Prevalence of Respiratory Viral Infections in Korean Adult Asthmatics With Acute Exacerbations: Comparison With Those With Stable State

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Introduction

Asthma is a highly prevalent, chronic respiratory condition characterized by reversible airflow obstruction, airway hyperresponsiveness and airway inflammation producing frequent exacerbations. There are 300 million people worldwide affected by asthma. The public health burden of asthma has increased over the past 2 decades, and acute exacerbation of asthma is a particularly important and costly problem, because morbidity and mortality due to asthma are closely related to the frequency and severity of the exacerbations. Identification of causal factors is vital for prevention and management of exacerbations. In Western countries, viral infections are responsible for up to 80%-85% of exacerbations in childhood asthma. In contrast, viral infections are involved in <50% of asthma exacerbations.

Key Words: Asthma; virus; exacerbation; season; sputum

INTRODUCTION

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tions among adult asthmatics.7-12
Among various respiratory tract viruses, including rhinovirus (RV), influenza virus (IFV), adenovirus (ADV), human metapneumovirus (hMPV), parainfluenza virus (PIV), coronavirus, and respiratory syncytial virus (RSV), RV, and IFV trigger exacerbations in children with asthma most frequently.14 In Korea, the prevalence of respiratory viruses is reported to be similar to that in Western countries. Of the respiratory viruses that cause asthma exacerbations, up to 60%-70% are RV, while IFV and RV are responsible for a substantial proportion of exacerbations in children with asthma.15,16 However, there have been few reports on the prevalence of viruses related to asthma exacerbations in Korean adult asthmatics.

This prompted us to evaluate the prevalence of respiratory viruses in the sputum of asthmatics with lower respiratory tract illnesses (LRTIs) and to compare the frequencies and types of viruses detected in patients with exacerbations (exacerbated LRTIs) with those in subjects without exacerbations (stable LRTIs) to evaluate the contribution of respiratory viruses to asthma exacerbation.

MATERIALS AND METHODS

Study subjects

The study was prospectively carried out in a tertiary hospital in Korea from June 2009 to June 2014. Asthma was previously diagnosed based on the Global Initiative for Asthma guidelines (GINA report: global strategy for asthma management and prevention 2011 May 4. Available at: http://www.ginasthma.org/uploads/users/files/GINA_Report2011_May4.pdf). All subjects had a clinical diagnosis of asthma supported by at least one of the following criteria: 1) an increase in the forced expiratory volume in 1 second (FEV1) of >12% or 200 mL after inhalation of 400 µg albuterol, 2) a reduction in the FEV1 of 20% in response to a provocative concentration of <10 mg/mL inhaled methacholine (PC20), and 3) an increase in the FEV1 >20% over 14 days after inhalated or systemic corticosteroid use. The subjects underwent a standardized assessment, which included analyses of induced sputum specimens, complete blood cell count with differential counts, total immunoglobulin E (IgE) measurement, chest radiography, body mass index (BMI) measurement, and allergy skin-prick tests at the initial visit. Twenty-four common inhalant allergens, including dust mites (Derma-topagoides farina and Dermatophagoides pteronyssinus), cat fur, dog fur, cockroaches, grass pollens, tree pollens, ragweed pollens, and Aspergillus species (Bencard Co., Brentford, UK) were used in the skin-prick tests. This study was prospectively performed by including the patients of the Soonchunhyang asthma cohort (n=1,843), and their characteristics were summarized in our previous publication.17 Among them, we recruited those who complained the aggravation of the lower respiratory symptoms and were able to expectorate good quality sputum samples. Exclusion criteria were the presence of parenchymal lung diseases, such as pulmonary tuberculosis, bronchiectasis, lung cancer, idiopathic interstitial lung diseases, and abnormal lung infiltrations on chest radiography.

