Respiratory Tract Viral Infections in Inner-City Asthmatic Adults

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Background: Respiratory tract viral infections (RTVIs) have been identified frequently in association with asthma exacerbations in children, but few studies have shown similar rates of viral infections in adults with asthma. Further studies using newer diagnostic techniques to evaluate the frequency of RTVIs in adults with acute exacerbations of asthma need to be performed.

Methods: Twenty-nine asthmatic adults were recruited from the pulmonary clinic of an urban county hospital and were followed up in a longitudinal cohort study for signs and symptoms of asthma and RTVI. One hundred twenty-two asthmatic adults presenting to the emergency department (ED) of the same hospital with acute symptoms of asthma underwent evaluation for RTVI in a cross-sectional prevalence study. In both studies, respiratory secretions and paired serum samples were collected from subjects with acute wheezing episodes and evaluated using virus culture, serologic testing, and reverse transcription--polymerase chain reaction (RT-PCR).

Results: In the longitudinal cohort study, 138 respiratory illnesses, of which 87 were asthma exacerbations, were evaluated; 41% of all illnesses and 44% of asthma exacerbations were associated with an RTVI. In the ED study, 148 asthma exacerbations were evaluated; 55% were associated with an RTVI. An RTVI was identified in 21 (50%) of 42 of the subjects hospitalized in the ED study. Picornaviruses (rhinoviruses), coronaviruses, and influenza viruses were the most commonly identified causes of RTVI. Forty-six (60%) of the 77 picornavirus infections and 22 (71%) of the 31 coronavirus infections were identified only using RT-PCR.

Conclusions: Asthmatic exacerbations in adults are frequently associated with an RTVI. Identification of such infections often requires newer diagnostic methods, such as virus-specific RT-PCR. The high frequency of RTVIs identified in association with asthmatic exacerbations in adults from the inner city suggests that strategies for the prevention of RTVI should be targeted toward this population.

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Asthma IS a common disease, affecting 14 to 15 million Americans. Recent epidemiological studies of asthmatic patients report morbidity and mortality to be increasing. Certain subpopulations, such as racial and ethnic minorities who are poor and live in the inner city, appear to be at the highest risk for hospitalization and mortality. The economic burden associated with asthma also is great. In 1990, there were estimated to be more than 450,000 hospital admissions for asthma-related illnesses and approximately $6.2 billion spent for total health care costs.

Although the cause of asthma remains unknown, the association between respiratory tract viral infections (RTVIs) and exacerbations of asthma has been acknowledged for more than 25 years. Respiratory tract viruses have been identified in up to 80% of children with wheezing episodes and asthma exacerbations. The most commonly identified viruses have been rhinoviruses, coronaviruses, and parainfluenza viruses. Studies in adults with asthma have documented RTVIs less commonly, with the frequency of asthma exacerbations associated with RTVI ranging from 0% to 44% in 3 recent studies.

We conducted 2 studies evaluating the role of RTVIs in exacerbations of asthma in adults who used an urban public hospital for their medical care. In 1 study, adults with asthma were followed up longitudinally during 2 fall and winter seasons and underwent testing for viral infection for each reported asthma exacerbation. An additional study evaluated acute exacerbations of asthma in adults coming to the emergency department (ED) of the same urban public hospital. The role of RTVIs in the morbidity of these adults with asthma is described.
SUBJECTS, MATERIALS, AND METHODS

STUDY GROUPS

Longitudinal Cohort Study

Asthmatic adults were recruited for a longitudinal cohort study (December 6, 1991-May 3, 1994). Asthma was defined by a history of multiple episodes of wheezing and documented by at least a 15% improvement in forced expiratory volume in 1 second following bronchodilator therapy or positive results of a methacholine chloride challenge test. Study subjects were recruited from patients at an urban public hospital pulmonary clinic (Ben Taub General Hospital, Houston, Tex). Subjects with a greater than 5 pack-years smoking history and those receiving daily systemic steroid therapy were excluded from participation.

ED Study

A convenience sample of adults presenting to the ED of Ben Taub General Hospital for acute care of an asthma exacerbation was recruited for this cross-sectional prevalence survey (October 9, 1992-March 22, 1994). Asthma was defined by a history of multiple episodes of wheezing. Adults with known chronic obstructive lung disease or a history of cigarette smoking (>5 pack-years) were excluded from participation.

