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Research Article

Investigation of Respiratory Viruses by the Multiplex PCR Method in the Community Suggested Reciprocal Virus Transmission between Children and Adults

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ABSTRACT

The observational study of respiratory virus infection for children has already been reported, but there are few reports that examined both children and adults simultaneously in the community.

We performed a multiplex PCR (Polymerase Chain Reaction) assay of respiratory viruses by using Film Array Respiratory Panel version 2.1 for 248 children (under 15 years old) and 5,354 adults (15 years old or older) who were admitted or visited our hospital with respiratory symptoms and/or fever from December 2020 to November 2021. During this period, a seasonal outbreak of Respiratory Syncytial Virus (RSV) and Para Influenza Virus 3 (PI3) was found as well as a pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In adults, viruses were detected in 6.8% (364/5,354), and SARS-CoV-2 accounted for 228 cases. Only two cases presented multiple viruses. In children, viruses were detected in 64.9% (161/248), while 75.4% (135/179) were under 5 years old. Multiple viruses were detected in 26 cases, all of which were under 5 years old. SARS-CoV-2 was positive in six children. RSV prevailed both in children and adults simultaneously, while the PI3 outbreak was found first in children, followed by adults three weeks later. Rhinovirus/Enterovirus (RV/EV) was detected throughout the year with some fluctuation and was mostly found in children under 5 years old and in adults aged between 20s and 30s, which suggested reciprocal virus transmission between the children and the parents. This study indicates the importance of vaccines in the community as a whole to prevent the transmission of the respiratory pathogens.

Keywords: FARP; Multiplex PCR; Respiratory virus infection; SARS-CoV-2

Abbreviations: FARP: Film Array Respiratory Panel; RSV: Respiratory Syncytial Virus; PI: Para Influenza; Flu: Influenza; RV/EV: Rhinovirus/Enterovirus; hMPV: Human Metapneumovirus; AdV: Adenovirus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

INTRODUCTION

Respiratory virus infection is a major cause of morbidity in children and accounts for most hospitalized patients, especially those younger than 5 years old [1-3]. A rapid and easy diagnostic method is mandatory for proper management and improves outcomes. The Point-Of-Care (POC) antigen test for respiratory syncytial virus, influenza A, B, and human metapneumovirus is widely available and has been used for primary clinical care [4-6]. However, there are many other important respiratory viruses to care for clinically. Recently, multiplex PCR (Polymerase-Chain Reaction) testing for respiratory viruses has been applied for clinical use, and an observational study has been reported [7-9]. Furthermore, multiplex PCR testing revealed that respiratory viruses common in infants

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were also found in adults with severe respiratory symptoms [10]. Infectious respiratory diseases such as influenza, COVID-19, and pertussis spread from person to person. Now days the vaccination is the most effective way to help prevent the spread of certain respiratory virus diseases. However, few studies have investigated respiratory viruses by multiplex PCR in both children and adults simultaneously and have discussed the interrelation of viral infection and transmission between both generations. Here there are fewer chances for a vaccine to prevent the spread of diseases, if enough people are vaccinated, keeping everyone healthier.

MATERIALS AND METHODS

Multiplex PCR testing for respiratory virus was performed by using Film Array Respiratory Panel (FARP) version 2.1 (BioMerieux Japan, Tokyo). This panel covers 18 viruses, Adenovirus (AdV), coronavirus HKU1, 229E, OC43, NL63, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), Influenza (Flu) A, A/H1, A/H1 2009, A/H3, B, Para Influenza Virus (PI) 1,2,3,4, Respiratory Syncytial Virus (RSV), Rhinovirus/Enterovirus (RV/EV), Human Metapneumovirus (hMPV), and three bacteria; *Bordetella pertussis*, *Chlamydia pneumonia* and *Mycoplasma pneumonia*.

From December 2020 to November 2021, 248 children (under 15 years old) and 5,354 adults (15 years old and older) were included.

Principally, FARP testing was performed for the patients who presented fever and respiratory symptoms at admission. For adults, it was also performed for outpatients who visited the emergency unit presenting fever and/or respiratory symptoms. Nasopharyngeal swab samples were obtained and assayed immediately according to the manufacturer's protocol.

