C-reactive protein (CRP) levels are not generally associated with viral infections. This study investigated the changes in the CRP level caused by an infection from respiratory virus (RV). Nasopharyngeal samples from hospitalized patients with suspected RV infection were used to measure the CRP levels, virus load, virus–virus co-infection, age, sex, and length of hospital stay (LOS). Abnormal CRP levels were detected in 62.3% (3,608 out of 5,788) of all RV-positive samples. The percentage of patients with abnormal CRP levels tended to increase with age. Furthermore, LOS in patients with abnormal CRP levels was significantly longer than that in patients with normal CRP levels. The frequency of elevated CRP levels differed according to the causative virus and the frequency of abnormal levels increased with age. Moreover, LOS was longer in those with abnormal CRP levels. These data provide important insights into the role of CRP levels in RV infection.

Key words: Coinfection, C-Reactive Protein, Length of stay, Respiratory virus, Virus diseases

INTRODUCTION

Respiratory virus (RV) infection is the most common disease in humans, accounting for approximately 50% of all diseases [1]. Additionally, RV is one of the most contagious communicable pathogens and can rapidly infect a large number of individuals [2]. Because different RVs cause similar symptoms and signs, it is difficult to differentiate the causative virus solely based on clinical features [2–5].

However, recent developments in molecular biology-based diagnostic techniques for identification of viruses, such as sequencing or multiplex reverse transcription polymerase chain reaction, have facilitated simultaneous examination of a number of respiratory viruses, allowing simple, accurate extraction of the causative virus.

As such, much effort has been focused on effective treatment and prevention of RV infection by improving our understanding of the symptoms and clinical features of infected individuals based on the causative RV; this has been done to prevent antibiotic abuse and the spread of the disease [6]. Various studies have reported haematological characteristics, including abnormal elevation or reduction in the number of haemoglobin molecules or platelets,
based on the causative virus [7,8]. For example, it was observed that patients with RSV pneumonia were more co-infected by bacteria than those with RSV bronchiolitis and bronchopneumonia, so that patients with RSV pneumonia had elevated levels of WBC count, CRP and erythrocyte sedimentation rate (ESR) than those with bronchiolitis or bronchopneumonia [9]. Among these proteins, CRP is the most representative of acute phase reactants [10,11]. Although elevated CRP levels indicate bacterial infection [12], it was reported to be still insufficient to conclude as direct evidence that virus causes elevation of CRP levels [13]. However, infection of RV such as RSV is prone to co-infection with other bacteria or viruses, and CRP level increases together [9], so that identification of levels of biomarker proteins such as CRP can be an important indirect indication for viral infection. However, similar levels may be observed in patients with inflammatory diseases or in those infected with certain viral strains (e.g., adenovirus and influenza) [14,15].

In comparison to adults, children are more susceptible to viruses or bacteria, and show more cold-related symptoms accompanied by fever or coughing that are caused by these sources of infection. In case of these symptoms, various tests and studies have been performed to identify the cause. Although biomarkers are not recommended in primary examination to identify presence of pathogens [16,17], CRP can be used as a biomarker for inflammation, which enables to trace progress of infection or monitor treatment effect, so that studies on various sources of infection and CRP have been actively conducted to date. Nevertheless, RV and CRP have been relatively unexplored. Infection by RV can cause various symptoms accompanied by fever, and secondary infection to subjects with declined immunity causes many complications during viral prevailing season, even resulting in death sometimes. As such, it is highly critical to find biomarkers to diagnose specific viruses.

Although a recent study showed that CRP may be used to detect various viruses in the clinical setting [18], studies applying CRP levels for diagnosis of RV infections are lacking. Therefore, in this retrospective study, we compared and analysed the correlations between RV infection and CRP levels. We examined 12 types of RVs (Table 1) and the associations of these RVs with CRP levels, while considering patient age, sex, and length of hospital stay (LOS), to investigate the characteristics of each virus. In addition, while considering various factors such as gender and age, changes in CRP level are compared when infected by RV, by which it is expected that this study can investigate close relationships of CRP level with specific RVs.

