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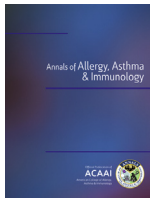

Detection of respiratory viruses in adult patients with perennial allergic rhinitis

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ABSTRACT

Background: The symptoms of allergic rhinitis may be worsened by a viral respiratory infection. However, there are few data on the presence of respiratory virus in patients with allergic rhinitis.
Objective: To evaluate whether patients with allergic rhinitis have an increased frequency of respiratory virus detection in a prospective case–control study.
Methods: Fifty-eight adult patients diagnosed with perennial allergic rhinitis were evaluated from September 2011 through June 2012. A control group of 61 adult patients without allergy was included. Multiplex polymerase chain reaction was used to detect respiratory viruses in nasal lavage samples.
Results: Respiratory viruses were detected in 25 of 58 patients (43.1%) with perennial allergic rhinitis, but in only 15 of 61 control patients (24.6%). In virus-positive samples, multiple viruses were detected in 9 of 25 patients (36.0%) with perennial allergic rhinitis but in only 2 of 15 control patients (12.5%). Rhinovirus was the most common virus in patients without allergy and those with allergic rhinitis. There were significant differences in the detection rates of overall and multiple respiratory viruses and rhinovirus between the 2 groups ($P < .05$). However, in patients with allergic rhinitis, there was no statistically significant association between the detection of respiratory viruses and symptom scores.
Conclusion: This study shows that there is a high prevalence of respiratory viruses, especially rhinovirus, in patients with allergic rhinitis. Subsequent studies are needed to determine the clinical significance of highly prevalent respiratory viruses in patients with allergic rhinitis.

Introduction

Symptoms of viral upper respiratory infection (URI) usually overlap with those of allergic rhinitis (AR), such as nasal obstruction, rhinorrhea, and sneezing. Patients with AR mistakenly believe they have repeated viral infections that manifest as the common cold. Furthermore, it is difficult to differentiate the symptoms of AR aggravation from viral URI symptoms. Although the 2 are clearly distinctive, they share common clinical features. The pathogenetic relation between the 2 conditions is not fully understood. It is theoretically possible that allergen-induced inflammation and mucosal swelling in the nasal cavity and paranasal sinuses of patients with AR may make them more susceptible to viral or bacterial infections.[1,2] Despite these assumptive inter-relations between the 2 conditions, the role of respiratory viruses as an aggravating factor of AR has not been established. Some previous studies have focused only on the severity of URI symptoms in patients with AR. In 1 cohort study, adult patients with AR showed not only a higher incidence of respiratory infections, especially in the number of severe episodes, but also a longer total duration of respiratory infection compared with adult patients without allergy.[3] In contrast, a prospective study has reported that there are no significant differences in the frequency and duration of URI between adult patients with AR and patients without allergy.[4]

Viral respiratory infections act synergistically with allergen sensitization and exposure to exacerbate allergic asthma.[5,6] Domestic exposure to allergens acts synergistically with viruses in sensitized patients, increasing the risk of hospital admission.

Allergic rhinitis and asthma have similar clinical, epidemiologic, and pathogenetic features.[7] Furthermore, because viral respiratory infections are more frequent in the upper airway than in the lower airway,[8] the presence of respiratory viruses in the upper respiratory tract may result in more severe nasal mucosal inflammation and nasal symptoms in patients with AR. However, to date, the exact pathogenetic role of respiratory viruses present in the upper airway of patients with AR has not been elucidated. Therefore, in this study, as a basic step for better understanding the role of respiratory viruses in the pathogenesis of AR, the detection rate of respiratory viruses in patients with perennial AR (PAR) without URI symptoms was compared with that in control patients, and the symptom severity of AR was evaluated according to the presence of respiratory viruses.

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Methods

Study Population

One-hundred nineteen adult patients were enrolled from September 2011 through June 2012 at the Department of Otolaryngology, Asan Medical Center (Seoul, Korea). Recruitment was based on a history of nasal-ocular symptoms, endoscopic examination, and a skin prick test or allergen-specific IgE test. None of the patients had chronic illnesses, such as hypertension and diabetes mellitus, evidence of a viral URI within 4 weeks before screening, or a history of chronic rhinosinusitis and/or a nasal polyp. URI symptoms were distinguished from AR symptoms in cases with associated symptoms, including cough, malaise, throat discomfort, fever or chills, and headache, in addition to nasal discharge, nasal congestion, and sneezing.[9] The study was approved by the institutional review board of the Asan Medical Center and all participants provided written informed consent before enrollment.