Sputum was obtained within 2 weeks from the time when respiratory tract infections were suspected if the common symptoms of upper respiratory tract illnesses (URTIs), such as cough, sore throat, runny nose, post-nasal drip, nasal congestion, and low-grade fever, or those of LRTIs, such as shortness of breath, weakness, fever, coughing, sputum production, and wheezing were present. An exacerbation was diagnosed when pre-existing dyspnea and wheezing became aggravated within 14 days before the study, together with a post-bronchodilator FEV1 <80% of the personal best.18 We divided them into exacerbated LRTI and stable LRTI. Informed written consent was obtained from all subjects and all procedures were approved by the Ethics Committee of Soonchunhyang University Bucheon Hospital (SCH-2017-01-009).

Viral RNA extraction and multiplex reverse transcription-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from 300 µL of the total sputum samples diluted in 8× Dulbecco’s phosphate-buffered saline (DPBS) using the Viral Gene-spin™ Kit (iNtRON Biotechnology, Seoul, Korea) as recommended by the manufacturer. The isolated RNA was reverse-transcribed into cDNA following the protocol of the RevertAid First Strand cDNA Synthesis Kit (Thermo-Scientific, Waltham, MA, USA): 8 µL of purified RNA, 1 µL of 0.2 µg/µL random hexamer primer and 3 µL of diethylpyrocarbonate (DEPC)-treated water were mixed and heated at 80°C for 3 minutes. To this mixture, 4 µL of 5× reaction buffer, 1 µL Ribonuclease inhibitor (20 U/µL), 10 mM dNTP mix and 1 µL of RevertAid M-MulVRT (200 U/µL) were added, followed by incubation at 37°C for 90 minutes and 94°C for 2 minutes. Respiratory viruses were identified using the Seeplex® RV 7 Detection Kit (Seegene, Seoul, Korea) according to the manufacturer’s protocol, as follows: 3 µL of cDNA, 5× RV2 primer, 8-me-
thoxypsoralen solution and 2×multiplex Master Mix were mixed and heated at 94°C for 15 minutes. Forty amplification cycles were carried out in a thermal cycler (94°C for 0.5 minutes, 60°C for 1.5 minutes, and 72°C for 1.5 minutes). Amplification was completed by a final extension step at 72°C for 10 minutes. RT-PCR products were visualized by electrophoresis on an ethidium bromide-stained 2% agarose gel to identify ADV, hMPV, PIV 1/2/3, IFV A, IFV B, RSV A/B, and RV A.

**Statistical analysis**

The statistical analyses were performed using SPSS 13.0 (IBM, Armonk, NY, USA). Comparisons of clinical and physiological parameters between the exacerbation and stable groups were conducted using Pearson’s χ² test and an independent t test for discrete and continuous variables, respectively. Fisher’s exact test was applied for comparisons of the detection frequencies between the exacerbation and stable groups. Data are expressed as means ± standard error (SE) of the mean. Values of P < 0.05 were deemed to indicate statistical significance.

**RESULTS**

**Clinical characteristics of the subjects**

We obtained 353 sputum samples from 283 asthmatics with URTI or LRTI symptoms, including an increased amount of sputum without asthma exacerbation (stable state) or with asthma exacerbation (exacerbated state). Among them, 30 sputum samples were discarded because of inadequate quality. This, PCR detection of respiratory viruses was performed in 323 samples from 259 asthmatics (259 samples from subjects in an exacerbated state and 64 from those in a stable state). The clinical profiles of subjects in both states are presented in Table 1. The exacerbated cases had significantly lower FEV1, forced vital capacity (FVC), and FEV1/FVC values compared with those in a stable state (P < 0.001). The total IgE level and eosinophil percentage in the peripheral blood and sputum were significantly higher in the exacerbated cases than in the stable cases (P = 0.010-0.001).

**Respiratory viruses detected**

Respiratory viruses were detected in 68 (26.3%) of the 259 exacerbated-state sputum samples and in 12 of the 64 stable-state samples (17.0%; P = 0.213; Fig. 1A). RV was the most frequently detected virus in both the exacerbated and stable cases (32.4% vs 41.7%; P = 0.529; Table 2). In the exacerbated cases, IFV A/B was the second most frequently detected virus (20.6%), followed by PIV 1/2/3 (16.2%), RSV A/B (11.8%), ADV (11.8%), and hMPV (11.8%). In the stable cases, RSV A/B (25.0%) was detected. In the stable cases, RSV A/B (25.0%) was detected. In the stable cases, RSV A/B (25.0%) was detected. In the stable cases, RSV A/B (25.0%) was detected.
the second most frequently detected, followed by PIV 1/2/3 (16.7%) and IFV A/B (16.7%) (Table 2 and Fig. 1B). There was no difference in the virus distribution between the exacerbated and stable groups ($P=0.664$; Fig. 1B).

