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Fecal Respiratory Viruses in Acute Viral Respiratory Infection and Nasopharyngeal Diarrheal Viruses in Acute Viral Gastroenteritis: Clinical Impact of Ectopic Viruses Is Questionable

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Our aim was to determine the detection rate of respiratory viruses (RVs) in feces of patients with acute viral respiratory infection (AVRI) and the detection rate of diarrheal viruses (DVs) in nasopharyngeal samples from patients with acute viral gastroenteritis. The relationships between the presence of fecal RVs or nasopharyngeal DVs and their impacts on the clinical severity were also investigated. A total of 144 fecal specimens were collected from AVRI patients and 95 nasopharyngeal specimens were collected from acute viral gastroenteritis patients. Clinical characteristics and laboratory profiles were compared between subgroups on the basis of the presence or absence of virus in the specimens. The detection rate of RVs in feces was 17.4% (25/144), whereas the detection rate for viruses identical to the respiratory pathogen was 10.4% (identical group, 15/144). Within the identical group, adenovirus (86.7%, 13/15) was most commonly found. Patients in the identical group showed statistically higher values for C-reactive protein, mean age, increased frequency of vomiting, and decreased frequency of chest film involvement and cough ($p < 0.05$). The detection rate of nasopharyngeal DVs among acute viral gastroenteritis patients was 19.0% (18/95), and in the identical group it was 15.8% (15/95). Norovirus group II and enteric adenovirus were the major pathogens detected in the identical group. There were no significant differences in clinical characteristics and laboratory profiles between the subgroups. In conclusion, the major pathogens of fecal RV and nasopharyngeal DV were adenovirus and norovirus group II, respectively. However, their relationship with the clinical symptoms or disease severity is unclear.

Keywords: Respiratory virus, diarrheal virus, fecal virus detection, nasopharyngeal virus detection

Introduction

Acute respiratory infection (ARI) is the most common disease in all age groups [1]. Viruses comprise a significant proportion of ARI pathogens, and, in acute lower respiratory infection, 60%–90% of pathogens are viruses [2]. In Korea, the most common respiratory viruses (RVs) in children with acute lower respiratory infection are respiratory syncytial virus (RSV, 22.2%), parainfluenza virus (PIV, 15.3%), influenza virus A and B (FluA and FluB, 9.4%), human rhinovirus (HRV, 8.0%), adenovirus (AdV, 7.2%), and metapneumovirus (MPV, 4.9%) [2]. Many patients suffering from acute viral respiratory infection (AVRI) have gastrointestinal (GI) symptoms, such as nausea, vomiting, diarrhea, and/or abdominal pain. It remains unclear whether these GI symptoms are due to the clinical course of RV infection, to the direct infection of RVs to the GI tract, or to antiviral agents or other medications used to treat the respiratory symptoms [3]. Because RVs are sometimes found in patient fecal specimens [3–6], investigating the

detection rates of RVs in the stool and their association with disease severity may be helpful toward understanding the pathogenesis and propagation patterns of RVs. However, studies on RV detection in stool specimens in patients with AVRI are limited.

Acute viral gastroenteritis is another common disease among infants and children. Known causative viral pathogens are group A rotavirus (RotV), astrovirus (AstV), calicivirus, enteric adenovirus (EAdV), enterovirus, and norovirus (NoV) [7, 8]. Like RVs, diarrheal viruses (DVs) are found in nasopharyngeal specimens obtained from patients with acute GI infection [9, 10]. Investigating the presence of DVs in nasopharyngeal specimens can provide information in regard to their pathogenicity outside of the GI tract and their transmission pattern.

In this study, our objective was to determine the detection rate of fecal RVs among patients with AVRI and of nasopharyngeal DVs among patients with acute viral gastroenteritis. In addition, we compared laboratory profiles and clinical symptoms between patients with and without fecal RVs or nasopharyngeal DVs in order to understand viral pathogenicity in systems outside of their primary targets.