### MATERIALS AND METHODS

The present study was approved by the institutional review board (IRB) of Dankook University (Date of IRB approval: 2015.10.13; IRB approval No.: 2015-09-009).

#### 1. Subject and sample collection

A total of 9,204 patients admitted at Dankook University Hospital for respiratory symptoms between December 2006 and February 2014 were included in this study. Patients were admitted via the emergency room with symptoms of acute respiratory illness or via outpatient services. General blood tests and tests examining for the

| Table 1. Material and diagnostic method |
|------------------------------------------|
| **Details**                             |
| **Virus type**                          |
| human adenovirus [hADV]; human coronavirus [hCoV] 229E/ NL63, and OC43; human metapneumovirus [hMPV]; human rhinovirus [hRV]; influenza virus [INF] A and B; parainfluenza virus [PIV] types 1, 2 and 3; and respiratory syncytial virus [RSV] A and B |
| **Diagnostic method (RT-PCR)**          |
| PTC 200 PCR system (MJ Research, Watertown, MA, USA) with a program of 40 cycles of: 30 s at 94°C, 90 s at 60°C, and 90 s at 72°C, followed by 1 cycle of 10 min at 72°C |
| **Diagnostic method (CRP)**             |
| MODULAR P auto-analyser with specialized reagents (Roche Diagnostics, Mannheim, Germany) |
| **Type of diagnostic kit**              |
| Seeplex RV detection kit-1 (Seegene, Seoul, Korea) |
| **Material**                            |
| A total of 9,204 patients blood sample treated at Dankook University Hospital for respiratory symptoms |
presence of RV were performed on all patients. Within 24 hours of admission, nasopharyngeal fluids were collected by inserting a mucus extractor connected to a sterile 8-French catheter 5~7 cm into the nostril and suctioning with a pressure of 60~80 mmHg. Samples were stored at 4°C until nucleic acid extraction was performed. The extracted nucleic acids were stored at −70°C until the tests were performed.

2. Analysis of CRP levels

Blood samples were drawn for CRP analysis. Sera were stored at −20°C until use. CRP levels were measured using particle-enhanced immunoturbidimetric assays using a MODULAR P auto-analyser with specialized reagents (Roche Diagnostics, Mannheim, Germany). CRP samples were also analysed by means of an immunoturbidimetric method using an analyser and reagents from the same manufacturer. A CRP level of 0.5 mg/dL or higher was considered abnormal.

3. Extraction of RV nucleic acids and RV detection

Nucleic acid extraction was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol with a QIAcube instrument (Qiagen). RNA was isolated from nasopharyngeal fluid and reconstructed as complementary deoxyribonucleic acid (cDNA). The cDNA was then amplified using a Seeplex RV detection kit-1 (Seegene, Seoul, Korea) to test for one DNA virus (human adenovirus [hADV]) and 11 RNA viruses that cause respiratory infection (human coronavirus [hCoV] 229E/NL63, and OC43; human metapneumovirus [hMPV]; human rhinovirus [hRV]; influenza virus [INF] A and B; parainfluenza virus [PIV] types 1, 2 and 3; and respiratory syncytial virus [RSV] A and B) PCR was performed using the PTC 200 PCR system (MJ Research, Watertown, MA, USA), and the following program was implemented: 40 cycles of 30 s at 94°C, 90 s at 60°C, and 90 s at 72°C, followed by one cycle of 10 min at 72°C. The amplified PCR products were analysed following 30 min of