### Virus detection during follow-up

Of the subjects, 210 (44 stable and 166 exacerbated cases) underwent one sputum examination, and the remaining 49 underwent 2 to 4 sputum examinations. Seventy samples were obtained from 35 subjects during 2 exacerbations (exacerbated/exacerbated). A further 28 samples were obtained from 14 subjects with exacerbations and a stable LRTI state (exacerbated/stable) (Table 3). Virus was detected at both examinations in 3 of the exacerbated/exacerbated cases ($+/+$, 8.6%), at one examination in 6 cases ($+/-$, 17.1%) and 8 cases ($-/+$, 22.9%), and at neither examination in 18 cases ($-/-$, 51.4%). Among the exacerbated/stable cases, virus was detected at one examination in 11 cases (78.6%): 10 cases at the first examination ($+/-$, 71.4%) and 1 case at the second examination ($-/+$, 7.1%); it was not detected in 3 cases ($-/-$, 21.4%). There was a significant difference in the virus-positive and -negative rates between the 2 groups ($P=2.00 \times 10^{-4}$). Interestingly, among the 14 exacerbated/stable cases, 10 (71.4%) had virus-positive initial sputum samples (exacerbated state) but virus-negative second sputum samples (stable state). Accordingly, the exacerbated/stable group had a significantly higher frequency of an initial positive sample and a follow-up negative sample than did the exacerbated/exacerbated group (17.1%; $P=0.003$; Table 3).

![Fig. 1. Virus detection rates (A) and frequencies (B) in cases with lower respiratory infections with exacerbated (n=259) and those with stable state (n=69). $P$-values calculated by Pearson’s $\chi^2$ test (A) and Fisher’s exact test (B). $P$-values for the rate of virus detection in the exacerbated and stable states were 0.213 (A) and 0.664 (B), respectively.](image-url)
Seasonal variation in virus detection

A seasonal pattern was noted in the rates of detection of respiratory virus. RV was prevalent in September to December, IFV in January to April, PIV in May to September, and RSV A/B in September to April (Fig. 2).

DISCUSSION

In the present study, of the viruses evaluated, RV was most frequently related to exacerbations of asthma in Korean adult asthmatics. RV was responsible for one-third (32.4%) of the viral infections in exacerbated cases. This frequency is in good agreement with previous reports on Caucasians. RV URTI and LRTI were frequently associated with asthma exacerbations in child and adult Caucasian asthmatics. Indeed, viruses were detected in ~80% of child asthmatics with acute exacerbations; RV caused 70%-90% of these episodes. Interestingly, the frequency of RV detection was higher in non-exacerbated stable LRTI cases than in exacerbated LRTI cases in our study (45.5% vs 32.4%), although the difference was not significant. Thus, because RV was detected in almost half of the stable LRTI cases, the relationship between RV infection and asthma exacerbations seems to be inconsistent: detection of RV in sputum does not always induce an asthma exacerbation. Other studies of adult asthmatics have reported similar results. In an 11 month longitudinal study of 31 atopic asthmatics aged 15-56 years, 30 viruses were detected, 18 (60%) of which were associated with asthma exacerbations. In these populations, RV was detected in 14/30 (46.6%) asthmatics and 3/9 (33.3%) non-asthmatics. A longitudinal study of adult asthmatics reported that one-third of symptomatic respiratory tract viral infections were not associated with worsening of asthma exacerbations in both Korean and Caucasian adult asthmatics. The prevalence of respiratory viruses differs markedly according to age and geography. The frequency of RV detection is significantly lower in adult asthmatics than in child asthmatics. Indeed, viruses were detected in ~80% of child asthmatics with acute exacerbations; RV caused 70%-90% of these episodes.8