Materials and Methods

Patients and Specimens

The study was performed at Chung-Ang University Hospital, Seoul, South Korea from December 2010 to February 2012. We attempted to detect fecal RV in 144 stool specimens that we obtained from 144 AVRI patients with GI symptoms, which included nausea, vomiting, and diarrhea. The patients included 89 males and 55 females with ages ranging from one month to 14 years old. Clinical symptoms of the subjects were fever (92.4%), coughing (86.8%), sputum (72.2%), sore throat (6.3%), rhinorrhea (45.1%), nausea (22.2%), vomiting (31.9%), and diarrhea (70.8%). Clinical diagnoses were pneumonia (45.1%), bronchitis (20.8%), bronchiolitis (26.4%), and other upper respiratory diseases (15.3%). The mean duration of hospitalization was 6.5 days. Stool specimens for fecal RV detection were obtained on the same day as the nasopharyngeal specimen collection for AVRI diagnosis. Patients were grouped according to RV presence (detected group) or RV absence (non-detected) in the feces. The detected group was subdivided according to whether the virus was identical to that obtained from the nasopharyngeal aspirates (identical and non-identical groups).

The presence of DVs in nasopharyngeal specimens was assessed from 95 acute viral gastroenteritis patients with respiratory symptoms during the same period described above. The subjects included 59 males and 36 females with ages ranging from two months to 14 years old. Clinical symptoms of the subjects were nausea (43.8%), vomiting (46.9%), diarrhea (77.5%), fever (83.3%), cough (42.3%), sputum (31.5%), sore throat (5.2%), and rhinorrhea (46.9%). The mean duration of hospitalization was 6.1 days. These subjects were also grouped as described above.

Target Pathogens and Detection Method

RV detection was performed using the multiplex reverse transcription polymerase chain reaction (RT-PCR) with the SeePlex RV7 Detection Kit (Seegene Inc., Korea), which can detect seven different RVs, including AdV (adenovirus types A to E), FluA, FluB, MPV, HRV, RSV, and PIV. DV detection was conducted using multiplex RT-PCR with the SeePlex Diarrhea-V ACE Detection Kit (Seegene Inc.). The targets detected using this kit were EAdV (adenovirus types F40 and F41), RotV, NoV Group I (NoV-GI), NoV Group II (NoV-GII), and AstV. Amplification was performed with a GeneAmp PCR System 9700 thermal cycler (Thermo Fisher Scientific Inc., USA). All analyses were performed according to the manufacturer's instructions. Specimens were stored at 4°C and analyzed within 48 h of collection.

Clinical and Laboratory Features of Study Subjects

The clinical medical records of the study subjects were reviewed in order to compare related symptoms (nausea, vomiting, diarrhea, fever, coughing, sputum, sore throat, and rhinorrhea) and laboratory findings, including chest X-ray results, between the study groups. The laboratory profile values compared were the presence of leukocytosis, neutrophilia, and eosinophilia, and the levels of hemoglobin (Hb), blood urea nitrogen (BUN), C-reactive protein (CRP), aspartate aminotransferase (AST), and alanine transaminase (ALT). Reference values for all laboratory data were based on patient age.

Statistical Analysis

Independent t-tests, one-way ANOVA, and Pearson's chi-square tests with a 2 by 3 crosstab were conducted in order to compare the clinical and laboratory profiles among the three groups. A p value of <0.05 was considered statistically significant. The SPSS statistical software program ver. 21.0 (IBM, USA) was used for all statistical calculations.

Ethics Statement

The study protocol was approved by the Institutional Review Board of Chung-Ang University Hospital (IRB No. C2012169 [864]).

Results

Fecal RV Detection in AVRI Patients

The overall detection rate of RVs in the stool was 17.4% (25 of 144, detected group, regardless of the presence of DVs in stool specimens); 10.4% (15 of 144, identical group) had a fecal RV identical to the virus detected in nasopharyngeal aspirates, whereas 6.9% (10 of 144, non-identical group) had a fecal RV different from the one detected in the

Table 1. Detection rates of respiratory viruses in fecal specimens from acute viral respiratory infection patients, and of diarrheal viruses in nasopharyngeal specimens from acute viral gastroenteritis patients.

