Incidence of Recurrent Wheezing in Under 5-Year-Old Human Bocavirus Infection during One Year Follow-up†

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Background: *Human bocavirus (HBoV) is a newly identified virus that can cause acute wheezing in young children. The present study aimed to evaluate the incidence and factors associated with recurrent wheezing during 1 year after HBoV lower respiratory tract infection (LRI).*
Material and Method: *Children younger than 5 years old who were admitted to King Chulalongkorn Memorial Hospital between February 1, 2006 and September 30, 2008 due to LRI were recruited. Their nasopharyngeal aspirates were evaluated for respiratory virus by polymerase chain reaction (PCR) assays. Those who had positive PCR for HBoV and had no underlying diseases were studied. Their clinical presentations were evaluated and their clinical data about recurrent wheezing as well as pulmonary function tests were followed-up for 1 year.*
Results: *Fifteen patients with HBoV-LRI were regularly followed-up. Seven patients (47%) had co-infection with other respiratory viruses. Generalized wheezing was the most common lung sign detected in 73% of cases (11 cases) and 36% (4 cases) of them responded well to bronchodilators. During one year follow-up, serial pulmonary function tests were normal in all cases and most patients were doing well. However, 27% of HBoV infected patients (4 cases) developed recurrent wheezing associated with respiratory tract infections. Two of them had to be re-hospitalized. Compared to patients without recurrent wheezing, eosinophil count tended to be higher in those with recurrent wheezing and isolated HBoV infected patients tended to develop recurrent wheezing more than those with co-infection*
Conclusion: *Acute wheezing is a common presenting lung sign in HBoV-LRI. Although the pulmonary function tests of all patients were normal, more than a quarter of patients suffered from recurrent wheezing during one-year follow-up.*

Keywords: *Human bocavirus, Recurrent wheezing, Children, LRI*

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Lower respiratory tract infection (LRI) is one of the most common causes of hospitalization in children younger than 5 years of age. The most frequent causative agents of LRI are respiratory viruses, including respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, rhinovirus, adenovirus, and human metapneumovirus. Human bocavirus (HBoV) is a recently discovered parvovirus associated with respiratory tract infections in children. The prevalence of HBoV infection reported in several countries worldwide varying from 1.5 to 19%[1]. There have been few reports concerning prevalence of HBoV and clinical manifestations of HBoV infection in the Thai population[2,3]. The majority of patients positive for HBoV were young children[4-6]. Recent studies in young children suggested that HBoV could play an important role as a causative agent in children with acute wheezing[6-8]. However, the correlation between HBoV and subsequent airway hyperresponsiveness or recurrent wheezing has not been evaluated. The objective of the present study was to determine the incidence and factors associated with airway hyperresponsiveness or recurrent wheezing during one year after HBoV lower respiratory tract infection.

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Material and Method

A cohort study was performed on children younger than 5 years old who were admitted to the Department of Pediatrics, King Chulalongkorn Memorial Hospital (KCMH) between February 2006 and September 2008. They were evaluated for HBoV

infection and the correlation between HBoV and bronchial hyperresponsiveness was subsequently performed. The exclusion criteria included patients with preterm birth or neonatal respiratory distress, previously diagnosed asthma or reactive airway diseases or chronic lung diseases, congenital heart diseases, and Down syndrome. Their nasopharyngeal aspirates during the first three days of hospitalization were examined for respiratory viruses including human bocavirus, respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, adenovirus, human metapneumovirus (hMPV), and rhinovirus. Patients who had positive PCR for HBoV and could be followed-up regularly at the chest clinic every one to three months for one year were enrolled in the present study. During follow-up, clinical data about recurrent wheezing were interviewed and pulmonary function tests were performed at one to three months and six to nine months after their HBoV infection episodes resolved. For each patient, tidal breathing flow volume loops were analyzed. The presenting symptoms and clinical data on recurrent wheezing during follow-up visits, as well as the result of pulmonary function tests were analyzed.

The present study protocol was approved by the ethics committee, Faculty of Medicine, Chulalongkorn University. Prior to enrollment, all legal guardians of the patients were informed about the present study and their consent was obtained.

