Research paper

Human bocavirus infections in hospitalized Greek children

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Abstract

Introduction: The epidemiology of human bocavirus (HBoV) infections has not been described in Greece, a south-eastern European country. To define the epidemiological profile and the clinical characteristics associated with HBoV infection in a population of children hospitalized with respiratory tract infection.
Material and methods: During a one-year period throat swab samples were collected from 370 previously healthy children, aged 14 days to 13 years, admitted to two different paediatric wards because of respiratory tract infection. Samples were tested for HBoV by PCR amplifying a part of the NS1 gene.
Results: Human bocavirus was detected in 12 children (3.2%). Four of the 12 cases were co-infections, 3 of them with influenza A and 1 with coronavirus OC43. Cases were observed only during the cold months. The mean age of children was 1.8 years (range 2 months to 4 years). The most common symptoms were fever, cough and various degrees of respiratory distress. All children were clinically diagnosed as having lower respiratory tract infections, mainly pneumonia and acute laryngotracheobronchitis, and recovered uneventfully.
Conclusions: HBoV infections occur in Greece mostly among very young children. They accounted for 3.2% of children hospitalized with acute respiratory disease. Cases were observed only in late autumn to early spring.

Key words: respiratory infections, children, respiratory viruses, human bocavirus.

Introduction

Human bocavirus (HBoV) (genus Bocavirus, family *Parvoviridae*) has been recently identified in children with respiratory tract infection (RTI), first in Sweden [1], and subsequently in different parts of the world [2-10]. However, most studies so far have only retrospectively studied virus prevalence and only a few have addressed whether HBoV infection is associated with respiratory disease symptoms.

The aim of the present study was to define the epidemiological profile and the clinical characteristics associated with HBoV in hospitalized children with respiratory tract infection (RTI) in Greece.

Material and methods

During a one-year period (October 2006 to September 2007) throat swab samples were collected from 370 previously healthy children, aged 14 days to 13 years (mean age ± SD 17 ±13 months) admitted to two dif-

Table I. Clinical (A) and laboratory findings (B) in 8 patients in whom HBoV was the sole pathogen detected

A. Clinical findings	No. (%)
Cough	8 (100)
Rhinorrhoea/pharyngitis	7 (87.5)
Fever	7 (87.5)
Respiratory distress	6 (75)
Wheezing	5 (62.5)
Stridor	1 (12.5)
Diarrhoea	2 (25)
Otitis media	1 (12.5)

B. Laboratory findings	Mean ± SD	Range
White blood [cells/mm^3]	13744 ±5441	3200-20 380
Granulocytes/mm^3	8103 ±5245	480-15 672
Granulocytes %	53 ±25.6	15-77
Lymphocytes/mm^3	3613 ±1657	921-5892
Lymphocytes %	31.5 ±19.3	11.5-48.7
Haemoglobin [g/dl]	12.3 ±0.7	11.3-13.4
Platelet count/mm^3	428 714 ±138 816	235 000-643 000
C-reactive protein [mg/dl]	2.9 ±2.8	0.1-6.75
Creatinine [mg/dl]	0.4 ±0.6	0.33-0.49
Na^+ [mEq/l]	136.1 ±3.4	131.1-139.4
K^+ [mEq/l]	3.9 ±0.9	3.6-4.8
SGOT [IU/l]	47.7 ±14.8	30-74
SGPT [IU/l]	33.4 ±23.9	17-81

ferent large hospitals of Thessaloniki, Northern Greece. Samples were taken on the first day of admission and kept at –70°C until use.

All children were admitted because of acute infection of the respiratory tract. Demographic data and clinical diagnosis including acute infection of the upper respiratory tract, croup, bronchitis, bronchiolitis and pneumonia were obtained from a computer-generated discharge diagnosis based database. The case notes of children were reviewed using a standardised clinical data extraction form. The following data were recorded: sex, age, initial presenting symptoms, signs, discharge diagnosis and routine laboratory examinations upon admission and during hospitalization (complete blood count, C-reactive protein, chest X-ray).

Extracted DNA was subjected to polymerase chain reaction (PCR) which targets the NS1 gene of the HBoV genome [8]. All PCR products were sequenced and nucleotide sequences were compared with respective HBoV sequences retrieved from GenBank. In addition, all samples were tested by molecular methods for *Mycoplasma pneumoniae*, respiratory syncytial virus, coronaviruses, influenza viruses, human metapneumovirus and adenoviruses.

Results

HBoV DNA was detected in samples of 12 children (3.3%), 6 of them males. Sequencing and phylogenetic analysis revealed that HBoV sequences of 11 cases were identical to each other and to the Swedish strain st2 (NC007455), differing by 1 nucleotide from the 12th case (GR186), which was identical to strain CHSD4 (DQ471814) from USA.

All cases had clinical evidence of lower RTI (fever, tachypnoea, hypoxia, retractions, and abnormal auscultation findings). Four of the 12 cases were co-infections, 3 of them with influenza A virus and 1 with coronavirus OC43.

Concerning the 8 patients in whom only HBoV was detected, they were 3 males and 5 girls, aged 2-33 months (mean age ± SD: 17 ±9 months), hospitalized with the clinical diagnosis of 2 each: laryngotracheobronchitis, bronchiolitis, pneumonia, and asthma exacerbation.