	Detected			Non-detected
	Identical[a]	Non-identical[a]	Total	
RV in fecal specimens	10.4% (15/144)	6.9% (10/144)	17.4% (25/144)	82.6% (119/144)
DV in nasopharyngeal specimens	15.8% (15/95)	3.2% (3/95)	19.0% (18/95)	81.0% (77/95)

[a]Identical to the RV detected in the nasopharyngeal specimen (for RV in the fecal specimen), or identical to the DV detected in the fecal specimen (for DV in the nasopharyngeal specimen).
RV, respiratory virus; DV, diarrheal virus.

nasopharyngeal aspirates (Table 1). In the identical group, AdV was the most commonly found virus (86.7%, 13 of 15). The remaining two cases were one case each of FluA and HRV (6.7%, 1 of 15 for each). Among the 15 cases in the identical group, 8 patients (53.3%) were found to have RVs in their fecal specimens without DVs. In the non-identical group, AdV (60.0%, 6 of 10), FluA (20.0%, 2 of 10), HRV (10.0%, 1 of 10), and PIV (10.0%, 1 of 10) were detected in fecal specimens. The details of fecal RV detection are presented in Table 2.

Comparisons of laboratory profiles and clinical features among the three groups for RVs in fecal specimens confined

Table 2. Respiratory viruses detected in fecal specimens from acute viral respiratory infection patients.

Groups	Subgroups	RVs in fecal specimens	RVs in nasopharyngeal specimens	DVs in fecal specimens	No. of detections	
					N	%
Detected	Identical	AdV	AdV	EAdV	2	1.4
				NoV-GII,	3	2.1
				RotV	1	0.7
				N	6	4.2
			AdV, HRV	N	1	0.7
		FluA	FluA	RotV	1	0.7
		HRV	HRV	N	1	0.7
	Non-identical	AdV	FluA	N	1	0.7
			FluB	N	1	0.7
			MPV	N	1	0.7
			RSV	RotV	1	0.7
				N	1	0.7
			RSV, HRV	RotV	1	0.7
		FluA	MPV	NoV-GII,	1	0.7
			RSV	N	1	0.7
		HRV	RSV	RotV	1	0.7
		PIV	HRV	N	1	0.7
Non-detected		N	Various[a]	Various[b]	56	38.9
				N	63	43.8
Total					144	100.0

[a]Includes AdV, FluA, FluB, HRV, MPV, PIV, and RSV.
[b]Includes AstV, EAdV, NoV-GI, NoV-GII, and RotV.
AdV, adenovirus; AstV, astrovirus; EAdV, enteric adenovirus; DV, diarrheal virus; FluA, influenza A virus; FluB, influenza B virus; HRV, human rhinovirus; MPV, metapneumovirus; N, none; NoV-GI, norovirus group I; NoV-GII, norovirus group II; PIV, parainfluenza virus; RotV, group A rotavirus; RSV, human respiratory syncytial virus; RV, respiratory virus.

to cases in which patients were revealed to have no DVs in fecal specimens are listed in Table 3. Among GI-related symptoms, vomiting was significantly more frequently reported in the identical group (50.0%, 16.7%, and 17.5% for the identical, non-identical, and non-detected groups, respectively; $p < 0.05$). There were no significant differences in nausea or diarrhea reported for any of the three groups. The difference in mean age was statistically significant among the three groups (51.75, 37.00, and 21.08 months for the identical, non-identical, and non-detected groups, respectively; $p < 0.05$), and the mean level of CRP was statistically higher in the identical group (58.29, 3.73, and 3.47 mg/l for the identical, non-identical, and non-detected groups, respectively; $p < 0.05$). The frequency of coughing or chest film involvement was significantly lower in the identical group than in any other group (50.0%, 83.3%, and 87.3% for the identical, non-identical, and non-detected groups, respectively; and 50.0%, 83.3%, and 85.5% for the identical, non-identical, and non-detected groups, respectively; $p < 0.05$ each).

Table 3. Clinical features and laboratory profiles of acute viral respiratory infection patients without fecal diarrheal viruses according to detection of fecal respiratory viruses.