Fig. 2. Seasonal variation of respiratory virus in study cases. RV was prevalent in the fall, and IFV A was detected predominantly in January, February, and March. PIV was detected predominantly from May to September and RSV from September to April. RV, rhinovirus; IFV, influenza virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.
symptoms. In a recent longitudinal cohort study of couples (1 with asthma and 1 without) that evaluated the presence of RV in nasal secretions at 2-week intervals over a 3-month period, the incidence of RV infection was similar in those with asthma and those without (10.1% vs 8.5%). In a study of children, the rate of RV isolation was almost identical in those with wheezy bronchitis (28.6%) and those with upper respiratory illness (29.5%). These studies and our data suggest that RV is related to both acute exacerbations of asthma and stable LRTI without asthma exacerbations.

Although the prevalence of virus infections is similar in asthmatic children with vs without exacerbations, respiratory symptoms are more severe in the presence of viral infections, in that cold and asthma symptoms are more than 2-fold longer in duration and loss of control is more frequent in virus-positive compared with virus-negative respiratory tract illness in children (47% vs 22%). Adults with asthma are not at increased risk of RV infection, but those infected with RV have more severe and longer-lasting LRTI symptoms and greater decreases in peak expiratory flow rate than do healthy individuals.

In the present study, IFV A was the second most frequently detected virus. Thus, RV and IFV constituted 51.5% of the viral infections in subjects with exacerbated asthma. A similar frequency has been reported in adult asthmatics. However, RV and IFV infections are more prevalent among subjects with asthma exacerbations compared with those without exacerbations in Korean children.

In the present study, respiratory viruses were detected in the sputum of subjects with exacerbated (26.3%) and stable (17.2%) asthma. This detection rate is lower than that initially expected. In a 1979 study of children, the detection rate was 26.4%. In that study, virus isolation was performed by cell culture. In contrast, respiratory viruses can now be identified by virus culture, serology, immunofluorescence antigen detection, and PCR-based tests. Since the development of PCR assays in the 1990s, their sensitivity has improved markedly. Thus, our detection rate is relatively low, likely due to inadequate sputum samples. Virus detection is usually performed in the upper airway because collection of sputum from young children and some adult asthmatics is not feasible. In our study, viruses were detected in sputum samples, not in nasopharyngeal washings or swabs. Use of this method may have resulted in the low frequency of RV detection in our study, because RV usually infects the upper respiratory tract. However, in our comparison study of sputum and nasal swabs in asthmatics with LRTIs, the concordance rate of virus detection was 95.2%, and the detection rate was higher in sputum than in nasal swabs. Thus, use of sputum did not seem to be a cause of the low frequency of RV detection in our study. The second reason is the delayed sampling of sputum in our study. An exacerbation was defined as aggravation of pre-existing symptoms of dyspnea and wheezing within 14 days before the study and a post-bronchodilator FEV1 <80% of the predicted value or the personal best. RV has an incubation period of 2 days and is shed for 7 days after development of symptoms. FEV1 decreases significantly after infection, reaching a minimum at 2 days after experimental RV inoculation. Because our study was a cross-sectional design, we did not analyze the lag time between the appearance of LRTI symptoms and sputum virus analysis. Therefore, a lag time of >7 days in some patients may have resulted in the low detection rate.

