Association of specific viral infections with childhood asthma exacerbations

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Abstract: *Introduction:* Asthma exacerbations may occur due to a variety of triggers including respiratory viruses. The aim of this study was to determine the role of particular viral infections in asthma exacerbations in children. *Materials and methods:* The study was performed at Dr. Daneshvari Hospital Pediatric Emergency Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran between 2014 and 2015. A nasopharyngeal aspirate or swab was obtained from each patient during admission. All samples were maintained at 4 °C until submission to the virology laboratory and were tested for respiratory viruses by nucleic acid testing. *Results:* A total of 60 patients with asthma exacerbations were recruited for this study. Of the 60 samples collected from the patients with acute asthma exacerbations, rhinovirus was detected in 12 patients (20%), respiratory syncytial virus in 5 (8%), adenovirus in 5 (8%), and influenza virus in 1 (1.6%). Respiratory pathogens were not detected in 37 (61%) samples. All the samples investigated showed single viral infection. *Conclusions:* To conclude, the most common viruses detected were rhinovirus followed by respiratory syncytial virus (RSV) and adenovirus. RSV was more commonly associated with more severe attacks. Both the study design (e.g., time of sampling, age of the patients, etc.) and also the method used for viral detection influence the frequency of detection of the respiratory viruses.

Keywords: asthma, exacerbations, viral infections, childhood

Introduction

Asthma is the most common chronic respiratory disease associated with an increasing prevalence characterized by airway inflammation, mucus production, and airway hyperresponsiveness [1]. Bronchial hyperresponsiveness may occur in response to many common environmental triggers [2, 3] leading to asthma exacerbations. Asthma exacerbations are acute or subacute worsening episodes of shortness of breath, coughing, wheezing, and chest tightness [4], which are classified as mild, moderate, severe, or life-threatening. These exacerbations may be associated with a variety of respiratory viruses, including human rhinovirus (HRV), respiratory syncytial virus (RSV), influenza virus, parainfluenzavirus, human metapneumovirus, adenovirus, bocavirus, and coronavirus [5], of which the first two mentioned groups are most importantly relevant to asthma development [6]. Viral respiratory infections are usually self-limiting illnesses [4]. However, they may exert profound effects on important aspects of asthma [7]. Studies suggest a substantial role for viral infections in about 50% of asthma exacerbations

in adults and a greater involvement in childhood asthma exacerbations. Wheezing episodes in early life are associated with respiratory infections, which in most instances may diminish as the child ages [4].

The purpose of this study is to investigate the role of particular viral infections in asthma exacerbations in children with a previous history of allergy.

Material and Methods

Patients

The study was performed at Dr. Daneshvari Hospital Pediatric Emergency Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran between 2014 and 2015. Patients included in the study were between 1 month and 17 years of age presenting with a wheezing episode severe enough to require hospitalization and a previous history of two or more wheezing episodes diagnosed by a physician and a personal and/or familial allergic background. Because of the difficulty in differentiation of asthma from wheezing episodes of other origin in very young children, the patients enrolled in the study needed to comply with the diagnostic criteria of bronchial asthma according to the GINA guidelines [8]. Children with a history of premature birth and the presence of the pulmonary or cardiovascular system, metabolic disorders, immunosuppression, genetic, or neurologic disorders were excluded from the study.

Respiratory samples

Nasopharyngeal aspirates were obtained from patients during admission. Those patients, in whom respiratory secretions could not be collected by this method, had a nasopharyngeal Dacron tip swab. All samples were maintained at 4 °C until submission to the virology laboratory and were tested for respiratory viruses by nucleic acid testing. In virology laboratory, the samples were processed and the secretions were maintained at −20 °C for the upstream procedures.

Genome extraction and cDNA synthesis

Viral RNA and DNA were extracted together using the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) from respiratory samples according to the manufacturer's recommendations, and 100 µl of nucleic acids was eluted. For RNA viruses, cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA). The extracted genome elutes and cDNA were stored at −20 °C for further studies with conventional and real-time polymerase chain reaction (PCR).