PROCEDURES

Longitudinal Study

A brief medical history was obtained and a physical examination was performed at enrollment. Baseline information included current and past use of medications, history of cigarette smoking, and complete influenza virus and pneumococcal vaccination history. Spirometric studies were performed with and without bronchodilators. Any subject who could not perform spirometry was excluded. At the time of the initial visit and in the fall of each year during the study, each subject was approached from October 9, 1992, to May 31, 1993, for participation in the study were enrolled; the principal reasons for nonparticipation were inability to confirm the diagnosis of asthma and unwillingness to participate. One hundred twenty-two participants were enrolled; the principal reasons for nonparticipation were inability to confirm the diagnosis of asthma and unwillingness to participate. One hundred twenty-two participants were enrolled; the mean age was similar to that of participants in the longitudinal study, but a significantly larger percentage of black subjects participated in the ED study (P = .03).

ILLNESS DEFINITIONS

Upper respiratory tract illnesses (URTIs) were defined by the presence of symptoms of acute rhinitis and/or pharyngitis. Of the 17 culture-positive picornavirus infections, 12 were rhinoviruses, 2 were enteroviruses, and 3 were not further identified. Seven picornavirus and 6

RESULTS

DEMOGRAPHICS

The demographic characteristics of the subjects of both studies are described in Table 1. Thirty-six subjects were enrolled in the longitudinal cohort study, and 29 continued in the study past the initial (enrollment) visit and were eligible for evaluation. The mean and median durations of follow-up were 19.5 and 22.4 months, respectively (range, 2.5-28.5 months). The documented influenza virus vaccination rates were 64% (7/11), 78% (21/27), and 64% (14/22), respectively, for the 3 influenza seasons from December 1, 1991, to April 30, 1994. The ED study was conducted from October 9, 1992, until March 22, 1994. Fifty-seven percent of the subjects ap-
gitis (ie, sore throat, rhinorrhea, or sneezing). Lower respiratory tract illnesses were defined by increased cough and sputum production or increased wheezing. An exacerbation of asthma was defined by an increase in wheezing and/or dyspnea.

VIRUS CULTURES

Specimens were inoculated (usually within 12 hours) onto the following 4 different cell culture tubes: human diploid lung fibroblasts (WI-38), Madin-Darby canine kidney cells, human tracheal carcinoma cells (HEp-2), and monkey kidney cells (LLC-MK2) (BioWhittaker, Walkersville, Md). The cell culture tubes were incubated on a roller drum at 33°C. Standard detection and identification methods for respiratory tract viruses were used.17 Rhinoviruses were distinguished from enteroviruses using acid lability or RT-PCR,28 and some type A influenza viruses were subtyped using RT-PCR.19

SEROLOGIC TESTS

Serum samples were stored at −20°C until tested. Hemagglutination inhibition and microneutralization tests were used to measure antibodies against influenza virus types A and B using strains representative of those circulating in the community.20 Microneutralization tests were used to measure antibodies to parainfluenza viruses 1, 2, and 3; coronavirus 229E; and respiratory syncytial viruses (RSV) using previously published techniques.21-23 A serologic rise was defined as a 4-fold or greater rise in antibody titer between acute and convalescent serum samples or a 4-fold (influenza virus only) or greater antibody rise in hemagglutination-inhibition and microneutralization tests. Enzyme-linked immunosorbent assay antibody tests for coronavirus OC43 were used as described previously.24

REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION

Viral nucleic acids were extracted from respiratory secretions using RNAzol (Biotecx, Houston) as previously described.12,13,24,25 Primers used for amplification were as follows: complementary DNA (cDNA) synthesis (R1) 5′-ACGGACACCCAAAGTA-3′, upstream primer (R2) 5′-AGCACCTCTGTGTTCCC-3′ for picornavirus26; cDNA synthesis (FAM1) 5′-CAGAGACTGAGATGCTTGC-3′, upstream primer (FAM2) 5′-GCTCCTGCTACGTTATTTG (GA)AT-3′ for influenza virus type A26,27, and cDNA synthesis (CVP3) 5′-IIAAATTGCTIITCTGTGTC-3′ (1 indicates inosine), downstream primer (CVP4) 5′-CCAAAAIITCTGATTGCGGCTCTC-3′ for coronavirus OC43.28 Complementary DNA was synthesized for 1 hour at 43°C in a reaction mix containing 10-mmol/L tris(hydroxymethyl)amino methane hydrochloride (pH, 8.3), 50-mmol/L potassium chloride, 1.5-mmol/L magnesium chloride, 3.3-µmol/L cDNA synthesis primer, 667-µmol/L deoxynucleoside triphosphates, 20 U of RNase inhibitor (RNasin; Promega Corporation, Madison, Wis), and 5 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc, St Petersburg, Fla). The PCR amplification was performed in a solution containing 10-mmol/L tris(hydroxymethyl)amino methane hydrochloride (pH, 8.3), 50-mmol/L potassium chloride, 1.5-mmol/L magnesium chloride, 1-µmol/L each of the cDNA synthesis and upstream primers, 200-µmol/L deoxynucleoside triphosphates, and 5 U of Taq polymerase (Perkin-Elmer Corporation, Norwalk, Conn) using a thermal cycler (PTC-100, MJ Research, Inc, Cambridge, Mass). After an initial 4-minute heat denaturation at 94°C, 40 cycles of heat denaturation at 94°C for 1 minute, primer annealing at 49°C (picornavirus), 55°C (influenza virus type A), or 58°C (coronavirus) for 1 minute 30 seconds, and primer extension at 72°C for 1 minute were followed by a final primer extension step at 72°C. Amplified products were 394, 212, and 186 base pairs in length for picornavirus, influenza virus type A, and coronavirus OC43, respectively. Positive results were confirmed by slot-blot hybridization using the following digoxigenin-labeled oligonucleotides: 5′-TCCTCCGCGGCCTGAATG-3′ (R4) for picornavirus; 5′-TCTGTACACCTGACTAAGGGGATTGTTGCTGCC-3′ (AH2) for influenza A virus; and 5′-AAGCAAITGCCCCAATAGTCAGICAGAAATTTTATT-3′ (CVPP) for coronavirus OC43.

STATISTICAL ANALYSIS

Discrete variables were compared using χ² test or Fisher exact test. Parametric data were analyzed using Student t test.
3 infections were enteroviruses, and 1 infection was not further identified. More than 1 associated respiratory tract viral pathogen was identified in 11 illnesses (13%); 3 of these resulted in hospitalization.

**RESPIRATORY ILLNESSES**

Of 138 respiratory illnesses in the longitudinal study, 137 were evaluated at an illness visit (Table 3). Of these, 41% of the illnesses and 44% of the asthma exacerbations were associated with a documented RTVI. Viral infections were documented more frequently from November through April than from May through October (39/79 [49%] vs 18/59 [30%], respectively; P=.03, χ²).

Symptoms of a URTI plus asthma exacerbation were seen in 52%, a URTI alone in 36%, an asthma exacerbation alone in 11%, and bronchitis alone in 1%. An RTVI was as likely to be identified with a URTI illness (19/50 URTIs only) as with asthma exacerbations (32/72 URTIs with asthma exacerbations) as with an asthma exacerbation (6/15) alone. Eleven ED visits resulted in 3 hospitalizations. An RTVI was documented in 6 ED visits (3 picornaviruses, 1 influenza virus type A, 1 parainfluenza virus 2, and 1 parainfluenza virus 3) and in 1 hospitalization (rhinovirus).

There were 148 illness visits in the ED study (Table 3). An RTVI was identified in 82 visits (55%). The number of illness visits per month ranged from 1 (April 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993), and the number of RTVIs identified per month ranged from 0 (August 1993) to 22 (February 1993, P=.23). No deaths occurred in any of the hospitalizations.

In our 2 studies, RTVs were identified frequently in association with asthma exacerbations in adults. An RTVI was documented in 44% of exacerbations in a small cohort of patients with asthma who were followed up longitudinally and in 55% of those undergoing evaluation in an acute-care setting (ED). Picornaviruses, coronaviruses, and influenza viruses were the most commonly identified viruses, with parainfluenza viruses and RSV being recognized less frequently. Most of the picornaviruses characterized were rhinoviruses, and it is likely that most of the uncharacterized picornaviruses also were rhinoviruses. Asthma exacerbations associated with RTVs were identified throughout the year, but the presence of an RTVI did not increase the likelihood or the duration of hospitalization for an asthma exacerbation.

One previous study also found a similar frequency of RTVs associated with worsening asthma in adults (Table 4). Nicholson et al13 reported RTVs in

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### Table 1. Demographics of Asthmatic Adults With Respiratory Tract Viral Infections and Illnesses*

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal Study</th>
<th>Emergency Department Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>122</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>37.8 (9.1)</td>
<td>38.5 (13.9)</td>
</tr>
<tr>
<td>Age range, y</td>
<td>19-50</td>
<td>17-77</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>6:23</td>
<td>37.85</td>
</tr>
<tr>
<td>Race, No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Hispanic</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>Black</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Length of follow-up, mean (SD), mo</td>
<td>19.5 (7.4)</td>
<td>. . .</td>
</tr>
</tbody>
</table>

*Study groups are described in the “Study Groups” subsection of the “Materials and Methods” section. Data are given as number (percentage) of total cases.