A subset of asthmatics is particularly susceptible to recurrent exacerbations. In our study, the initial sputum samples (exacerbation) were positive for virus, while the second sputum samples (stable state) were negative in 10 of 14 cases (71.4%). This frequency was higher than that in the exacerbated/exacerbated group (17.1%; P = 2.50 × 10⁻⁴). These data indicate that a subset of patients is susceptible to asthma exacerbation in the presence of viral LRTI. In addition to viruses, asthma exacerbation can be caused by other agents, including allergens (dust mites, pollen, and animal dander), occupational exposure (grains, flours, cleaning agents, metals, irritants, and woods), hormones (menstrual asthma), drugs (acetylsalicylic acid [ASA], nonsteroidal anti-inflammatory drugs [NSAIDs], and beta-blockers), exercise, stress, smoking exposure, and air pollutants. The factors that trigger exacerbations differ among individuals. Thus, exacerbation of asthma may be a result of the complex interplay among respiratory viruses, host airway susceptibility factors, and environmental modifiers. A case-control study of 60 adult patients compared those hospitalized with acute asthma with 2 control groups: patients with stable asthma and patients hospitalized for non-asthma conditions. Compared with the controls, a significantly higher proportion of acute asthmatics were both sensitized and exposed to allergens, including dust mites, cat, and dog allergens. Intriguingly, the combination of high exposure to 1 or more allergens and virus detection significantly increased the risk of hospitalization for asthma compared with controls with stable asthma. These results indicate synergism among allergen sensitization, exposure to a high level of a sensitizing allergen, and viral infection in inducing asthma deterioration. In our study, the frequency of virus detection was not different between atopics and non-atopics (data not shown). Although all of these factors are expected to predispose asthmatics to viral infections, determining whether the exacerbation is due to viral infection or other causes is not feasible at present.

The predominant respiratory virus depends on the season. In our study, the number of cases was highest in late fall and early winter. However, the virus detection rate was ~45% in February and March and ~20% in May, July, and August. These data indicate that symptoms of LRTI with asthma exacerbation in early spring may be due mainly to viral infections, and that those in late spring to summer may be due mainly to other environmental factors. In addition, virus prevalence varied mark-
Viruses were detected in approximately one-fifth of the subjects. In summary, the presence of respiratory viruses was analyzed in 323 sputum samples from asthmatics with manifestations of LRTI to evaluate their contribution to asthma exacerbations. This work was supported by a Research Program funded by the Korea Centers for Disease Control and Prevention (fund code 2015-ER7402-00) and by Soonchunhyang University Research Fund grant to CS Park. The sputum samples for this study were provided by the Soonchunhyang University Hospital Biobank, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare, and Family Affairs, Korea. This work was supported by a Research Program funded by the Korea Centers for Disease Control and Prevention (fund code 2015-ER7402-00) and by Soonchunhyang University Research Fund grant to CS Park.