Laboratory testing

Nasopharyngeal aspirates from all patients were tested for HBoV, RSV, influenza A, B virus, para influenza virus, adenovirus, hMPV, and rhinovirus by using the polymerase chain reaction (PCR) assays. The detail process of DNA and RNA extraction, reverse transcription and detection of HBoV as well as other viruses are previously described by Chieochansin T et al[3], Thanasugarn W et al[9] and Linsuwanon P et al[10]. A SensorMedics 2600 Pediatric Pulmonary CartR (SensorMedics Corp., Yorba Linda, CA) was used to obtain tidal breathing flow-volume (TBFV) loops. An appropriately sized transparent facemask with an air-inflated cuff (Vital Signs, Inc.) was connected to the pneumatic slide valve (used for passive occlusion measurement) and a 0 to 30-L/min pneumotachograph (4500 Series^R, Hans Rudolph). Airflow was measured and integrated to yield the volume. Calibration of flow and volume signals was performed daily prior to measurements. Pulmonary function tests were performed while patients were in a supine and neutral head position. Arterial oxygen saturation (SpO_2) and heart rate were continuously monitored during the study by pulse oximeters. Four acceptable TBFV loops with stable volumes and shapes were selected for measurement. The respiratory parameters obtained from TBFV included the ratio of volume until peak expiratory flow volume to total expiratory volume (V_{PTEF}/V_E), the ratio of time until peak tidal expiratory flow to total expiratory time (T_{PTEF}/T_E) and the ratio of tidal expiratory flow at 25% remaining expiration to peak expiratory flow ($T_{EF}25/P_{TEF}$). Obstructive pattern was determined if the V_{PTEF}/V_E ratio was less than 0.4 or the T_{PTEF}/T_E ratio was less than 0.2 or the $T_{EF}25/P_{TEF}$ ratio was less than 0.6.

Statistical analysis

All parametric values were expressed as means±SD. Demographic data and clinical data were compared between groups by using the Chi-square, Fisher's exact test for qualitative data and unpaired t-tests, or Mann-Whitney U test for quantitative data. The first and second pulmonary function tests were compared by paired t-test. A value of $p<0.05$ was considered statistically significant.

Results

During the present study period, 15 patients with HBoV infection were enrolled into the study. The male:female ratio was 4:1 with the mean age of 22.3 months (5 months-43 months). Nine patients (60%) were less than 2 years of age. Two patients (13%) had a family history of asthma and nine patients (60%) had history of passive smoking. The most common presenting symptoms in all patients in the present study were cough (100%) and dyspnea (100%). Fever was found in 87% of the patients with a mean duration of 3.9 days. Other common symptoms were rhinorrhea (67%), vomiting (33%), and diarrhea (27%). Generalized wheezing was the most common chest symptom and sign (73%) and 36% of these responded to bronchodilators. Crepitations were heard in 53% of the patients. The mean initial SpO_2 at room air was 94.6% (85-98%). Perihilar infiltration was the most common finding on chest x-ray among all patients. Other chest x-ray findings were hyperinflation (33%), patchy infiltration (13%), and enlarged hilar lymph nodes (27%). The mean duration of oxygenation was 2.7±1.3 days and the mean duration of admission was 3.2±1.5 days. No serious complications were found.

During one year follow-up, most patients were doing well. However, four patients (27%) had

recurrent episodes of wheezing. All were male patients. The first patient reported to have two episodes of recurrent wheezing with acute respiratory tract infection (ARI) at four and six months after HBoV infection, he had to be readmitted to a provincial hospital and received nebulized bronchodilator for three days during both admissions. However, his physical examination during follow-up at the chest clinic was normal and his lung function tests were normal. The second patient had recurrent wheezing with ARI presented with cough and dyspnea one month after HBoV infection. He was readmitted to KCMH and was treated with nebulized bronchodilator for two days. After that admission, he had a few brief episodes of wheezing with ARI, which responded well to oral bronchodilator. The other two patients had two or three episodes of cough and wheezing with ARI. These two patients needed only nebulized bronchodilator at the outpatient clinic and responded well; both of them were treated as reactive airway diseases. None of the patients with recurrent wheezing had a history of asthma in the family and no clinical factors were significantly associated with recurrent wheezing (Table 1).

Pulmonary function tests of all patients were normal at one to three months and six to nine months after admission and there were no significant change of PFT between the two tests (Table 2). No differences in pulmonary function were found among the patients with and without recurrent wheezing (Table 1).

