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Short Communication

Coronavirus Infections in Pediatric Outpatients with Febrile Respiratory Tract Infections in Hiroshima, Japan, over a 3-Year Period

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SUMMARY: Previously, we conducted a 3-year prospective study to determine the viral causes of acute respiratory tract infections among 495 febrile pediatric outpatients. We collected 495 nasopharyngeal aspirate specimens, and used both real-time PCR assays and viral culture to test each for respiratory viruses other than coronavirus. Here, we used real-time PCR to test the 495 archival specimens for four human coronavirus strains. We identified 15 coronavirus-positive specimens: eight with OC43, 5 with NL63, 2 with HKU1, and none with 229E. Of the 15 children (5 boys) infected with human coronavirus, the mean age was 3.5 years, and the age range was 1.1 to 5.8 years; one child was diagnosed with lower respiratory infection; the other 14 were diagnosed with upper respiratory infection. Of these 15 patients, none were hospitalized, 5 were infected with coronavirus alone, 8 were co-infected with another virus, and 2 were co-infected with 2 other viruses. The multi-virus infections involved 6 adenoviruses, 3 respiratory syncytial viruses, 2 parainfluenza viruses, and 1 rhinovirus. In conclusion, the burden of human coronaviruses was relatively light among this cohort of 495 pediatric outpatients, and the incidence of these infections was low.

Human coronaviruses (HCoVs) were initially identified as causes of upper respiratory tract infections (URTI) in the 1960s based on tissue culture assays (1,2). Two of the HCoV strains (HCoV-OC43 and HCoV-229E) were detected predominantly in individuals with common colds (3–6). Thereafter, three new HCoV strains were identified via molecular and tissue culture assays: severe acute respiratory syndrome coronavirus (SARS-CoV) (7), coronavirus strain noted in The Netherlands (HCoV-NL63) (8,9), and coronavirus strain noted in Hong Kong (HCoV-HKU1) (10). SARS-CoV was associated with an outbreak of severe pneumonia that spread from Asia to other parts of the world, but since then has not caused any outbreaks. HCoV-NL63 and HCoV-HKU1 are reportedly associated with respiratory tract infection (RTI). Furthermore, after the appearance of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, epidemiological studies of HCoVs have been performed with interest (11). The true burden of disease from the non-SARS and non-MERS HCoVs has not been clearly documented, especially among pediatric outpatients. The objective of this study was to use archival specimens from a previous 3-year prospective study to determine the prevalence and clinical features of RTIs due to four non-SARS and non-MERS HCoVs among febrile pediatric outpatients with acute RTI.

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The previous prospective study was conducted over a 3-year period between September 2008 and August 2011 at the Hara Pediatric Clinic, a primary care clinic, and the Health and Environment Center of the Hiroshima Prefectural Technology Research Institute (12). Each nasopharyngeal aspirate (NPA) specimen was collected prospectively from a child ($\leq$15 years old) who presented with febrile RTI at the clinic; each patient had to have both a fever lasting $\geq$3 days and a peak temperature $\geq$39.0°C during those 3 days to be enrolled in this study. Informed consent was obtained from parents or guardians of each participant. A pediatrician made a diagnosis at the time of sample collection, and a chest radiograph was ordered at the discretion of the pediatrician. Together with a cough, each of four clinical features: i) wheezing; ii) tachypnea; iii) dyspnea; and iv) abnormal breath sounds on auscultation were considered to be signs of lower respiratory tract infection (LRTI). Bronchitis was defined as a LRTI characterized by cough and the presence of local rales on auscultation. Wheezy bronchitis was defined as LRTI characterized by cough, diffuse wheezing, and rales on auscultation. Pneumonia was defined as LRTI with the presence of focal infiltrate on a chest radiograph. URTIs were categorized as: i) an URTI with cough (URTI-C) was defined as acute respiratory disease presenting with cough, but no evidence of any LRTI; and ii) tonsillitis, which was defined as an URTI characterized by the presence of exudates on the tonsils and the absence of cough. Notably, URTI-C was the only URTI diagnosis that included cough. Previously, viral culture and real-time PCR assays were used to test each specimen for respiratory viruses: real-time PCR assays were used to test each specimen for 9 specific respiratory viruses: respiratory syncytial virus (RSV); human metapneumovirus (HMPV); parainfluenza virus type 1, 2, or 3 (PIV1-3);