REFERENCES

1. Masoli M, Fabian D, Holt S, Beasley R; Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469-78.
2. Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. Clin Exp Allergy 2009;39:193-202.
3. Murray CS, Simpson A, Custovic A. Allergens, viruses, and asthma exacerbations. Proc Am Thorac Soc 2004;1:99-104.
4. Tan WC. Viruses in asthma exacerbations. Curr Opin Pulm Med 2005;11:21-6.
5. Oleneck JP, Kim WK, Lee WM, Yang F, Pappas TE, Salazar LE, et al. Weekly monitoring of children with asthma for infections and illness during common cold seasons. J Allergy Clin Immunol 2010;125:1001-1006.e1.
6. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. BMJ 1995;310:1225-9.
7. Beasley R, Coleman ED, Hermon Y, Holst PE, O’Donnell TV, Tobias M. Viral respiratory tract infection and exacerbations of asthma in adult patients. Thorax 1988;43:679-83.
8. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307:982-6.
9. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. Lancet 2002;359:831-4.
10. Atmar RL, Guy E, Guntupalli KK, Zimmerman JI, Bandi VD, Baxter BD, et al. Respiratory tract viral infections in inner-city asthmatic adults. Arch Intern Med 1998;158:2453-9.
11. Sokhandan M, McFadden ER Jr, Huang YT, Mazanec MB. The contribution of respiratory viruses to severe exacerbations of asthma in adults. Chest 1995;107:1570-4.
12. Teichtahl H, Buckmaster N, Pertnikovs E. The incidence of respiratory tract infection in adults requiring hospitalization for asthma. Chest 1997;112:591-6.
13. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunity. Clin Microbiol Rev 2010;23:74-98.
14. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. J Allergy Clin Immunol 2005;116:267-73.
15. Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clin Infect Dis 2006;43:585-92.
16. Kim WK, Gern JE. Updates in the relationship between human rhinovirus and asthma. Allergy Asthma Immunol Res 2012;4:116-21.
17. Kim TB, Jang AS, Kwon HS, Park JS, Chang YS, Cho SH, et al. Identification of asthmatic clusters in two independent Korean adult asthma cohorts. Eur Respir J 2013;41:1308-14.
18. Park SW, Kim DJ, Chang HS, Park SJ, Lee YM, Park JS, et al. Association of interleukin-5 and eotaxin with acute exacerbation of asthma. Int Arch Allergy Immunol 2003;131:283-90.
19. Park SW, Lee YM, Jang AS, Lee JH, Hwanqbo Y, Kim DJ, et al. Development of chronic airway obstruction in patients with eosinophilic bronchitis: a prospective follow-up study. Chest 2004;125:1998-2004.
20. Horn ME, Brain EA, Gregg I, Inglis JM, Yealland SJ, Taylor P. Respiratory viral infection and wheezy bronchitis in childhood. Thorax 1979;34:23-8.
21. Minor TE, Dick EC, Baker JW, Ouellette JJ, Cohen M, Reed CE. Rhinovirus and influenza type A infections as precipitants of asthma. Am Rev Respir Dis 1976;113:149-53.
22. Kwon JM, Shim JW, Kim DS, Jung HL, Park MS, Shim JY. Prevalence of respiratory viral infection and wheezing in adult asthmatics. Korean J Pediatr 2014;57:29-34.
23. Park JS, Kim JN, Kim MS, Seo KH, Uh ST, Kim YH, et al. Comparison of virus detected in sputum and nasal washing fluids from exacerbated asthmatics. Proceedings of the 2016 KAAACI Annual Spring Congress; 2016 May 6–7; Seoul. Seoul: Korean Academy of Asthma, Allergy and Clinical Immunology; 2016. 185 p.
24. Douglas RG Jr, Cate TR, Gerone PJ, Couch RB. Quantitative rhinovirus shedding patterns in volunteers. Am Rev Respir Dis 1966;94:159-67.
25. Harris JM 2nd, Gwaltney JM Jr. Incubation periods of experimental rhinovirus infection and illness. Clin Infect Dis 1996;23:1287-90.
26. Grünberg K, Timmers MC, de Klerk EP, Dick EC, Sterk PJ. Experimental rhinovirus 16 infection causes variable airway obstruction in subjects with atopic asthma. Am J Respir Crit Care Med 1999;160:1375-80.
27. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. BMJ 2002;324:763.
28. Weiss ST, Utell MJ, Samet JM. Environmental tobacco smoke exposure and asthma in adults. Environ Health Perspect 1999;107 Suppl 6:891-5.
29. Sunyer J, Spix C, Quénel P, Ponce-de-León A, Pönka A, Baruman-dzadeh T, et al. Urban air pollution and emergency admissions for asthma in four European cities: the APHEA Project. Thorax 1997;52:760-5.
30. Goldsmith CA, Kobzik L. Particulate air pollution and asthma: a review of epidemiological and biological studies. Rev Environ Health 1999;14:121-34.
31. Lim JS, Woo SI, Kwon HI, Baek YH, Choi YK, Hahn YS. Clinical characteristics of acute lower respiratory tract infections due to 13 respiratory viruses detected by multiplex PCR in children. Korean J Pediatr 2010;53:373-9.
32. Griswold SK, Nordstrom CR, Clark S, Gaeta TJ, Price ML, Camargo CA Jr. Asthma exacerbations in North American adults: who are the “frequent fliers” in the emergency department? Chest 2005;127:1579-86.
33. Rodrigo GJ, Rodrigo C, Hall JB. Acute asthma in adults: a review. Chest 2004;125:1081-102.
34. Seo YB, Song JY, Choi MJ, Kim IS, Yang TU, Hong KW, et al. Epidemiology and clinical outcomes of acute respiratory virus infection in hospitalized adults. Infect Chemother 2014;46:67-76.
35. Seo YB, Cheong HJ, Song JY, Noh JY, Kim IS, Song DJ, et al. Epidemiologic differences of four major respiratory viruses between children, adolescents, and adults in Korea. J Infect Chemother 2014;20:672-7.