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Respiratory viruses and atypical bacteria triggering severe asthma exacerbation in children

Key Messages

1. Respiratory viruses and atypical bacteria were detected in 51.0% of Hong Kong children with asthma exacerbations, which was significantly higher than the detection rate of 27.3% in children with chronic stable asthma.
2. Co-infections of two or more respiratory pathogens were more commonly found in children with asthma exacerbations (10.7%) than in patients with stable asthma (2.6%).
3. Human rhinovirus infection was a significant risk factor for asthma exacerbations.
4. There was no significant association between the severity of asthma exacerbations and respiratory viral or atypical bacterial infections.
5. Routine use of macrolide antibiotics in the treatment of childhood asthma exacerbations should be discouraged.

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Introduction

Asthma is the most common chronic respiratory disorder in childhood, affecting about 10% of Hong Kong children. Asthma exacerbations in children commonly result in hospitalisation, which accounts for a major fraction of the total cost of asthma care. Increasing evidence supports the importance of respiratory infections in asthma exacerbations. Prospective epidemiologic studies show that up to 80% of childhood asthma attacks are associated with viral upper respiratory infections of human rhinovirus (HRV), respiratory syncytial virus (RSV), adenovirus, human metapneumovirus (HMPV), and influenza viruses.[1] Beside respiratory viruses, atypical bacteria such as *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* have also been linked to asthma exacerbations in children. In 46 asthmatic patients seropositive for *C pneumoniae* treated with oral macrolides, asthma-related symptoms resolved or significantly improved in about half of them. *M pneumoniae* was found to be present in >50% of asthmatics. Therefore, understanding the roles of respiratory pathogens in precipitating asthmatic attacks may provide ways to reduce the health care burden associated with asthma-related hospitalisation.

Methods

This case-control study was conducted from November 2006 to April 2008. We investigated the epidemiology of respiratory viruses and atypical bacteria in respiratory secretions collected from children with asthma exacerbations (as cases) and those with chronic stable asthma (as controls). Associations between asthma exacerbations and presence of these organisms were analysed.

Patients aged 3 to 18 years with physician-diagnosed asthma and having exacerbations were recruited. Most of them were hospitalised for severe exacerbations, and assessed within 48 hours of hospitalisation. Asthmatic children with stable disease (controls) were recruited from our paediatric clinics. They had been free of symptoms of respiratory infection for ≥4 weeks. Patients who received antimicrobial agents in the 2 weeks before assessment were excluded. Patients' parents gave informed written consent, and the Joint CUHK-NTEC Clinical Research Ethics Committee approved this study.

The baseline characteristics, asthma status, and treatments of patients were recorded. The severity of asthma exacerbations was evaluated according to the Global Initiative for Asthma guideline. Patients in both groups underwent exhaled nitric oxide measurements followed by spirometry. Each patient with an acute asthma was reassessed 3 weeks after the episode to collect samples for microbiological studies.

In accordance with the Hospital Authority policy on Infection Control, nasopharyngeal aspirates were collected from each subject in negative-pressure isolation rooms. Deep nasal swabs were obtained in situations where such infection control facility was not available. These specimens were put in viral transport medium at 4 to 10ºC, and nucleic acids were extracted for molecular studies on the same day. In patients with asthma exacerbations, paired serum samples were also obtained, if clinically indicated, to determine antibody titres

to respiratory pathogens.

Both RNA and DNA in respiratory samples were extracted by PureLink Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Extracted RNA was converted to cDNA by reverse transcriptase, and all DNA and cDNA were used immediately for the five groups of multiplex nested PCR assays as described previously.[2] Twenty respiratory pathogens could be simultaneously detected by these PCR assays. Group 1 comprised influenza A and B group-specific and subtypes H1N1, H3N2, H5N1-specific primers; group 2 comprised parainfluenza viruses (PIV-1, PIV-2, PIV-3, PIV-4a and PIV-4b); group 3 comprised RSV A and B, HRV and enterovirus (EV); group 4 comprised human coronavirus (HCoV)-OC43, -229E and SARS-CoV and HMPV; and group 5 comprised *M pneumoniae, C pneumoniae*, human bocavirus, and adenovirus. Both the first and second rounds of PCR were conducted in 20-μl reaction mixtures using 'fast' thermal cycler (Applied Biosystems, Foster City [CA], USA). For the first round of PCR, 2 μL of the cDNA preparation was used as the template for groups 1 to 4, whereas 8 μL of the extracted preparation was used for group 5. In the second round of PCR, a 0.2-μL aliquot of the first-round PCR product was used as a template. Following PCR reactions, the products were prestained by SYBR Safe and visualised by electrophoresis in 1.5% agarose gels. Four corresponding positive controls and one negative control (sterile water) samples were included for each group simultaneously. The preparation of reagents, processing of samples, and nested PCR assays were performed in separate rooms away from the area where amplified products were analysed in order to prevent PCR contamination. Aerosol-resistant pipette tips were used throughout the experiments.