		Fecal RV detected		Fecal RV Non-detected ($N = 63$)	p Value
		Identical[a] ($N = 8$)	Non-identical[a] ($N = 6$)		
Mean age (months)		51.75 [8–168][b]	37.00 [6–84]	21.08 [1–108]	0.04[c]
Sex					
	Male	5 (62.5%)	6 (100.0%)	37 (58.7%)	0.14
	Female	3 (37.5%)	0(0.0%)	26 (41.3%)	0.14
Laboratory profile					
	Leukocytosis	2 (25.0%)	0 (0.0%)	15 (23.8%)	0.40
	Neutrophilia	0 (0.0%)	0 (0.0%)	8 (12.7%)	0.37
	Lymphocytosis	0 (0.0%)	0 (0.0%)	1 (1.6%)	0.89
	Eosinophilia	1 (12.5%)	0 (0.0%)	4 (6.3%)	0.64
	Mean Hb (g/dl)	12.4 [10.7–14.4]	12.1 [11.0–13.2]	12.0 [10.3–13.2]	0.83
	Mean CRP (mg/l)	58.3 [3.8–199.5]	3.7 [0.42–10.14]	3.5 [0.06–42.65]	<0.01[d]
	Mean BUN (mg/dl)	8.5 [4–18]	8.3 [4–13]	9.0 [1–20]	0.90
	Mean AST (IU/l)	42.5 [17–122]	63.8 [23–143]	45.1 [20–240]	0.47
	Mean ALT (IU/l)	22.0 [8–65]	24.3 [13–38]	31.1 [8–382]	0.88
	Chest film involvement	4 (50.0%)	5 (83.3%)	53 (85.5%)	0.04[c]
Symptoms					
	Fever	8 (100.0%)	6 (100.0%)	60 (95.2%)	0.71
	Cough	4 (50.0%)	5 (83.3%)	55 (87.3%)	0.04[d]
	Sputum	3 (37.5%)	5 (83.3%)	48 (76.2%)	0.06
	Sore throat	2 (25.0%)	0 (0.0%)	5 (7.9%)	0.21
	Rhinorrhea	1 (12.5%)	1 (16.7%)	29 (46.0%)	0.09
	Nausea	3 (37.5%)	0 (0.0%)	7 (11.1%)	0.07
	Vomiting	4 (50.0%)	1 (16.7%)	11 (17.5%)	0.03[c]
	Diarrhea	6 (75.0%)	5 (83.3%)	46 (73.0%)	0.86
Duration of hospitalization (days)		5.3 [3–8]	5.0 [3–7]	7.0 [2–15]	0.06

[a]Identical to the RVs detected in the nasopharyngeal specimen.

[b]Numbers in the brackets represent minimum and maximum values of the data.

[c]All groups showed significantly different values.

[d]Identical group showed significantly different values from all other groups.

ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C reactive protein; DV, diarrheal virus; Hb, hemoglobin; RV, respiratory virus.

Nasopharyngeal DV Detection in Acute Viral Gastroenteritis Patients

The detection rate of DVs in nasopharyngeal specimens was 19.0% (18 of 95, detected group). Identical pathogens were detected in the nasopharyngeal and fecal specimens from 15 patients (15.8%, identical group), whereas different viruses in each sample type were detected in three patients (3.2%, non-identical group) (Table 1). In the identical group, the most commonly detected DVs in nasopharyngeal specimens were NoV-GII (80.0%, 12 out of 15) and EAdV (20.0%, 3 out of 15). In the non-identical group, NoV-GII, EAdV, and NoV-GI were each detected in one specimen from patients with NoV-GII or RotV GI tract infections. Detailed results on the nasopharyngeal DV detection are shown in Table 4.

The clinical features, patients' characteristics, and laboratory profile were not statistically different among the groups, when the analyzed specimens were confined to those from patients without RVs in their nasopharyngeal specimens. Details on the comparisons are presented in Table 5.

Discussion

Our study determined the detection rate of RVs in fecal specimens from patients with acute RV infection. The total detection rate of RVs in the stool was 17.4%. AdV, FluA, or HRV viruses were detected in both nasopharyngeal aspirates and stool samples from 10.4% of the patients with acute RV infection. AdV was the most frequently detected virus in the identical group, comprising 9.0% (13/144) of all infections. AdV is typically detected in up to 3.8% of the general pediatric population [11], which suggests that we achieved a significantly high detection rate.