Patients with PAR were defined by persistent allergic symptoms for at least 1 year and a positive response to *Dermatophagoides farina* and/or *Dermatophagoides pteronyssinus*, which are the most prevalent allergens in Korea,[10] using a skin prick test or allergen-specific IgE test. A positive skin prick test result was defined as a wheal diameter at least 3 mm larger than the negative control. Patients included in the study must have experienced symptoms that were severe enough to require continuous treatment before and during the study. In other words, they experienced moderate or severe AR symptoms according to the 2008 Allergic Rhinitis and its Impact on Asthma guideline.[11] Their allergic symptoms were controlled using only oral antihistamines. Patients using intranasal topical steroids were excluded. Patients presenting with a respiratory disorder other than AR, including asthma and chronic obstructive pulmonary disease, also were excluded from the study. Control patients were healthy volunteers or volunteers who required planned surgery for thyroid masses. All control patients had no history of allergy and had a negative response to allergy tests.

Total Nasal Symptom Score

Allergic rhinitis symptoms were recorded based on the patient's expression of symptoms. Symptoms were recorded before the collection of nasal samples at enrollment. Watery rhinorrhea, sneezing, itching, and nasal obstruction were graded according to severity within a range of 0 to 3: 0, none; 1, mild; 2, moderate; 3, severe. The scores were summed to produce the total nasal symptom score (TNSS).

Sample Collection

Nasal lavage samples were collected from all patients in the outpatient clinic. The sample size was approximately equally distributed over the study period. Lavage fluid was obtained from washing the nasal mucosa with 15 mL of saline using a syringe and a plastic container. Patients were asked to forcibly expel the nasal contents into a plastic container after saline washing. Then, the specimens were transported immediately to the laboratory for multiplex polymerase chain reaction (PCR).

Multiplex PCR Analysis

Respiratory virus detection was facilitated by multiplex PCR. RNA samples were extracted from 140 μL of each respiratory specimen using the QIAamp Viral RNA kit (Qiagen, GmbH, Hilden, Germany). After reverse transcription to synthesize cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada), each cDNA sample was subjected to multiplex PCR using the Seeplex RV15 ACE Detection kit (Seeplex RV15, Seegene Inc, Seoul, Korea) according to the manufacturer's instructions. Briefly, 3 μL of synthesized first-strand cDNA, 5$\times$ RV15 primer mix, 2$\times$ multiplex master mix (Taq DNA polymerase and dNTPs are included), and 3 μL of 8-methoxypsoralen solution were added. The primer mixes contained the internal control template and the primer pair to validate the PCR. Three reactions (primer mixes A, B, and C) were set up for each sample according to the kit instructions. Specific primer targets for each respiratory virus are listed in Table 1. PCR was carried out using the following reaction conditions: initial denaturation at 94°C for 15 minutes; 40 cycles at 94°C for 30 seconds, 60°C for 1 minute 30 seconds, and 72°C for 1 minute 30 seconds; and a final extension phase at 72°C for 10 minutes. The multiplex PCR products were visualized by electrophoresis on a 2% agarose gel.

Statistics

Detection rates of respiratory viruses in the 2 groups were analyzed with the χ^2 test. Between-group differences in age were analyzed using the Student t test. In the PAR group, differences in the TNSS according to the detection of respiratory virus were analyzed using the paired t test. Numeric data were expressed as mean $\pm$ standard deviation. SPSS 16.0 (SPSS, Inc, Chicago, Illinois) was used for statistical analysis. A P value less than .05 was considered statistically significant.

Results

Nasal lavage samples from 58 patients with PAR and 61 control patients were evaluated using multiplex PCR. The sex distribution and mean age of patients with PAR and control patients were similar. The PAR group consisted of 29 men and 29 women and their mean age was 41.0 years (range 18–69). The control group without allergy consisted of 33 men and 28 women and their mean age was 36.8 years (range 17–65).