RESULTS

In 248 children (under 15 years old), viruses were detected in 64.9% (161/248) and 75.4% (135/179) of children under 5 years old (Table 1). RV/EV was the most common, followed by RSV and PI3. Furthermore, among virus-positive children, multiple viruses were detected in 15.8% (26/161), and all were under 5 years old.

In adults, viruses were detected in 6.8% (364/5354), and SARS-CoV-2 accounted for more than 60% of those (228/364), followed by RV/EV, RSV, PI3 and NL63. Multiple viruses were detected in only two cases in adults. The old coronavirus OC43 was detected mainly in children, and NL63 was detected both in children and adults. During the observational period, seasonal outbreaks of RSV and PI3 were found in both children and adults (Figure 1). While an outbreak of RSV occurred simultaneously in both groups, the PI3 outbreak occurred first in children and three weeks later in adults (Figure 2a).

RV/EV was detected through the observational period with fluctuations both in children and adults. In regard to the infected age group, RV/EV was mainly found in children under 5 years old and in adults aged between 20s and 30s (Table 1), which suggested virus transmission between the children and the parents.

No patients were positive for *Bordetella pertussis*, *Chlamydia pneumonia* or *Mycoplasma pneumonia*.

Table 1: A summary of the FARP assay.

Age	n	RV/EV	CoV-2	PI3	RSV	AdV	NL63	OC43	PI4	total	Positive (n)	Positive (%)
0-4	179	62	2	26	59	7	4	5	4	169	135	75.42
05-09	36	9	2	0	2	2	2	0	0	17	17	47.22
10-14	33	6	2	0	1	0	0	0	0	9	9	27.27
15-19	74	2	7	0	0	0	0	0	0	9	9	12.16
20-29	403	10	41	1	3	1	4	1	2	63	63	15.63
30-39	463	24	22	3	5	1	3	0	2	60	60	12.96
40-49	413	6	28	3	2	2	0	0	0	41	41	9.93
50-59	551	6	43	2	3	2	0	0	0	56	54	9.8
60-69	648	5	24	3	3	0	0	0	0	35	35	5.4
70-79	1060	9	35	3	5	0	2	0	1	55	55	5.19
80-89	1273	6	22	2	2	1	3	0	1	37	37	2.91
90-99	455	3	6	1	0	0	0	0	0	10	10	2.2
100-	14	0	0	0	0	0	0	0	0	0	0	0

Positive: Patient with one or more than one virus detected.

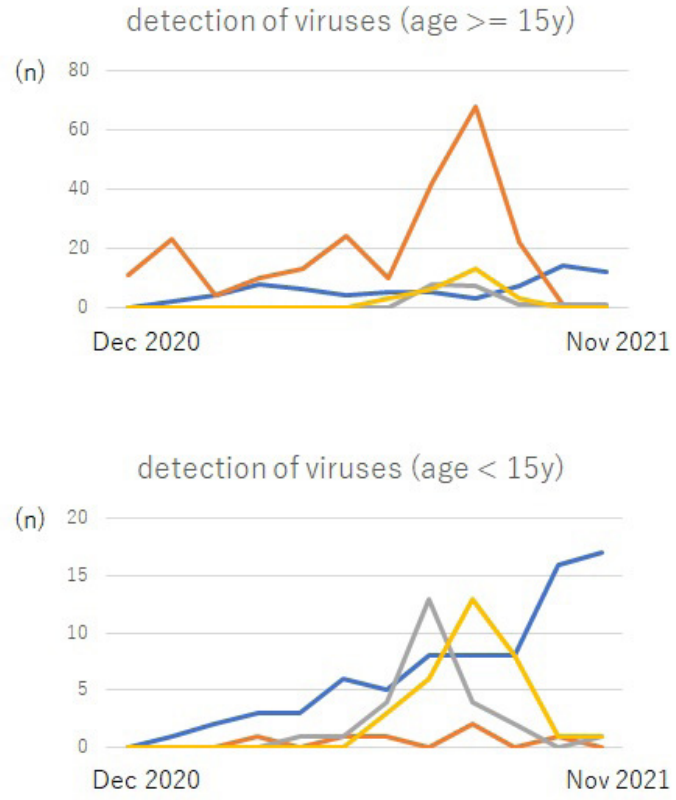

Figure 1: DA seasonal trend of RV/EV-, SARS-CoV-2-, PI3-, and RSV-infected patients in adults (above) and children (below) is presented. During this period, a seasonal outbreak of RSV and PI3 infection was found in addition to the pandemic of SARS-CoV-2. **Note:** (—) RV/EV; (—) SARS-Cov-2; (—) P13; (—) RSV