In the current study, fecal FluA was detected in three cases (2.1%), with one case from the identical group and two from the non-identical group. FluB was never detected in fecal specimens. In contrast to our study, FluB is known to be related to the presence of GI symptoms and is typically more frequently detected than FluA in the gastric mucosa of infected patients [12]. In one study, FluB was detected in 8 of 63 fecal samples (12.70%) from patients with influenza virus in their respiratory aspirates, whereas FluA was detected in only two cases (3.16%; subtypes H1N1 and H3N2) [3]. One reason for the low detection levels of FluB in fecal specimens in our study was because of the small number of influenza infections. In our study, among the 144 AVRI patients who were tested fecal RVs, 20 cases had influenza viruses in their nasopharyngeal specimens; 12 and 8 cases for the FluA and FluB, respectively. Another study that focused only on FluA found that 0.92% of cases (6/651) were positive in corresponding stool specimens, and half of these were of the H3N2 subtype [4].

Table 4. Diarrheal viruses detected in nasopharyngeal specimens from acute viral gastroenteritis patients.

Groups	Subgroups	DVs in nasopharyngeal specimens	DVs in fecal specimens	RVs in nasopharyngeal specimens	No. of detections	
					N	%
Detected	Identical	EAdV	EAdV	N	3	3.2
		NoV-GII	NoV-GII	AdV	1	1.1
				FluB	1	1.1
				RSV	7	7.4
				RSV, HRV	1	1.1
				N	1	1.1
			NoV-GII, NoV-GI	RSV	1	1.1
	Non-identical	EAdV	NoV-GII, RotV	RSV	1	1.1
		NoV-GI	NoV-GII	RSV	1	1.1
		NoV-GII	RotV	N	1	1.1
Non-detected		N	Various[a]	Various[b]	52	54.7
				N	25	26.3
Total					95	100.0

[a]Includes AstV, EAdV, NoV-GI, NoV-GII, and RotV.

[b]Includes AdV, FluA, FluB, HRV, MPV, PIV, and RSV.

AdV, adenovirus; AstV, astrovirus; EAdV, enteric adenovirus; DV, diarrheal virus; FluA, influenza A; FluB, influenza B; HRV, human rhinovirus; MPV, metapneumovirus; N, none; NoV-GI, norovirus group I; NoV-GII, norovirus group II; PIV, parainfluenza; RotV, group A rotavirus; RSV, human respiratory syncytial virus; RV, respiratory virus.

Table 5. Clinical features and laboratory profiles of acute viral gastroenteritis patients without nasopharyngeal respiratory viruses according to detection of nasopharyngeal diarrheal viruses.

		Nasopharyngeal DV detected		Nasopharyngeal DV Non-detected (N = 25)	p Value
		Identical[a] (N = 4)	Non-identical[a] (N = 1)		
Mean age (months)		37.5 [12–98][b]	34.0	40.1 [2–128]	0.61
Sex					
	Male	3 (75.0%)	1 (100.0%)	14 (56.0%)	0.55
	Female	1 (25.0%)	0 (0.0%)	11 (4.0%)	0.55
Laboratory profile					
	Leukocytosis	2 (50.0%)	0 (0.0%)	6 (24.0%)	0.46
	Neutrophilia	3 (75.0%)	1 (100.0%)	17 (68.0%)	0.77
	Lymphocytosis	1 (25.0%)	0 (0.0%)	5 (20.0%)	0.86
	Eosinophilia	0 (0.0%)	0 (0.0%)	1 (4.0%)	0.90
	Mean Hb (g/dL)	13.1 [12.2–14.0]	12.5	12.5 [10.4–14.5]	0.45
	Mean CRP (mg/l)	11.7 [0.4–34.7]	8.5	14.1 [0.1–65.4]	0.92
	Mean BUN (mg/dL)	11.3 [6–21]	11.0	11.0 [4–18]	0.78
	Mean AST (IU/l)	62.0 [33–118]	18.0	43.0 [13–171]	0.26
	Mean ALT (IU/l)	33.0 [17–59]	9.0	34.1 [9–208]	0.74
	Chest film involvement	3 (75.0%)	1 (100.0%)	11 (44.0%)	0.31
Symptoms					
	Fever	3 (75.0%)	1 (100.0%)	19 (76.0%)	0.85
	Cough	2 (50.0%)	1 (100.0%)	17 (68.0%)	0.60
	Sputum	1 (25.0%)	1 (100.0%)	10 (40.0%)	0.39
	Sore throat	0 (0.0%)	0 (0.0%)	2 (8.0%)	0.81
	Rhinorrhea	1 (25.0%)	0 (0.0%)	11 (44.0%)	0.55
	Nausea	2 (50.0%)	1 (100.0%)	10 (40.0%)	0.45
	Vomiting	2 (50.0%)	1 (100.0%)	13 (52.0%)	0.63
	Diarrhea	2 (50.0%)	1 (100.0%)	18 (72.0%)	0.54
Duration of hospitalization (days)		4.3 [2–6]	11.0	5.6 [2–10]	0.10

[a]Identical to the DVs detected in the fecal specimens.