Of the 15 patients infected by HBoV, seven patients (47%) had co-infection with other respiratory viruses. RSV was the most frequently detected as co-infecting virus (3 cases). Other co-infecting viruses included parainfluenza virus (2 cases), influenza virus (2 cases), adenovirus (1 case), and human metapneumovirus (1 case). One patient in the present study was found co-infected with three other viruses

Table 1. Clinical characteristics associated with recurrent wheezing in HBoV-infected patients

	Recurrent wheezing (n = 4)	No recurrent wheezing (n = 11)	p-value
Age (months)	18.50±11.60	23.60±10.30	0.42
Male	4 (100%)	8 (73%)	0.52
Family history of asthma	1 (25%)	1 (9%)	0.48
Passive smoking	3 (75%)	6 (54%)	0.60
Eosinophil count (/cu.mm) (median, min-max)	180.5 (42-536)	30 (0-326)	0.05
Pulmonary function test (first)			
V_{PTEF}/V_E	0.40±0.09	0.40±0.09	0.99
T_{PTEF}/T_E	0.43±0.09	0.36±0.12	0.34
$T_{EF}25/P_{TEF}$	0.77±0.07	0.81±0.10	0.54
Co-infection	1 (25%)	6 (54%)	0.33

V_{PTEF}/V_E = ratio of volume until peak expiratory flow volume to total expiratory volume; T_{PTEF}/T_E = ratio of time until peak tidal expiratory flow to total expiratory time; $T_{EF}25/P_{TEF}$ = ratio of tidal expiratory flow at 25% remaining expiration to peak expiratory flow

The normal ratios for V_{PTEF}/V_E, T_{PTEF}/T_E, $T_{EF}25/P_{TEF}$ are >0.4, >0.2, and >0.6 respectively.

Table 2. The result of the pulmonary function tests (PFT)

	First PFT at 1-3 months (mean±SD)	Second PFT at 6-9 months (mean±SD)	Mean change (95% CI)	p-value
V_{PTEF}/V_E	0.42±0.07	0.40±0.05	0.01 (-0.01 to 0.04)	0.24
T_{PTEF}/T_E	0.35±0.07	0.35±0.06	0.01 (-0.02 to 0.04)	0.54
$T_{EF}25/P_{TEF}$	0.77±0.10	0.85±0.09	-0.07 (-0.16 to 0.01)	0.08

V_{PTEF}/V_E = ratio of volume until peak expiratory flow volume to total expiratory volume; T_{PTEF}/T_E = ratio of time until peak tidal expiratory flow to total expiratory time; $T_{EF}25/P_{TEF}$ = ratio of tidal expiratory flow at 25% remaining expiration to peak expiratory flow

All respiratory parameters are normal in every patient.

Table 3. Comparing clinical characteristics between patients with HBoV single infection and patients with co-infection

Characteristics	HBoV, single infections (n = 8)	HBoV, co-infection (n = 7)	p-value
Presenting symptoms			
Rhinorrhea	7 (88%)	3 (43%)	0.07
Vomiting	3 (38%)	1 (14%)	0.33
Diarrhea	3 (38%)	0	0.08
Presenting signs			
Wheezing/rhonchi	6 (75%)	5 (71%)	0.95
Bronchodilator response	2/6 (33%)	2/5 (40%)	0.44
Crepitation	5 (63%)	3 (43%)	0.53
SpO_2	93.2±4.1	96.1±0.9	0.13
Recurrent wheezing during 1 year follow-up	3 (38%)	1 (14%)	0.33

HBoV = human bocavirus; SpO_2 = oxygen saturation

(adenovirus, RSV, and parainfluenza virus) as co-infecting virus. Other patients carried only one co-infecting virus.

When comparing patients with a single infection and co-infection on presenting signs and symptoms as well as laboratory findings, there were no significant differences on lung signs between the two groups. However, patients with a single infection tended to have more rhinorrhea and gastrointestinal symptoms than the co-infection group as shown in Table 3. Of the four patients who had recurrent wheezing during follow-up, three were in the single infection group and one was in the co-infection group.