Respiratory Viral Detection by Multiplex PCR

Respiratory viruses were detected in 25 of 58 nasal lavage samples (43.1%) from patients with PAR but in only 15 of 61 nasal lavage samples (24.6%) from control patients. The detection rate of respiratory viruses was higher in the PAR group than in the control group (P = .033; Table 2). In the PAR group, 16 of 25 positive samples (64.0%) were positive for only 1 respiratory virus, whereas

Table 1
Targets for detection of respiratory viruses using the Seeplex respiratory detection assay

RV15 ACE detection assay	Size in agarose gel (bp)
A set	
Internal control	850
Human adenovirus	534
Human coronavirus 229E/NL63	375
Human parainfluenza virus 2	264
Human parainfluenza virus 3	189
Human parainfluenza virus 1	153
Human parainfluenza virus 1	153
B set	
Internal control	850
Human coronavirus OC43	578
Human rhinovirus A, B, C	394
Human respiratory syncytial virus A	269
Influenza A virus	206
Human respiratory syncytial virus B	155
C set	
Internal control	850
Human bocavirus 1, 2, 3, 4	579
Influenza B virus	455
Human metapneumovirus	351
Human parainfluenza virus 4	249
Human enterovirus	194

Table 2
Detection rates of respiratory viruses in nasal lavage fluid

Respiratory viruses	Positive samples, n (%)		*P* value
	PAR (n = 58)	Control (n = 61)	
Overall viruses	25 (43.1)	15 (24.6)	.033
Single virus	16 (27.6)	13 (21.3)	.523
Multiple viruses	9 (15.5)	2 (3.3)	.027
2 species	6 (10.6)	2 (3.3)	
3 species	3 (5.2)	0 (0)	
Type of virus			
Rhinovirus	13 (22.4)	5 (8.2)	.040
Parainfluenza virus	9 (15.5)	5 (8.2)	.262
Influenza virus	6 (10.3)	1 (1.6)	.057
Respiratory syncytial virus	4 (6.9)	3 (4.9)	.713
Coronavirus	1 (1.7)	3 (4.9)	.619
Adenovirus	3 (5.2)	0	.113
Meta pneumovirus	1 (1.7)	0	.487
Enterovirus	0	0	N/A
Bocavirus	0	0	N/A

Abbreviations: N/A, not available; PAR, perennial allergic rhinitis.

9 samples (36.0%) tested positive for multiple viruses. In the control group, 14 of 15 positive samples (87.5%) were positive for a single virus, whereas 2 samples (12.5%) were positive for multiple viruses. Multiple viruses were detected more frequently in the PAR group than in the control group ($P = .028$).

Human rhinovirus (HRV) was the most commonly detected virus in nasal lavage fluid from the PAR and control groups. The detection rate of HRV was greater in patients with PAR than in control patients ($P = .040$). In contrast, the detection rate of parainfluenza viruses (PIVs), which are the second most common type of respiratory virus, was not significantly different between patients with PAR and control patients ($P = .215$). Moreover, the detection rates of other respiratory viruses in nasal lavage samples were not statistically significant between the 2 groups (Table 2).

Symptom Score Comparison of PAR Groups According to Detection of Respiratory Viruses

The TNSS of the respiratory virus–positive PAR group was 5.20 ± 3.65 and that of the respiratory virus–negative PAR group was 6.12 ± 2.92. There was no significant difference in the allergic symptom score for the PAR groups according to the detection of respiratory viruses ($P = .290$). The TNSS of patients with HRV-positive PAR was higher than that of patients with HRV-negative PAR, whereas the TNSS of patients with PIV-positive PAR was lower than that of patients with PIV-negative PAR. However, there were no statistical significances based on the detection of HRV and PIV ($P > .05$ for each, respectively; Table 3).

Discussion

In the present study, respiratory viruses were detected by multiplex PCR in 43.1% of patients with PAR, which was higher than in patients without allergy. In particular, detection rates of overall and multiple viruses and HRV were significantly higher in patients with PAR than in patients without allergy. However, there was no difference in the severity of AR symptoms based on the presence of a respiratory virus.

Table 3
TNSS according to detection of a respiratory virus in patients with perennial allergic rhinitis

Type of virus	Detection of virus	TNSS, mean ± SD	*P* value
Overall	positive (n = 25)	5.20 ± 3.65	.290
	negative (n = 33)	6.12 ± 2.92	
HRV	positive (n = 13)	6.31 ± 3.92	.469
	negative (n = 45)	5.56 ± 3.07	
PIV	positive (n = 9)	4.00 ± 3.46	.144
	negative (n = 49)	6.04 ± 3.16	

Abbreviations: HRV, human rhinovirus; PIV, parainfluenza virus; TNSS, total nasal symptom score.