Nucleic acid testing

Influenza virus (types A and B) was detected by real-time PCR method based on WHO information for molecular diagnosis of influenza virus updated on March 2014 [9]. To screen for the human bocavirus (hBoV) genome, a nested PCR was performed using primers in the NS-1 coding region, as previously described [10]. Laboratory investigation of parainfluenzavirus types 1, 2, 3, 4; RSV A and B; HRVs; human coronaviruses OC43 and 229E; human metapneumoviruses; and adenoviruses were conducted by multiplex nested PCR assays that were described earlier [11]. The positive clinical samples and the Vircella AmpliRun® PCR control (Vircell, Spain) were used as positive controls in our assay.

Statistical analysis

Fisher's and Pearson's χ^2 tests were used to assess the associations. The probability level to determine statistical significance was considered to be 0.05 and the statistical analyses were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Ethics

The study protocol was approved by the ethics committee of the hospital. Legally authorized representatives of the children provided informed consent.

Results

Between 2014 and 2015, a total of 60 patients with asthma exacerbations were recruited in this study. The mean age of the patients was 7 years. The majority of the patients were male (71.7% male vs. 28.3% female) *(Table I)*.

Of the 60 samples collected from the patients with acute asthma exacerbations, rhinovirus was detected in 12 patients (20%), RSV in 5 (8%), adenovirus in 5 (8%), and influenza virus in 1 (1.6%). *Figure 1* demonstrates the frequency of detected viruses during asthma exacerbations.

Table I The sexual distribution of patients' ages and attacks

Patients' characteristics	Male	Female
Total (%)	43 (71.7)	17 (28.3)
Mean age (years)	7.2	6.5
Moderate attacks (%)	34 (64.4)	15 (30.6)
Severe attacks (%)	9 (81.8)	2 (18.2)

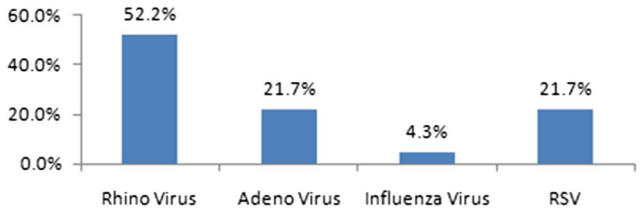

Fig. 1. The frequency of detected viruses in asthma exacerbations

All the samples that are investigated showed single viral infection. A total of 37 (61%) samples did not have any respiratory pathogens. The patients were also evaluated in three distinct groups according to their age. Three (5%) of the patients studied were under 1 year of age, 20 (33.3%) were between 1 and 5 years of age, and 37 (61.7%) were older than 5 years of age. The majority of the patients with documented viral infections in this study were between 1 and 5 years of age. Rhinovirus was the most common vial agent detected in all three age groups followed by both RSV and adenovirus. In children between 1 and 5 years of age, RSV (five patients) followed by rhinovirus (four patients) were the most common respiratory pathogens detected.

Thirty-one patients had multiple (more than one) admissions. All of them except one were associated with rhinovirus detection. The only one reported was due to RSV.

RSV had been detected more significantly in patients with severe attack than those with moderate exacerbations of the disease. *Figure 2* demonstrates the relationship between asthma severity and viral infections.

The patients were also evaluated for any relationship between specific symptoms with the viral agents. There were no significant differences when evaluating symptoms with a specific viral agent. Viral infections detected in the patients were compared according to their frequencies and also the clinical manifestations and complications.