Table 2. Analysis of cases, positive rates and infection types

| No. of samples | Age (years) | Ratio | Average CRP (mg/dL) |
|----------------|-------------|-------|---------------------|
|                |             | Average | Median |                 |
| Submitted      | 9,205       | 14.1   | 2.0    | 100.0%            |
| Positive       | 5,788       | 7.6    | 1.4    | 62.9% 2.80        |
| Male           | 3,394       | 7.9    | 1.4    | 36.9% 2.83        |
| Female         | 2,394       | 7.3    | 1.6    | 26.0% 2.76        |
| Sex ratio (M/F)| 1.41:1      |        |        |                   |
| Negative       | 3,417       | 25.1   | 5.6    | 37.1% 5.25        |
| Single         | 4,610       | 8.5    | 1.4    | 50.1% 2.82        |
| Multiple       | 1,178       | 4.2    | 1.5    | 12.8% 2.76        |
| Double         | 1,053       | 4.1    | 1.5    | 11.4% 2.71        |
| Triple or more | 125         | 4.4    | 1.6    | 1.4% 3.17         |
| Virus          | 7,096       | 7.6    | 1.4    | 100.0% 2.80       |
| hADV           | 1,085       | 5.0    | 2.2    | 15.3% 3.66        |
| hCoV 229E/NL63 | 205         | 12.2   | 1.8    | 2.9% 3.80         |
| hCoV OC 43     | 226         | 10.4   | 1.6    | 3.2% 4.19         |
| hMPV           | 497         | 8.7    | 1.8    | 7.0% 3.48         |
| hRV            | 1,529       | 5.0    | 1.5    | 21.6% 2.61        |
| INF A          | 602         | 19.2   | 5.0    | 8.5% 4.08         |
| INF B          | 170         | 17.7   | 5.3    | 2.4% 3.51         |
| PIV 1          | 266         | 5.0    | 1.5    | 3.7% 2.12         |
| PIV 2          | 85          | 5.0    | 2.1    | 1.2% 2.44         |
| PIV 3          | 478         | 6.0    | 1.2    | 6.7% 2.36         |
| RSV A          | 1,106       | 3.3    | 0.5    | 15.6% 1.67        |
| RSV B          | 847         | 5.3    | 0.8    | 11.9% 1.95        |

Abbreviation: hADV, human adenovirus; hCoV, human coronavirus; hMPV, human metapneumovirus; hRV, human rhinovirus; INF, influenza; PIV, parainfluenza virus; RSV, respiratory syncytial virus.
electrophoresis at 100∼150 V in 2% agarose gels stained with ethidium bromide.

4. Statistical analysis

RV PCR and CRP levels were evaluated to analyse patterns and differences in CRP levels in relation to multiple infections, causative viruses, sex, and age. Regression analysis and Welch’s t-tests were performed. A p value of less than 0.05 was considered statistically significant.

RESULTS

1. Patient characteristics

Of the 9,205 cases included in this study, 5,788 (62.9%; 3,394 men and 2,394 women) patients tested positive for RV. The average age of RV-positive patients was 7.6 years, and the median age was 1.4 years. A total of 7,096 strains were detected in all samples and rhinovirus was the most frequently detected virus (1,529 cases; 21.5%), followed by RSV A (1,106 cases; 15.6%) and hADV (1,085 cases; 15.3%; Table 2).

2. Analysis of viral infection and CRP levels

In RV-positive cases, the rate of abnormal CRP levels was 62.8% (3,633 of 5,788 cases). In cases of single RV infection, the rate of abnormal CRP levels was 61.9% (2,855 of 4,610 cases), whereas in cases of viral co-infection (two or more infections), the rate of abnormal CRP levels was 66.0% (778 of 1,178 cases). Abnormal CRP levels were observed in 66.0% (695 of 1,053 cases) of double-infection cases and in 66.4% (83 of 125 cases) of ≥ triple-infection cases (Table 2).

The differences in abnormal CRP rates between overall positive cases and cases of co-infection were not statistically significant (p=0.28), and no significant differences were observed between single infection and ≥double infections (p=0.48 and p=0.37, respectively). The average CRP levels were 2.80±4.87 mg/dL in all RV-positive patients, 2.82±4.94 mg/dL in patients with single infections, and 2.71±4.56 mg/dL in patients with double infections.