Discussion

In the present study, 60% of HBoV infected patients were younger than two years. The high incidence of HBoV infections in children younger than two years old has been reported[4-6]. The common presenting symptoms of children with HBoV-LRI were cough, dyspnea, fever, and rhinorrhea. Perihilar infiltration was the most common x-ray finding. These clinical presentations and x-ray findings are similar to other studies[11-15]. Generalized wheezing was the most common lung sign in HBoV-infected children and about one third of them had clinically significant responses to inhaled bronchodilators. The high occurrence of wheezing in HBoV-infected children has been previously reported[2,6-8] and the incidence of HBoV infection was reported to be higher in children with wheezing[7,8]. These findings suggested that HBoV could be an important causative agent for acute wheezing. In addition, in a study by Vallet C et al, HBoV was detected in 13% of children who were hospitalized for severe asthma exacerbation and was found in only 2% in children with stable asthma, suggesting a significant effect of HBoV on development of acute exacerbations in asthmatic children[16]. However, this was not relevant to the findings in the present study because very few children in the present study had history of asthma.

In this cohort study, the authors found that 27% of patients had recurrent wheezing with response to bronchodilators during 1-year follow-up. Compared to the non-recurrent wheezing group, eosinophil count tended to be higher in patients with recurrent wheezing but not significantly different. Eosinophilia had been reported to be a predicting factor for childhood reactive airway disease after RSV bronchiolitis[17]. For pulmonary function measurements using tidal breathing flow volume loop at one to three months and six to nine months after admission, the results in all patients were normal. No obstructive pattern was detected in all patients. This suggested that no patients in this small cohort study had underlying lower airway diseases. Recurrent wheezing, found during a follow-up visit, led us to speculate that HBoV could be a significant cause of acute and recurrent wheezing similar to other previously reported viruses such as RSV. However, it had been demonstrated that HBoV-infected patients were often co-infected with other respiratory viruses[2,7,19-22], suggesting that HBoV infection might induce other viruses to cause wheezing or require other viral pathogens to cause wheezing. It remained possible that wheezing might be simply caused by other viruses[20,22]. When clinical features of HBoV- infected patients with and without co-infections were compared, rhinorrhea and gastrointestinal symptoms tended to be more common presenting symptoms in the isolated HBoV-infected group, though no significant differences were demonstrated.

These gastrointestinal symptoms had been reported as common in HBoV-infected patients in previous studies[12,15,23,24]. Wheezing and bronchodilator response as presenting symptoms were found in similar rates in both groups. However, at 1-year follow-up, the rate of recurrent wheezing tended to be higher in the isolated HBoV-infected group (37% vs. 14%). This suggested that bronchial hyperresponsiveness might occur in children previously infected with HBoV. Recurrent wheezing post viral-LRI was common as evidenced by 42 to 71% of RSV-infected patients developing recurrent wheezing years later[25].

TBFV loops have been used to assess obstructive airway diseases in young children[26,27]. The T_{PTEF}/T_E and V_{PTEF}/V_E ratios have been reported to decrease in children with obstructive airway diseases[27,28] and have been demonstrated to be useful in differentiating asymptomatic individuals with recurrent wheeze[29] or asthmatic subjects[27] from non-asthmatic controls and in predicting who will wheeze during early childhood[30,31]. However, previous studies revealed different results and some studies could not demonstrate the clinical values of these parameters[32,33]. The findings in the present study that every patient had normal TBFV loops in both tests at one to three months and six to nine months after HBoV infection and no parameter of TBFV was associated with acute wheezing or subsequent wheezing in patients with HBoV infection suggested that performing TBFV in patients with HBoV-LRI did not add significant value in predicting recurrent wheezing in clinical practice.

The limitations of the present study were the small number of patients, which might limit the statistical power in demonstrating the significant association between possible factors and recurrent wheezing after HBoV-LRI. Some data obtaining by interviewing the caregivers about the past clinical symptoms might be missed or less accurate. However, the information about recurrent wheezing needed nebulized bronchodilator or hospitalization could be accounted for its reliability.

In conclusion, acute wheezing was a common presenting lung sign in HBoV-infected children. In those children, 36% of which had a clinically significant response to bronchodilators. Although the pulmonary function tests of all patients were normal, recurrent wheezing occurred in 27% of HBoV-infected children during one-year follow-up. Neither clinical factors nor parameters of TBFV loops were associated with recurrent wheezing after HBoV infection.