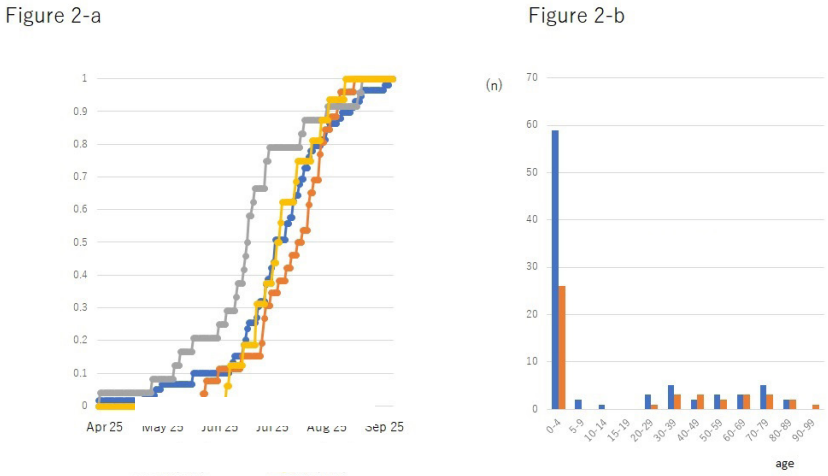

Figure 2: A cumulative ratio of RSV and PI3-infected patients in adults and children is plotted (Figure 2-a). RSV infection occurred simultaneously both in children and adults. In contrast, PI3 infection in children proceeded three weeks earlier than that in adults. The number of RSV- and PI3-infected patients among the designated age groups is presented (Figure 2-b). There was no difference in the infected age group between RSV and PI3. **Note:** Figure 2-a (—) RSV (child); (—) RSV (adult); (—) P13 (child); (—) P13 (adult), Figure 2-b (■) RSV; (■) P13.

DISCUSSION

Substantial pathogen epidemiological studies of respiratory virus infection in children have reported that respiratory viruses were detected in more than 50%-70% [11-15]. Children easily have viral respiratory infections because of the prematurity of immune function and respiratory function physiologically and anatomically. However, a report that viruses were detected in 20% of severely affected adult patients (ICU inpatients) has been reported so far about the examination of the pathogen epidemiology of respiratory virus infection in adults [10].

When considering respiratory virus epidemiology from social and regional points of view, it is important to observe both children

and adults in a certain restricted community and during the same period.

Our hospital is located in West Tokyo, Japan, designated as an infection and emergency medical center institute in the south of north-Tama area, which covers approximately 1 million residents.

During the designated period from December 2020 to November 2021, a seasonal outbreak of RSV and PI3 infection was found, as well as a pandemic of SARS-CoV-2 infection (Figure 1).

It is well known that a seasonal outbreak of flu was not found internationally in the 2020-2021 season, probably because of the changes in public life and behavior that promote mask wearing and hand washing in addition to the restriction of people flow against the global pandemic of SARS-CoV-2 [16-19]. To date, no seasonal outbreak of influenza has been found in the 2020-2021 and early 2021-2022 seasons in Japan [19]. In our cohort, no influenza virus was detected.

In Japan, a substantial lockdown was performed from April to August 2020, just before the regular outbreak season of RSV, and the seasonal outbreak of RSV was not found in the 2020 season; however, it came back in 2021 as a larger wave than the standard regular wave [20]. However, the closely related virus hMPV did not prevail for two consecutive years. In our study, hMPV was also not detected at all.

The differences in the impact on a seasonal outbreak between flu and RSV may be explained by the fact that the main target of RSV infection is a child under 2 years old, and it is quite difficult to prevent contact and droplet transmission of the virus in the nursery without social lockdown. In contrast, school children, who are the main target of Flu infection, can obey and maintain hygiene management and procedures individually.