adenovirus (AdV); rhinovirus; enterovirus; and influenza C virus (Flu-C) (11,12). Additionally, to detect respiratory viruses, viral culture or real-time PCR assay was also used to identify patients infected with influenza A or B viruses (12,13), and each such patient was then excluded from the study. Ultimately, there were 495 children who met the definition of febrile RTI and were enrolled in the current study. Of these 495 children, 398 (80.4%) were positive for at least one virus; among the 398 virus-positive children, 320 had single-virus infections, and 78 had multi-virus infections. RSV was detected in 138 children, HMPV in 66, PIV1-3 in 73, AdV in 124, rhinovirus in 23, enterovirus in 38, and Flu-C in 11 based on viral culture and real-time PCR assays; patients with multi-virus infections were counted multiple times, once independently for each type of virus.

QIAamp Viral RNA Mini Kits (Qiagen, Hilden, Germany) were used to extract RNA and DNA from the 495 archival NPA specimens, which had been kept at −75°C. QuantiTect Virus Kits (Qiagen) were used to perform real-time reverse transcription-PCR (RT-PCR) for multiplex detection of HCoV-NL63, HKU1, OC43, and 229E (14). We used Student's *t*-test to compare between patient groups infected with HCoVs with respect to duration of fever, peak temperature, and clinical diagnosis.

Real-time RT-PCR assays identified 15 specimens with HCoVs among all 495 archival specimens; HCoV-OC43 was detected in 8 specimens, HCoV-NL63 in 5, HCoV-HKU1 in 2, and HCoV-229E in none (Table 1). The mean age of the 15 children (5 boys) was 3.1 years, with ages ranging from 1.1 to 5.8 years. Clinical diagnoses for the 15 children included 8 cases of URTI-C (upper respiratory tract infection with cough), 3 cases of URTI-C accompanied by tonsillitis, 3 cases of tonsillitis, and 1 case of bronchitis; of the 15 HCoV-infected patients, there was only one with LRTI (Table 1). Of the 15 children, 6 (patients No. 3, 4, 7, 10, 11, and 15) underwent chest radiography, and the results for each were normal. Five children had single-virus infections, and 10 children had multi-virus infections: 8 with double infections and 2 with triple infections (Table 1). The co-infecting viruses were 6 AdVs, 3 RSVs, 2 PIVs, and 1 rhinovirus; no patient was co-infected with two HCoV strains. Among the 6 children co-infected with AdV, 5 had a diagnosis of tonsillitis. Patients with single-virus infections did not differ from those with multi-virus infections with regard to fever duration, peak body temperature, or clinical diagnosis. Of the 15 diseases caused by HCoVs, 12 (80.0%) occurred during the cold months between November and March.

We used real-time RT-PCR assays to determine the incidence of HCoV infections in febrile outpatient children and then evaluated the burden of these infections. We tested archival NPA specimens that were prospectively collected in a 3-year study. HCoV was present in only 15 (3.0%) of 495 febrile pediatric outpatients with RTI. Several other studies, each conducted over a period ≥1 year, in various clinical settings, have found detection rates of 2.1% to 7.6% for these four HCoV strains (4,5,15–18). In the present study, the rate of HCoV infection was markedly lower than those for each of 8 other common respiratory viruses, but not lower than that of Flu-C. Of 4 other studies that used PCR to examine the incidence of common respiratory viruses (RSV, PIV1-3, AdV, and HMPV) and HCoVs (4,5,15,16), 3 found that the rates of HCoV detection were the lowest among the respiratory viruses tested (4,15,16). We could not compare the distribution of HCoV strains with that of other studies because the number of HCoV-infected patients in our study was too small and because of differences in the clinical settings; however, our finding that no patient was infected with HCoV-229E was consistent with previous findings indicating that HCoV-229E infection is the least common type of HCoV infection (4,15–18,19).