For serologic detection of respiratory infections, the titres of IgG antibodies to common respiratory viruses, *M pneumoniae* and *C pneumoniae* in paired serum samples were measured by complement fixation tests. Significant seroconversion was defined as having ≥4-fold change in antibody titre to the organism.

Results

A total of 209 children with asthma exacerbations (cases) and 77 children with stable asthma (controls) were recruited (Table 1). The patient age in the controls was significantly older (7.6±4.1 vs 11.1±4.5 years, P<0.001). This was mainly due to our inability to recruit one age-matched control for each participant with asthma exacerbation.

Sufficient respiratory samples were collected from 206 (98.6%) of the cases and all the controls. The samples consisted of 236 nasopharyngeal aspirates and 47 nasal

Table 1. Comparison of patients with asthma exacerbations and stable asthmatic controls

Clinical feature	Asthma exacerbation (n=209)	Stable asthma (n=77)
Mean±SD age (years)	7.6±4.1†	11.1±4.5
Male (% of patients)	68.4	75.3
Mean±SD hospitalisation (days)	3.6±1.9	-
Domestic tobacco smoke exposure (% of patients)	22.3†	13.0
Clinical status (% of patients)		
Fever	33.0	-
Shortness of breath on talking or at rest	8.7	-
Only able to talk in phrases or words	11.2	-
Altered consciousness (agitation or drowsiness)	0	-
Mean±SD duration of fever (days)	0.49±0.86	-
Received supplemental oxygen	23.0	-
Severity of exacerbations (No. [%] of patients)		
Mild	5 (2.4)	-
Moderate	101 (48.3)	-
Severe	103 (49.3)	-
Imminent respiratory arrest	0	-
Vital signs (mean±SD)		
Minimum SaO_2 (%)	94.1±2.4	-
Maximum pulse rate (/min)	135±22	-
Maximum respiratory rate (/min)	34±9	-
Systolic blood pressure (mm Hg)	110±15	-
Diastolic blood pressure (mm Hg)	69±10	-
Laboratory results (mean±SD)		
Fractional exhaled nitric oxide concentration (ppb)	57.1±43.0	77.2±59.6
Forced expiratory volume in 1 second (FEV_1) predicted (%)	73.2±21.7†	95.8±15.2
Forced vital capacity (FVC) predicted (%)	81.6±32.5*	94.2±18.8
FEV_1 to FVC ratio	0.81±0.25	0.86±0.10
Peak expiratory flow (l/min)	194±79†	344±132
Treatment and outcome (% of patients)		
Received systemic corticosteroid	75.4	-
Intensive care unit care	3.5	-
Death	0	-

* P<0.05
† P<0.001

swabs. Respiratory pathogens were identified in samples of 105 (51.0%) cases and 21 (27.3%) controls (P<0.001, Table 2). The presence of any virus with or without atypical bacteria was significantly associated with asthma exacerbations (P<0.001 for both). Specifically, HRV infection was significantly more common among cases than controls (26.2% vs 13.0%, P=0.018), whereas other pathogens were not related to asthma exacerbations. In addition, co-infections of ≥2 pathogens was associated with asthma exacerbations (10.7% vs 2.6%, P=0.030). Nonetheless, none of the respiratory pathogens, or their co-infections, was associated with the severity of asthma exacerbations as defined by the Global Initiative for Asthma guideline (P>0.15 for all).

Patients with asthma exacerbations caused by respiratory viruses were significantly younger than those without identifiable viral infections (P<0.05, Table 3). This was mainly attributed to RSV, influenza A, and HMPV infections (P<0.05).

Regarding the seasonality of respiratory infections in 2007, patients with asthma exacerbations were infected with HRV throughout the whole period, but the peak seasons appeared to be spring (March to June) and autumn to winter (September to December). The low detection rates for all other organisms precluded analysis of their seasonality.