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Potential conflicts of interest

None.

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อุบัติการณ์ของการเกิดเสียงหวีดซ้ำภายในระยะเวลา 1 ปีหลังการติดเชื้อโบค่าไวรัสในทางเดินหายใจในเด็กอายุต่ำกว่า 5 ปี

จิตลัดดา ดีโรจนวงศ์, อารยา ศรัทธาพุทธ, นวลจันทร์ ปราบพาล, สุชาดา ศรีทิพยวรรณ, รุจิภัตต์ สำราญสำรวจกิจ

ภูมิหลัง: *โบค่าไวรัสเป็นไวรัสที่ค้นพบใหม่ และอาจก่อให้เกิดเสียงหวีดเฉียบพลันในเด็กเล็ก*

วัตถุประสงค์: *การศึกษานี้มีวัตถุประสงค์ที่จะประเมินอุบัติการณ์ และปัจจัยที่มีความเกี่ยวข้องกับการเกิดเสียงหวีดซ้ำ ภายในระยะเวลา 1 ปี หลังการติดเชื้อโบค่าไวรัสในทางเดินหายใจส่วนล่าง*

วัสดุและวิธีการ: *ศึกษาในเด็กอายุต่ำกว่า 5 ปี ที่รับไว้ในโรงพยาบาลจุฬาลงกรณ์ด้วยโรคติดเชื้อทางเดินหายใจส่วนล่าง ในระหว่างวันที่ 1 กุมภาพันธ์ พ.ศ. 2549 ถึงวันที่ 30 กันยายน พ.ศ. 2551 โดยการตรวจหาเชื้อไวรัสจากสิ่งคัดหลั่งในจมูกโดยวิธี polymerase chain reaction (PCR) assays ผู้ป่วยที่ตรวจพบเชื้อโบค่าไวรัสและไม่มีโรคประจำตัวจะได้รับการตรวจสมรรถภาพปอด และติดตามอาการต่อเนื่องเป็นเวลา 1 ปี*

ผลการศึกษา: *ผู้ป่วยที่ติดเชื้อโบค่าไวรัส 15 ราย พบมีการติดเชื้อไวรัสชนิดอื่นร่วมด้วยร้อยละ 47 (7 ราย) ฟังเสียงปอดแรกรับพบเสียงหวีด ร้อยละ 73 (11 ราย) และร้อยละ 36 (4 ราย) ของผู้ป่วยที่มีเสียงหวีด ตอบสนองดีต่อการให้ยาขยายหลอดลม ระหว่างการติดตามการรักษาเป็นเวลา 1 ปี ผลการตรวจสมรรถภาพปอดต่อเนื่องของผู้ป่วยทุกรายอยู่ในเกณฑ์ปกติ พบผู้ป่วยที่มีเสียงหวีดซ้ำจากการติดเชื้อทางเดินหายใจ 4 ราย (ร้อยละ 27) ทุกรายตอบสนองต่อการให้ยาขยายหลอดลม แต่ผู้ป่วย 2 ราย ต้องเข้ารับการรักษาในโรงพยาบาลด้วยอาการหอบร่วมกับเสียงหวีด เมื่อเปรียบเทียบลักษณะทางคลินิกแรกรับ พบว่าผู้ป่วยที่เกิดเสียงหวีดซ้ำมีแนวโน้มที่จะมีจำนวนเม็ดเลือดขาวชนิดอีโอสิโนฟิลสูงกว่าผู้ป่วยที่ไม่เกิดเสียงหวีดซ้ำ และผู้ป่วยที่ติดเชื้อโบค่าไวรัสเพียงชนิดเดียวมีแนวโน้มที่จะเกิดเสียงหวีดซ้ำได้บ่อยกว่าที่ติดเชื้อไวรัสอื่นร่วมด้วย*

สรุป: *เสียงหวีดเป็นอาการแสดงแรกรับที่สำคัญในเด็กที่ติดเชื้อโบค่าไวรัส แม้ว่าผลการติดตามสมรรถภาพปอดในผู้ป่วยทุกรายจะปกติ แต่พบการเกิดเสียงหวีดซ้ำได้มากกว่าหนึ่งในสี่ของผู้ป่วยในระหว่างติดตามอาการเป็นเวลา 1 ปี*