The positive detection of a respiratory virus in nasal lavage fluid may imply subclinical persistence of a respiratory virus from previous infections. HRV infection can persist in the lower respiratory tract for longer than 1 year.[12] PIV also can be detected for up to 3 months in nasal epithelial cells of patients with postviral olfactory dysfunctions.[13] In the present study, the detection rates in patients with AR and control patients were high despite no evidence of recent URI. It has been reported that the Seeplex detection kit has high sensitivity and specificity comparable to the conventional viral culture. Coinfection was better detected by the multiplex PCR kit than by viral culture and immunofluorescence.[14] The overall detection rate of a respiratory virus was higher in patients with PAR than in controls. Specifically, a larger proportion of patients with respiratory virus–positive PAR had multiple viruses in their nasal lavage fluid compared with control patients without allergy. Although the clinical significance of multiple virus detection is debatable, the impaired innate immune response in patients with allergy could lead to multiple viral infections. Some researchers have reported that patients with allergic asthma and/or AR have impaired innate immune responses to respiratory viral infections.[15–18] Impaired interferon production associated with allergic sensitization may increase susceptibility to respiratory viral infection and compromise viral clearance, leading to long-term viral shedding.

Human rhinovirus triggers acute asthma exacerbations by increasing lower airway inflammation and bronchial hyperresponsiveness.[19,20] Moreover, persistent infection with HRV may be associated with the clinical features of more severe asthma.[21] In the present study, HRV was detected in 22.4% of patients with PAR, which is similar to the 26.1% detection rate in nasal lavage fluid from patients with chronic rhinosinusitis in the authors' previous multiplex PCR study.[22] Furthermore, the authors found that HRV, the most common respiratory virus in patients with PAR and control patients without allergy, was the only virus showing a significant difference between the PAR and control groups. These results suggest that HRV could play a major role in the pathogenesis of AR or exist for a long period in an inflammatory state. In addition to HRV, the detection rate of PIV was relatively high in nasal lavage samples from the PAR and control groups, showing no significant difference between the 2 groups. This finding may be due to tropism of PIV to epithelial cells.[23]

Viral respiratory infections induce inflammation of the nasal and sinus cavities.[24] Patients with seasonal AR inoculated with nasal HRV-16 outside the pollen season exhibited an increased responsiveness to the allergen, in part, through HRV-induced stimulation of eosinophil and neutrophil activities.[25,26] In addition, the combination of respiratory virus detection and allergen exposure increases the risk of acute asthma exacerbation.[5,6] Therefore, the authors sought to determine whether the persistence of a respiratory virus could aggravate mucosal inflammation in the patient with AR and subsequently cause more severe symptoms. However, in this study, there was no statistically significant association between AR symptom scores (TNSS) and the presence of respiratory viruses. This result suggests that patients with respiratory virus–positive PAR might not have more severe allergic symptoms compared with patients with respiratory virus–negative PAR. This result may be influenced by the use of oral antihistamine in the PAR group. Because discontinuation of oral antihistamine was not tolerable for the PAR group, this treatment could not be stopped in these study patients.

This study was conducted in patients with PAR and without evidence of a URI and patients without allergy and without evidence of a URI in the 4 weeks before the study to assess whether the presence of a respiratory virus could affect the clinical features

of PAR. The authors studied patients with PAR and not those with seasonal AR and enrolled roughly equal sample sizes over the months. Because the nasal allergy symptoms of patients with PAR can develop throughout the year, the authors tried to identify the association between the severity of allergic symptoms and respiratory viral infection.

A limitation of this study is that nasal lavage samples were used to detect respiratory viruses. Nasal lavage fluid might have higher false-positive rates owing to an increased risk of trapping viruses that are floating in the ambient air. However, it was a distinctive observation that overall and multiple virus detection rates in patients with PAR were higher than those in patients without allergy. Therefore, this study was still valuable in showing a relation between adult patients with PAR and the presence of respiratory viruses. To the authors' knowledge, there has been no previous study that has investigated the detection of respiratory viruses in patients with PAR who have no evidence of viral URI. The present results raise the possibility that patients with PAR are vulnerable to respiratory viral infection and have persistent respiratory viral infections, in particular HRV.

In conclusion, the present findings show a high prevalence of respiratory viruses in patients with PAR and suggest that respiratory viruses might play a role in the pathogenesis of AR. To confirm the clinical significance of these results, further research into the association between symptom aggravation and respiratory viral infection in a larger group of patients with AR and a parallel group of healthy control subjects is needed.

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