none of the exclusion criteria) mentioned above, of which 40 agreed to participate in the study. Nasal and pharyngeal swabs (NPS) were obtained from each participant at admission, together with clinical and epidemiological data recorded in ad-hoc forms (age, sex, patient's antecedents of rhinitis, number of episodes of wheezing during the previous year, family history of

atopy such as asthma, allergic rhinitis or atopic eczema, passive smoking -exposure to cigarette smoke at home, and antecedents of high or low ARI in one or more cohabitants anytime during a period of 7 days before hospitalization of the child. All samples were obtained at the hospital room by trained personnel and were kept refrigerated at 4°C until they were processed within 48 hours at the laboratory.

2.2. Processing of clinical specimens and detection of HBOV

Swabs placed in 1 ml PBS were vortexed 1 min using glass beads and an ice-cold bath. A 100 µl aliquot of suspension was treated with nucleic acid extraction buffer containing guanidinium thiocyanate and silica. Nucleic acid was resuspended in 25 ul TE buffer and 2 µl was used as template in the conventional PCR reactions for the detection of HBoV. The primers targeted a sequence spanning the coding region of non-structural protein NP1, nucleotides 2354 to 2684 of HBoV genome NC_007455.1 (Allander et al, 2005). The PCR mix contained 0.2 µM each primer, 0.8 mM dNTP mix, 2.5 mM MgCl2, and 0.02 U/µl Platinum Taq DNA polymerase (Invitrogen). Cycling conditions included incubation at 94°C for 2 min, 35 rounds of amplification (94°C-0.5 min, 48°C-0.5 min, 72°C-1 min) and a final extension at 72°C for 10 min. Negative and positive controls were included in each assay. The PCR products were visualized by electrophoresis in silver-stained 8.5-10% polyacrylamide gel (Ghietto et al, 2012a, Ghietto et al, 2012b).

2.3. Analysis of data

HBoV detection (HBoV+) was analyzed comparing groups of patients classified according to age and other epidemiological and clinical factors examined as listed in Table I. Continuous variables are described by mean values ± SD; discrete variables are described by percentages values, odds-ratio (OR) and 95% confidence interval (95%CI). Statistically significant differences between comparisons were identified using Fisher's exact test or Student's t test when appropriate (level of significance: 0.05).

3. Results

3.1. Description of the population studied

The study group included similar numbers of male and female patients. The majority (32/40, 80%, 95%CI 65.2-89.5) were school-aged children from 5 to 14 years old, among who the mean age was 7.8±2.8 years old; 8/40 (20%, 95%CI 10.5-34.8) patients were pre-school age children with a mean age of 3.5±0.5 years old.

Thirty out of 40 patients (75%, 95%CI 59.8-85.8) referred personal antecedents of rhinitis; 35/40 (87.5%, 95%CI 73.9-94.5) presented >3 wheezing episodes during the year previous to the current admission to hospital; 25/40 (62.5%, 95%CI 47.0-74.8) reported a history of asthma, rhinitis or atopic dermatitis in first-degree relatives. Twenty three out of 40 patients (57.5%, 95%CI 42.2-71.5) referred exposure to the smoke at home, and 12/40 (30%, 95%CI 18.1-45.4) had contact with cohabitant with upper or lower acute respiratory tract infection.

3.2. HBoV Frequency and association with risk factors involved in the development of wheezing

The presence of HBoV genome was detected in 22/40 (55%) patients, 95%CI 39.8-69.3%. Of them, 19 (86.3%, 95%CI 66.7-95.3) were detected in school-aged pediatric patients (5-14 years old), while 3/22 (13.6%, 95%CI 4.5-33.3) corresponded to pre-school age patients. There were no significant differences in the prevalence of HBoV when considering the following clinical-epidemiological variables: sex, age, antecedent of rhinitis, frequency of wheezing episodes during the previous year (patients with >3 episodes and patients with ≤3 episodes), and a family history of atopy (Table I). However, there was statistically significant association between the detection of HBoV genome and passive smoking, and also between HBoV detection and contact with cohabitant with ARI. As summarized in Table 1, 17/23 (74%) patients with exposure to cigarette smoke were HBoV+, while only 5/17 (33%) patients without exposure to cigarette smoke were HBoV+ ($p = 0.006$); on the other hand, 11/12 (92%) patients who reported contact with a cohabitant with ARI were HBoV+, while only 11/28 (39%) patients who had no contact with cohabitant with ARI were HBoV+ ($p = 0.002$).

4. Discussion

In this work we detected respiratory HBoV genome in pediatric patients (3 to 14 years old) hospitalized for acute wheezing. Then clinical and epidemiological risk factors of wheezing and it association to HBoV infection were analyzed.