Common symptoms of viral respiratory tract infection such as fever, cough, rhinorrhoea and pharyngitis were found in the majority of our patients (87.5-100%). Six patients (75%) presented with various degrees of respiratory distress. Those patients had tachypnoea (a respiratory rate of 45-90 breaths/min) and low haemoglobin oxygen saturation levels (SaO_2) in the range 90-94%. These children received oxygen supplementation until 1 day before discharge. Wheezing was the most common clinical finding (5 patients) while another 1 child presented with stridor. Hoarseness was noticed in 2 children. Difficulty in feeding was also a common complaint, reported by 5 patients. Other clinical findings included diarrhoea (2 patients, 25%), a symptom previously described in HBoV infections [11, 12], and otitis media (1 patient, 12.5%). The major clinical and laboratory findings in the 8 patients in whom HBoV was the sole pathogen detected are presented in Table I.

Chest X-rays were available for all 8 children. The most common finding, present in 6 patients, was bilateral interstitial infiltrates. Consolidation was detected in the other 2 children.

Clinical expression was not different in children with HBoV co-infection. The child with coronavirus OC43 co-infection also presented with conjunctivitis, while respiratory distress was the main concern in a child with influenza-A virus co-infection.

All children recovered uneventfully. The median hospitalization time was 5 days (range 4-9 days). For 1 patient a second throat swab sample, taken 20 days after the first one, was found negative for HBoV DNA.

Discussion

Prevalence of HBoV in our study (3.3%) was found to be relatively low compared to published data from other parts of the world [2, 4, 6, 9, 10, 13-16]. However, prevalence rates between 1.5 and 19% have been observed and it is possible that differences of study populations and sampling techniques account for the encountered discrepancies [17]. Although the number of studies since the first description of HBoV is increasing, many of these studies are retrospective on stored samples, or only on samples negative for other pathogens, or on samples collected during a few months and thus suffering from the bias that sampling may be guided from the occurrence of RSV or influenza epidemics. In the present prospective study samples were collected during 1 year and were tested regardless of positivity for another pathogen.

In previous whole year studies it was found that the vast majority of HBoV positive samples were collected in cold months [14, 18, 19]. We also found that positive cases were observed only during the cold months. The first HBoV case was observed in mid December 2006, and the last at the end of April 2007. Greece is a Mediterranean country in the Balkan Peninsula, with hot, dry summers and cold, damp winters. Nevertheless, the mean temperature of winter 2006-2007 in Greece (and worldwide) was above average (warmest winter on record), and the precipitation drier than normal (National Observatory of Athens), conditions which might have affected the prevalence of HBoV.

Previous studies have implicated HBoV as a cause of RTI, most commonly in children < 2 years old, a finding similar to our study, where 10/12 children were younger than 2 years, the youngest one being a 2-month boy with acute bronchiolitis [13, 14, 20-22]. However, frequency of HBoV infection was found to be relatively low in children younger than 6 months, and the possibility of passive protection due to maternal antibodies has been questioned. These findings together with the high prevalence rates in young children led to the hypothesis that HBoV may be an endemic virus with high attack rates in those susceptible; one would then expect that the majority of the population would be infected during childhood. In support of this hypothesis are the high seroprevalence rates against HBoV reported in the adult population [23]. The mere presence of HBoV in the airway may not be able to define its role as a pathogen; therefore it is anticipated that serology would be a useful diagnostic addition to the study of HBoV infection.

Four of the 12 cases were co-infections, 3 of them with influenza A virus and 1 with coronavirus OC43. Similar findings were reported in other studies, where co-infection was found in one third of patients [5, 24], while other investigators have reported even higher rates [8, 15, 25]. It has been hypothesized that detection of HBoV asymptomatic presence may be enhanced by the symptoms caused by another virus or that HBoV may aggravate the clinical course of symptoms due to other viral infections, so that it is frequently detected in hospitalized children [26]. Co-infection is frequently described with many respiratory viruses and the question whether this may result in more serious clinical outcomes has not been clarified [27].

The association of HBoV with acute wheezing, a symptom found in 62.5% of our patients, has been well described in recently published studies [5, 14, 18, 28]. Another 2 of our patients presented with laryngotracheobronchitis, a finding which is in accordance with the study of Rihkanen *et al*. [29]. Only 1 of our patients returned for follow-up and a second throat swab sample, taken 20 days after the first one, was found negative for HBoV DNA. Our findings suggest that HBoV may have similar clinical features to those of other respiratory viruses such as RSV. One may argue that the fact that HBoV is prevalent in samples from patients with respiratory tract infection does not guarantee a causative role for the symptoms, especially when – as in this case – it is frequently detected in combination with other respiratory viruses of known pathogenic potential. On the other hand, HBoV was rarely detected in asymptomatic children [16, 22].

In conclusion, in the present study we have provided a first insight into the epidemiology and clinical aspects of HBoV infections in hospitalized children in Greece. Our findings suggest a possible association of HBoV with lower RTI, manifested mainly by fever, cough and wheezing, most often in infants, and a seasonal pattern with HBoV cases occurring in cold months.

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