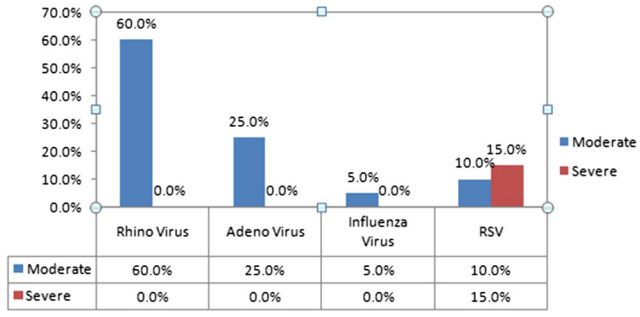

Fig. 2. The relationship between asthma attack severity and detected viruses

Discussion

This study is an evaluation of a bidirectional relationship between allergy and specific viral infections in increasing the risk for acute exacerbations of asthma in children.

The typical viruses to cause exacerbations are rhinovirus, RSV, influenza, coronavirus, and parainfluenzavirus [12]. Our previous study on hBoV in children with asthma exacerbation revealed that the detection rate of hBoV was 6% of studied subjects [9].

The role of viral infections in inducing an imbalance in the immune homeostasis has been well known for years. Among several mechanisms related to viral infection and allergic inflammation, epithelial damage and mucus production are the most important ones [13].

It has been suggested that host factors could alter susceptibility of asthmatic subjects to virus-induced exacerbations including age and gender [14]. Asthma has a greater prevalence in boys in early life. However, there is a sex reversal around puberty, which makes it higher risk in girls after adolescence [15]. Our findings indicated a male dominancy of 71% of the children presented with asthma attack, which is consistent with the previous studies.

The rate of viral detection in asthma acute exacerbations has been greatly varied in previous studies. While viruses have been detected in up to 85% of asthma exacerbations in different studies [7, 16], in a study performed by Liao et al. [17] later, the rate of positive viral detection using PCR in acute asthma exacerbation was 34.2%. In this study, we could identify a potential viral causative agent as the reason of acute asthma exacerbation in 23 (38%) of our patients. This rate is in agreement with the one reported by Liao et al. [17]. This wide variation among asthmatic individuals in different studies may denote the influence of additional factors, such as the possible effect of atmospheric pollution in association with viral infections in inducing asthma attack.

The most common viruses detected that cause wheezing illnesses during early life are RSV and HRV, with the former associated with a seasonal pattern and the latter occurring throughout the year [15]. Rhinovirus is common in all age groups; RSV is most important in infants and younger children. Children affected with HRV tend to be older than those affected with RSV [18]. Jartti et al. suggested the transition in dominance between HRV and RSV around 12 months of age [19, 20]. This is in accordance with the results we obtained in this study. Therefore, we could explain the preponderance of HRV-induced asthma exacerbations in this study with the longer duration of exposure and also the low number of children less than 1 year of age enrolled in this study. The same results were also obtained in the COAST study, in which HRV was found in 48% of the samples and RSV in 21% of the specimens [6].

RSV was more commonly associated with more severe attacks in this study that may be demonstrating an interplay between age at initial infection, genetic susceptibility, and virus strain, which altogether determine the outcome of the viral infection and the airway disease, which was first suggested by Saglani [20]. RSV infections, particularly the severe ones, are thought to be associated with Th2 polarization of the lung immune response with further sensitization of the host's allergic responses to other molecules [21].

To explain those patients without a viral agent being isolated in this study, we believe that both the study design and the method used for viral detection influence the frequency of detection of the respiratory viruses. For instance, the highest rates of virus detection are found when samples are taken as soon as symptoms commence. Viral detection becomes difficult by delaying the sampling at a time when virus shedding has been falling. Another limitation is the difficulty of diagnosing exacerbations in young children based on the clinical assessment of symptoms by both parents and clinicians. In addition, seasonal variations may significantly influence the virus identification. Extensive immunologic, virologic, and physiologic investigations during viral infections may lead toward the development of targeted therapy as a goal to personalize therapy for each asthmatic patient in the future.

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Authors' contribution: MH, SAN, and SD: study design, drafting of the manuscript, literature review, and critical revision of the manuscript. SAN: technical support. MH, ST-N, MRB, SAM, NB, and HG: acquisition of data. AAV: study supervision. All authors take responsibility for the integrity and the accuracy of the data.

Conflict of interest: None.

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