Only 14 (6.7%) of our 209 recruited patients with asthma exacerbations had blood checking for detection of respiratory pathogens. Paired blood samples were not collected from the remaining patients because their parents did not give consent or the tests were not clinically indicated. Two of these 14 patients in whom paired serum samples were collected had HRV infection, and one each had HMPV and influenza A. With this small number of samples, further evaluation was not feasible.

Discussion

Respiratory tract infections can be caused by a

Table 2. Detection of different viral and bacterial pathogens in respiratory samples

Parameter	(%) of patients		P value (Chi-squared or Fisher exact test)
	Asthma exacerbation (n=206*)	Stable asthma (n=77)	
Organism			
Rhinovirus	54 (26.2)	10 (13.0)	0.018
Human metapneumovirus	12 (5.8)	2 (2.6)	0.265
Influenza A virus	16 (7.8)	4 (5.2)	0.452
Influenza B virus	3 (1.5)	0	0.287
Parainfluenza viruses types 1-4	14 (6.8)	2 (2.6)	0.173
Respiratory syncytial virus	8 (3.9)	1 (1.3)	0.270
Bocavirus	5 (2.4)	2 (2.6)	0.935
Adenovirus	5 (2.4)	0	0.168
Human coronaviruses OC43 or 229E	5 (2.4)	0	0.168
Enterovirus	2 (1.0)	0	0.386
Mycoplasma pneumonia	2 (1.0)	2 (2.6)	0.302
Chlamydia pneumonia	4 (1.9)	1 (1.3)	0.715
Presence of any virus	103 (50.0)	20 (26.0)	<0.001
Presence of *M pneumoniae* or *C pneumoniae*	5 (2.4)	1 (1.3)	0.558
Presence of any pathogen	105 (51.0)	21 (27.3)	<0.001
Co-infection by ≥2 pathogens	22 (10.7)	2 (2.6)	0.030

* Insufficient respiratory specimens in three patients

Table 3. Relationship between different respiratory pathogens and patient age in 206 evaluable patients with asthma exacerbations

Parameter	Mean±SD patient age (years)	
	Infection	No infection
Pathogen		
Rhinovirus	7.3±3.8	7.7±4.1
Human metapneumovirus	5.8±2.8*	7.7±4.1
Influenza A virus	6.0±3.0*	7.7±4.1
Parainfluenza viruses types 1-4	7.7±4.9	7.6±4.0
Respiratory syncytial virus	4.5±2.1‡	7.7±4.1
Bocavirus	7.4±2.8	7.6±4.1
Adenovirus	6.2±3.8	7.6±4.1
Human coronaviruses OC43 or 229E	8.3±4.3	7.6±4.1
Presence of any virus	6.9±3.5*	8.3±4.4
Presence of *Mycoplasma pneumoniae* or *Chlamydia pneumoniae*	6.3±4.0	7.6±4.1
Presence of any pathogen	6.9±3.5†	8.4±4.4
Co-infection by ≥2 pathogens	6.5±4.1	7.7±4.0

* P<0.05
† P<0.01
‡ P<0.005

heterogeneous group of viruses and bacteria that produce similar clinical presentations. The present study made use of our published multiplex nested PCR assays that could simultaneously detect 20 different respiratory pathogens.[2] Respiratory viral infections were significantly associated with asthma exacerbations in Hong Kong children, particularly HRV. This observation was consistent with studies that identified HRV as the most important viral aetiology of childhood asthma.[3,4] More studies are necessary to delineate the link between HRV infection and worsening of asthma status.

M pneumoniae and *C pneumoniae* are common pathogens associated with asthma exacerbations; 61% of adults with asthma attacks have evidence of infection with *C pneumoniae, M pneumoniae*, or both.[5] Telithromycin is beneficial for treatment. However, in the current study, *M pneumoniae* and *C pneumoniae* were only detected in respiratory secretions from 2.4% of children with asthma exacerbations and 1.3% of patients with chronic stable asthma. These results do not support the usefulness of macrolide treatment for asthma exacerbations.

A limitation of the current study related to its power. The number of recruited controls was much lower than the target of 180, as case-control matching was not feasible on many occasions when relatively 'stable' asthmatics also had non-specific upper respiratory symptoms. Thus, our sample size had a power of 97% for detecting any difference in the detection of any virus between cases and controls, but had a marginal power of 70% for HRV infections. Besides, the study was underpowered (<50%) for the detection of other respiratory pathogens. Thus, larger studies are needed to delineate the relationship between asthma exacerbations and other respiratory pathogens.

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