Furthermore, we found that 10 (66.7%) of the 15 children were simultaneously infected with multiple viruses. Four other studies employing PCR assays for detection of both HCoVs and common respiratory viruses have reported co-infection rates ranging from 31.6% to 51.5% (4,5,16,18). In addition, none of these 15

Table 1. Clinical and demographic features of 15 children with acute respiratory tract infections caused by coronaviruses

Patient No.	Sex[1)]	Age	Diagnosis[2)]	Duration of fever (days)	Coronavirus strain	No. of coronavirus copies/assay	Co-infected virus(es)[3)]	Month of diagnosis
1	F	1 yr 3 mo	URTI-C	3	OC43	2.62×10^3	None	Feb.
2	F	3 yr 4 mo	URTI-C	4	HKU1	2.52×10^4	None	Jan.
3	F	3 yr 8 mo	URTI-C + Tonsillitis	5	OC43	5.68×10^3	None	Nov.
4	M	4 yr 0 mo	Bronchitis	6	NL63	1.40×10^4	None	Feb.
5	F	5 yr 2 mo	URTI-C	4	OC43	1.86×10^5	None	Feb.
6	F	1 yr 1 mo	URTI-C + Tonsillitis	4	NL63	1.89×10^2	AdV	Mar.
7	M	1 yr 2 mo	URTI-C + Tonsillitis	3	NL63	8.80×10^4	AdV-2	Feb.
8	M	1 yr 2 mo	Tonsillitis	3	HKU1	2.43×10^2	AdV-2, RV	Feb.
9	F	1 yr 11 mo	Tonsillitis	4	NL63	5.03×10^2	AdV-2	Nov.
10	F	2 yr 9 mo	URTI-C	5	OC43	6.23×10^3	PIV-1	Jul.
11	F	3 yr 1 mo	URTI-C	5	OC43	3.94×10^4	RSV	Dec.
12	F	3 yr 5 mo	URTI-C	3	OC43	4.19×10^6	RSV	Apr.
13	M	4 yr 0 mo	Tonsillitis	7	NL63	4.04×10^5	AdV-2	Mar.
14	F	4 yr 0 mo	URTI-C	3	OC43	1.05×10^5	AdV-1	Jun.
15	M	5 yr 9 mo	URTI-C	5	OC43	1.32×10^4	RSV, PIV-2	Nov.

1): F, female; M, male. 2): URTI-C, upper respiratory tract infection with cough.
3): AdV-1, -2, adenovirus type 1, 2; RV, rhinovirus; PIV-1, -2, parainfluenza virus type 1, 2; RSV, respiratory syncytial virus.

patients were hospitalized, and only 1 patient was diagnosed with LRTI. Several other studies indicate that HCoV-NL63 frequently causes LRTI such as croup, bronchiolitis, or pneumonia (16,20–23), and that 3 HCoV strains other than HCoV-NL63 generally cause URTIs (6,15,23). However, we could not readily compare our findings on rates of LRTI or disease severity with those from other reports because most previous studies involved only or mostly hospitalized patients (6,15,17,20–22). Diseases due to HCoVs occurred in young children and mainly in the cold months. Many other studies describe demographic and seasonality findings similar to those from our study (4,6,15–17,19,23).

In summary, we conclude that the clinical burden of HCoV infection was light, and that the incidence of HCoV infection was low when compared with incidences of infections with other common respiratory viruses. A limitation of this study is the fact that we enrolled only febrile patients with RTI. Further studies that include afebrile patients with LRTI will be needed.

Conflict of interest None to declare.

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