Most of the RSV and PI3 were detected in the children under 5 years old; however, a small number of adult patients infected with RSV or PI3 were found throughout all ages. Of note, there is an interesting difference between the outbreak of RSV and PI3. In the former, it occurred simultaneously both in children and adults. In contrast, PI3 infection in children proceeded three weeks earlier than the outbreak in adults. There was no difference in the infected age group between RSV and PI3 (Figure 2b). Considering that the incubation periods of both viruses are the same of 4 to 6 days and that they both have an envelope and are similarly sensitive to alcohol, it is difficult to explain this difference from the virological point of view. Complicated social factors might be involved in this discrepancy.

For reference, we retrospectively analyzed the previous seasonal outbreak of Flu (2014-2015, 2015-2016, 2016-2017, 2017-2018) by using the laboratory database in our hospital, in which patients with rapid antigen positivity for influenza A were investigated. There was no difference in the cumulative ratio of Flu A infected patients between children and adults as of RSV in this study (Supplementary figure 1).

In this sense, it is also interesting to see the cumulative ratio of SARS-CoV-2-infected patients in the 5th wave of the pandemic in Tokyo from the public database (Supplementary figure 2) [21]. More than 200,000 were infected during the 5th wave in Tokyo, and infected patients aged between 20 s and 50 s preceded the infected children under 10 years old, which accounted for 5.8% of the total infected patients by 10 to 14 days. This indicates that virus transmission from adults to children in the community occurred in the 5th wave of SARS-CoV-2 infection in Tokyo as a whole.

RV/EV infection is observed during the year, and it was not affected during the pandemic era of SARS-CoV-2 [22,23], which was also true in our observation. Considering that a peak of RV/EV infection was found in children under 5 years old and adults of 20s to 30s years old (Table 1), virus transmission may occur in the family between the parents and the children. EV/EV has no envelope and is not sensitive to alcohol, which indicates resistance to the prevention of viral transmission by standard precautions. These findings may explain some of the reasons why RV/EV infection persists despite intensified social standard precautions for viral infection during the SARS-CoV-2 pandemic.

The limitation of this study is that we could not validate the clinical evidence of viral transmission between children and adults in detail. Additionally, the criteria for FARP testing were not tightened. Principally, FARP testing was performed at admission for the patients who presented fever and respiratory symptoms. However, it was also performed for adult outpatients who visited the emergency unit and presented with fever and/or respiratory symptoms. Furthermore, FARP testing was applied even for adults with mild cold symptoms to exclude SARS-CoV-2 when SARS-CoV-2 prevailed explosively as a social infection control strategy. In this sense, positivity of virus detection in adults in our study may be lower than that of previous reports. From another perspective, our study may cover community-acquired respiratory virus infections in adults more widely than previous reports. However, the viral detection rate in children was approximately equivalent to that in previous reports [11-15]. Additionally, the multiple viral detection rate was similar to that of previous reports [11,12,14,15]. This may be explained by the fact that FARP testing was principally applied to children who required admission, as in previous reports.

CONCLUSION

The multiplex PCR method is a powerful tool to detect respiratory viruses in children and adults and is also useful to monitor the trend of viral infection and transmission in the community. A COVID-19 vaccine, Influenza vaccine, Pneumonia vaccine helps in treating against these respiratory viruses. There are still many unsolved questions how the respiratory virus spreads, lies in latent, flares up, and changes in the community. Our study will also provide useful and significant epidemiological information for the strategy of infection control against the new pandemic infectious disease such as SARS-CoV-2.

DECLARATION

Data availability statement

Data available on request from the authors.

Ethics approval statement

This study was approved by the institutional review board of Musashino Red Cross Hospital as research No 3041.

Patient consent statement

Informed consent was secured by the opt-out method.

AUTHOR CONTRIBUTIONS

Masayuki Nagasawa: Conceptualization, writing-original draft, review and editing; formal analysis, Ryuichi Nakagawa: Data curation, Yoichiro Sugita: Data curation, writing-review, Emi Ono: Data curation, Yoshimi Yamaguchi: Data curation, Tomoyuki Kato:

Data curation, writing-review, Hideki Kajiwara: Data curation, Reiko Taki: Data curation, Naoshige Harada: Writing-review.

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CONFLICT OF INTEREST DISCLOSURE

The authors have no conflicts of interest to declare.

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