The majority of the patients studied referred recurrent wheezing (87.5%), antecedents of rhinitis (75%) and a family history of atopy (62.5%). These data, considered together with the age of the patients and the seasonal period in which the clinical samples were collected (fall season of the southern hemisphere), suggest high prevalence of atopy and possibly altered immune profile.

HBoV was highly prevalent (55%) in the study population during the period covered, particularly among school-age wheezing patients (5-14 years old). In this group HBoV was detected with a frequency of 59%, not significantly different from the frequency in pre-school age wheezing children (38%). This contrasts with previous reports (Maffey et al, 2008; Schildgen, 2013), where the virus was preferentially detected in children less than 5 years old. Others, however, have published more recently high detection rates of HBoV, not only among infants and children with upper or lower acute respiratory illness (Martin et al, 2010; Pettigrew et al, 2011; Wiertsema et al, 2011; Zaghloul, 2011) but also among adults and elderly with ARI (Ghietto et al, 2012a, Guido et al, 2011) and even healthy school-age children (Lu et al, 2008). Our results suggest that HBoV is a common virus in children with acute wheezing -including children above 5 years old. The accumulating evidence indicates that HBoV is a frequent virus in the respiratory tract of humans with acute affection of the airway tract. Moreover, in situations such as primary infection of infants, or when high viral loads occur, HBoV might actually be responsible respiratory pathology (Meriluoto et al, 2012; Zaghloul et al, 2011).

Table 1

Epidemiological and clinical aspects of HBoV-positive and negative pediatric patients hospitalized for acute wheezing. Children's Hospital (Hospital de Niños de la Santísima Trinidad), Cordoba, Argentina, April 1st - June 1st, 2010.

Epidemiological and clinical factors	Sub-groups	Number of patients in the whole sample studied (%)	Number of HBoV+ patients (%)	OR	95%CI	P (Fisher´s exact test)
Sex	Female	19/40 (48%)	13/19 (68%)	2.89	0.79-10.57	0.07
	Masculine	21/40 (53%)	9/21 (43%)			
Age	School-age (5-14 yr old)	32/40 (80%)	19/32 (59%)	2.4	0.49-12.01	0.17
	Preschool age (≤ 4 yr old)	8/40 (20%)	3/8 (38%)			
Patient´s antecedents of rhinitis	Yes	30/40 (75%)	18/30 (60%)	2.25	0.52-9.70	0.16
	No	10/40 (25%)	4/10 (40%)			
Number of wheezing episodes in the previous year	< 3	5/40 (13%)	4/5 (80%)	3.78	0.38-37.28	0.20
	≥ 3	35/40 (88%)	18/35 (51%)			
Family antecedents of atopy (first degree relatives)	Yes	25/40 (63%)	16/25 (64%)	2.77	0.71-9.95	0.09
	No	15/40 (38%)	6/15 (40%)			
Passive smoking	Yes	23/40 (48%)	17/23 (74%)	0.71	0.07-7.66	0.006
	No	17/40 (13%)	5/17 (33%)			
Antecedents of upper/lower ARI in cohabitants	Yes	12/40 (30%)	11/12 (92%)	17.00	1.92-150.85	0.002
	No	28/40 (70%)	11/28 (39%)			

The detection of HBoV was significantly associated with exposure to cigarette smoke and with contact with cohabitant with ARI, while other factors analyzed were not significantly associated to the detection of the viral genome. The association of HBoV infection with passive smoking (74%) and direct contact with someone else suffering from ARI (92%) is noticeable since these two variables are referred to as epidemiological risk factors

highly involved in the development of wheezing in childhood (Bacharier et al, 2008). The high frequency of detection of HBoV in children exposed to cigarette smoke and in children who had contact with a cohabitant with ARI allows hypothesizing that while one condition might damage the airway epithelium and thus favor the establishment of HBoV infection, the other may provide the source of transmission.

The role of the virus as a respiratory pathogen has been extensively discussed, mainly because complete prospective and systematic studies to unequivocally demonstrate causality still remain to be done. Specifically, on one hand HBoV has been detected in asymptomatic individuals. These detections, nonetheless, generally occur at lower frequency or lower viral load compared to patients with respiratory illness (Jansen et al, 2011; Wiertsema et al, 2011). In addition, among HBoV+ individuals the percentage of co-infections with other respiratory viruses of well-established pathogenic potential is high, often over 50% (Schildgen, 2013). In this regard, the significant association observed here between HBoV and direct contact with subjects with ARI as well as exposure to cigarette smoke in acute wheezing children is epidemiological evidence for the role of HBoV as an agent of respiratory disease. Even though the study population is small and the results should be reinforced by a control group, the selection of clinical situations and the prospective, individualized recording of each case allow reporting quality data.

5. Conclusion

HBoV was frequent among pre-school and school-age hospitalized wheezing children. HBoV genome detection in NPS was significantly associated with passive smoking and contact with cohabitant with ARI.

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