[b]Numbers in the brackets represent minimum to maximum values of the data.

ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; DV, diarrheal virus; Hb, hemoglobin; RV, respiratory virus.

This is a lower value than that observed in our study. Although there was only one FluA case from the identical group, it was 8.3% (1/12) of the total FluA-related AVRI cases.

Whether fecal RV can increase the susceptibility of DV infections in the GI tract is debatable. One study demonstrated that one or more DVs were detected in half of the acute diarrhea patients who tested positive for influenza in their stools [3]; however, we did not find any evidence of those findings. Rather, the DV infections of the GI tract were more commonly found in the non-detected group than in the detected group. In the detected group, 44.0% (11/25) of cases were found to have DVs in their fecal specimens, whereas 47.1% (56/119) of cases in the non-detected group were found to have DVs. Within the detected group, the prevalence of coinfections was 46.7% (7/15) in the identical group and 40.0% (4/10) in the non-identical group; however, these values were not statistically significant ($p > 0.05$). When we calculated coinfections by virus separately, 66.6% (2/3) of the fecal FluA-positive specimens were coinfected with other DVs, 42.1% (8/19) for AdV, and 50% (1/2) for HRV. However, these were also not statistically significant ($p > 0.05$).

When we compared clinical features and laboratory profiles

among the subgroups of AVRI patients according to fecal RV detection and we included only those patients without DVs in their fecal specimens, the identical group had significantly higher CRP levels than any other group. However, higher CRP levels could be due to differences in the prevalence of AdV between groups. The symptoms related to AdV infection were usually more aggressive than the symptoms from any other RV infection [13], and because the identical group had an increased prevalence of nasopharyngeal AdV compared with the other groups, the CRP levels may be higher than any other groups. Although the frequency of vomiting was higher in the identical group than in the other groups, the frequency of other GI symptoms (diarrhea and nausea) was not statistically higher than the other groups. In addition, the frequency of coughing or chest film involvement in this group was lower than in the other groups ($p < 0.05$). Thus, evidence for direct RV infection of the GI tract is insufficient and the impact of fecal RVs on disease severity is unclear.

The true pathogenicity of RVs in fecal samples has been debated [5, 6, 14–16]. For example, because HRVs are labile in the stomach's acidic environment, HRV detection in fecal samples or in sewage has generally been attributed to swallowing of respiratory secretions and not viral amplification within the gut. Experimentally, HRVs differ from enteroviruses, as they are inactivated at pH values <5 and at pH values above 9–10 [5]. Some authors claim that this is unlikely because the acid lability of HRVs destroys their infectivity, since passing through the stomach would lead to HRV RNA degradation [6]. Another claim is that dilution of HRV secretions by the large volume of gut contents would not support the high viral loads detected by PCR. For these reasons, it has been argued that HRVs detected in the stool would be evidence of direct GI infection and viral amplification within the gut. The same controversies exist in human influenza virus studies. Some studies have argued that FluA and FluB can bind to alpha 2,6-sialic acid receptors in the human GI tract, and infect and actively replicate within the cells [14]. However, several studies did not find evidence for human influenza virus receptors in intestinal tract epithelial cells [15, 16]. Therefore, confirmation of these results will require future studies.

NoV was one of the major DVs detected in nasopharyngeal specimens. In the current study, 14.7% (14/95) of symptomatic acute viral gastroenteritis patients had NoV in their nasopharyngeal specimen. Most of these patients (92.9%, 13/14, regardless of the type of NoV) also had NoV in their stool specimens. The nasopharyngeal detection rates we observed in this study were comparable to those of other studies [9]. Dábilla *et al*. [9] reported that in nasopharyngeal swab samples, NoV positivity was 11.4% (11/96) in symptomatic AGE patients. However, in that study, NoV was detected in both feces and nasopharyngeal swabs from the same child in only two cases. This finding differed from our study. Because the study populations were nearly the same, further investigation are needed for nasopharyngeal NoV detection.