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### Table 2. Virus-Associated Illnesses in Asthmatic Adults*

<table>
<thead>
<tr>
<th>Respiratory Viruses</th>
<th>Longitudinal Study</th>
<th>Emergency Department Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picornavirus†</td>
<td>24 (7)</td>
<td>53 (39)</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>10 (6)</td>
<td>21 (16)</td>
</tr>
<tr>
<td>Influenza viruses type A and B</td>
<td>11</td>
<td>12 (0)</td>
</tr>
<tr>
<td>Parainfluenza viruses 1-3</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

*Study groups are described in the “Study Group” subsection of the “Materials and Methods” section. Numbers in parentheses indicate number of infections documented using reverse transcription–polymerase chain reaction.

†Majority of these are rhinoviruses.

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### Table 3. Use of Medical Care by Asthmatic Adults*

<table>
<thead>
<tr>
<th>Illnesses associated with RTVI/total acute respiratory illnesses</th>
<th>Longitudinal Study</th>
<th>Emergency Department Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED visits associated with RTVI/total ED visits for asthma</td>
<td>6/11 (55)</td>
<td>82/148 (55)</td>
</tr>
<tr>
<td>Hospitalizations associated with an RTVI/total hospitalizations</td>
<td>1/3 (33)</td>
<td>21/42 (50)</td>
</tr>
</tbody>
</table>

*Study groups are described in the “Study Groups” subsection of the “Materials and Methods” section. RTVI indicates respiratory tract viral infection; ED, emergency department. Data are given as number (percentage) of total cases.
association with 44% of asthma exacerbations and upper respiratory tract symptoms in 80% of asthma exacerbations, with viruses identified in 57% of the subjects with symptomatic colds. The results from Nicholson et al and those of our studies contrast sharply with those of other investigators (Table 4). The reason for this difference is unexplained. Most of the parainfluenza virus infections between our studies. Picornavirus infections in our 2 studies were identified significantly, and the induction of several inflammatory mediators, including interleukin 8 and bradykinin, are increased. The induction of virus-specific immunoglobulin E, direct damage to respiratory tract epithelium, down-regulation of β-adrenergic function, induction of other cytokines and chemokines, and activation of cellular immune responses are other proposed mechanisms by which respiratory tract viruses may contribute to increased bronchial reactivity.

Several studies have shown that RTVIs are not invariably associated with airway obstruction in adults with asthma. One third of the symptomatic RTVIs in our longitudinal study were not associated with worsening of lower respiratory tract symptoms. Both host- and virus-related factors appear to contribute to the different clinical manifestations associated with RTVI in subjects with asthma. These factors remain to be defined.

The morbidity and mortality associated with asthma have been increasing since the late 1970s. Residents of the inner city are at increased risk for asthma-related hos-
pitalization and mortality. Potential reasons for the increased risk in this population include increased exposure to allergens such as mites and cockroaches, decreased access to or underuse of medical care, poor air quality, psychosocial problems, exposure to cigarette smoke, and crowding. Race and ethnicity appear to be a less significant risk factor than socioeconomic status. Although this study does not evaluate the contribution of any of the aforementioned factors to the morbidity associated with asthma, it demonstrates that RTVIs are commonly associated with asthma exacerbations in adults from the inner city. Thus, another potential target for the control of asthma is the prevention of RTVIs with vaccines or antiviral agents. Because there is no group for comparison, the occurrence of influenza virus infections in vaccinated individuals does not indicate that influenza virus vaccination was ineffective; however, it does suggest that there is room for improvement in currently licensed influenza virus vaccines.

We performed 2 clinical studies that found RTVIs to be frequently associated with asthma exacerbations in adults receiving medical care at an urban hospital. Although the longitudinal cohort study was small, the results obtained are similar to those of Nicholson et al and to the results seen in several pediatric studies. The use of new diagnostic technology (ie, RT-PCR) greatly increased the identification of RTVIs. Although picornaviruses identified using RT-PCR were not separated into genera, most of these were likely to have been rhinoviruses. Because we did not look for other infections (ie, those caused by Mycoplasma pneumoniae and Chlamydia pneumoniae) that have been associated with asthma exacerbations in the past, the relative importance of respiratory tract infections as potential precipitants of asthma exacerbations may have underestimated. The high frequency of RTVIs identified in association with asthma exacerbations suggests that strategies for the prevention of RTVIs should be targeted toward the population with asthma.

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