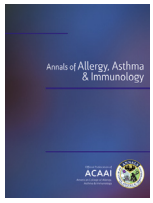
Ann Allergy Asthma Immunol 111 (2013) 508–511

Contents lists available at ScienceDirect



Detection of respiratory viruses in adult patients with perennial allergic rhinitis

Ji Heui Kim, MD, PhD; Byoung Jae Moon, MD; Chang-Hoon Gong, BSc; Nam Hee Kim, MSc; and Yong Ju Jang, MD, PhD

Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received for publication July 4, 2013.
Received in revised form August 16, 2013.
Accepted for publication August 25, 2013.

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.

Dr J.H. Kim and Dr Moon contributed equally to this work.
Reprints: Yong Ju Jang, MD, PhD, Professor, Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, Republic of Korea; E-mail: jangyj@amc.seoul.kr.
Disclosures: Authors have nothing to disclose.
Funding Sources: This study was supported by grant 2011-0506 from the Asan Institute for Life Sciences, Seoul, Republic of Korea.

1081-1206/13/$36.00 - see front matter
http://dx.doi.org/10.1016/j.anai.2013.08.024

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