Of the RV-positive patients, those in the group aged 70~79 years had the highest rate of abnormal CRP levels and the highest levels of CRP (10.67 mg/dL; Figure 1). Regression analysis showed that CRP levels tended to increase with age (p<0.001; Figure 2). Moreover, 67.6% of teenagers and 94.2% of individual aged 70~79 years exhibited abnormal CRP levels (Table 3).

The rate of abnormal CRP levels was highest in patients infected with hADV (79.2%) and lowest in patients infected with RSV A (47.2%). Based on virus type, the average CRP...
Table 3. Distribution of CRP levels by age in respiratory virus infection patients

| Age group (years) | Positivespecimens | Average CRP level (mg/dL) | CRP Abnormal count | CRP Abnormal rate |
|-------------------|-------------------|---------------------------|-------------------|-------------------|
| 0~9               | 5,136             | 2.10                      | 3,069             | 59.8%             |
| 10~19             | 143               | 3.53                      | 96                | 67.1%             |
| 20~29             | 25                | 6.20                      | 23                | 92.0%             |
| 30~39             | 29                | 7.46                      | 25                | 86.2%             |
| 40~49             | 36                | 8.02                      | 28                | 77.8%             |
| 50~59             | 72                | 9.81                      | 67                | 93.1%             |
| 60~69             | 110               | 10.14                     | 102               | 92.7%             |
| 70~79             | 171               | 10.67                     | 161               | 94.2%             |
| 80~89             | 57                | 9.76                      | 53                | 93.0%             |
| 90~99             | 9                 | 10.52                     | 9                 | 100.0%            |
| Total             | 5,788             |                           | 3,633             |                   |

Figure 3. Rate of abnormal CRP levels according to causative virus.

level was the highest in those infected with hCoV OC43 (4.19±7.22 mg/dL; \( p=0.004 \)) and the lowest in those infected with RSV A (1.67±3.75 mg/dL; Figure 3).

DISCUSSION

Viral infections can cause various abnormalities, including variations in CRP levels and platelet counts, which are the result of autoimmune responses and suppression of bone marrow progenitors [19]. In particular, CRP levels reflect the severity of the acute phase reaction [20]. Substantially increased CRP values are usually found in patients with pneumonia, and high CRP levels have been shown to be a strong predictor for disease in general practice [21].

The RV positivity rate was approximately 62.9%, which is similar to the results of Kim et al [1]. Moreover, the highest positive rate was in patients younger than 10 years of age, consistent with previous studies [1,3,22]. Our findings were also consistent with other reports in terms of the most commonly identified viruses (i.e., HRV and RSV A) [1,22]. We found that 62.8% of RV-positive patients had abnormal CRP levels, similar to the range (68~85%) reported in RV-infected patients in previous studies [23,24]. In contrast with the findings of some previous studies [25-27], co-infections in the current study were not more severe than single infections [28-30]. In addition, consistent with our study, a study by Guo et al [31] showed a gradual increase in CRP levels with age.

The increased severity of viral infections has recently been reported to prolong LOS [32]. Abnormal CRP levels in the present study were associated with longer LOS. As such, increased CRP levels may be used as a predictive factor for hospitalization in the intensive care unit or the requirement for mechanical ventilation [33]. Furthermore, our data provide evidence for differentiating between high- and low-risk patients, which has clear-cut clinical and therapeutic implications for decision-making on issues of patient management [34].

There were not significant increases in CRP levels during RV infection including co-infection by multiple viruses. However, when RVs were analysed individually, a significant increase in CRP level was observed during specific RV infections, with more prominent increases observed in elderly patients. Additionally, based on the higher proportion of elderly individuals with high CRP levels, in-depth studies on the biochemical and immunological relationships of different types of RVs in this
particular patient subset will be needed. Furthermore, to promote the timely diagnosis and treatment of patients, CRP tests should be carried out concurrently with virus identification tests.

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