There were no significant differences in the clinical features and laboratory profiles of acute viral gastroenteritis patients based on the presence of nasopharyngeal DVs. The study performed by Dábilla *et al*. [9] also showed that there were no significant differences in GI symptoms (vomiting, diarrhea, and fever) between the nasopharyngeal NoV-positive group and nasopharyngeal NoV-negative group. Thus, it seems that the presence of DVs in the nasopharynx does not correlate with disease severity and that they are detected because of GI secretory reflux or contamination. However, this finding cannot exclude the possibility of DV transmission by the respiratory route.

Our study had several limitations. First, we did not perform viral cultures or sequence analyses of the pathogens; we only conducted a multiplex RT-PCR assay. Because multiplex PCR assays have several drawbacks, such as low sensitivity due to PCR drift, competitive inhibition (PCR selection), or nonspecific interactions [17,18], additional confirmatory tests would have provided more accurate pathogen identification. Instead, positive and negative controls were included in each PCR to help us evaluate false positives. Nevertheless, because we did not perform sequence or subtyping analysis, the identity of the viruses is still unclear. For example, because the SeePlex RV7 Detection Kit can detect adenovirus genotypes A, B, C, D, and E, it cannot discriminate the genotypes. Thus, it is possible that the AdV genotypes detected in the respiratory tract and stool specimens may differ from one another. Similarly, NoV-GI and NoV-GII have several genotypes, and thus differences in NoV genotypes detected from the nasopharynx and the stool are not totally excluded. Another limitation is that the detection rates of RVs and DVs are likely to be reduced owing to inhibitory material present in the GI tract and in the stool. Lastly, because we used a multiplex RT-PCR assay, we were not able to assess the concentration of virus or viral loads in the specimens.

In conclusion, our study demonstrated that 10.4% of AVRI patients had identical RVs in their stool specimens, and that the relevant pathogens were AdV, HRV, and FluA. Moreover, 15.8% of acute viral gastroenteritis patients were

found to have identical pathogens in their respiratory tract, including the major pathogens NoV and EAdV. However, the simultaneous detection of RVs or DVs in the specimens from respiratory and stool specimens did not show evidence of additional virulence from AVRI or acute viral gastroenteritis.

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References

1. van den Hoogen BG, Osterhaus DM, Fouchier RA. 2004. Clinical impact and diagnosis of human metapneumovirus infection. *Pediatr. Infect. Dis. J.* **23:** S25-S32.
2. Park JS. 2009. Acute viral lower respiratory tract infections in children. *Korean J. Pediatr*. **52:** 269-276.
3. Arena C, Amoros JP, Vaillant V, Balay K, Chikhi-Brachet R, Varesi L, *et al*. 2012. Simultaneous investigation of influenza and enteric viruses in the stools of adult patients consulting in general practice for acute diarrhea. *Virol. J.* **9:** 116.
4. Chan MC, Lee N, Chan PK, Leung TF, Sung JJ. 2009. Fecal detection of influenza A virus in patients with concurrent respiratory and gastrointestinal symptoms. *J. Clin. Virol.* **45:** 208-211.
5. Blomqvist S, Savolainen-Kopra C, Paananen A, Hovi T, Roivainen M. 2009. Molecular characterization of human rhinovirus field strains isolated during surveillance of enteroviruses. *J. Gen. Virol.* **90:** 1371-1381.
6. Harvala H, McIntyre CL, McLeish NJ, Kondracka J, Palmer J, Molyneaux P, *et al*. 2012. High detection frequency and viral loads of human rhinovirus species A to C in fecal samples; diagnostic and clinical implications. *J. Med. Virol.* **84:** 536-542.
7. Ma SH. 2005. Acute infectious diarrhea in pediatric patients. *Korean J. Pediatr.* **48:** 235-250.
8. So CW, Kim DS, Yu ST, Cho JH, Kim JD. 2013. Acute viral gastroenteritis in children hospitalized in Iksan, Korea during December 2010-June 2011. *Korean J. Pediatr.* **56:** 383-388.
9. Dabilla N, Nunes Vieira Almeida T, Carvalho Reboucas Oliveira A, Kipnis A, Neres Silva T, Souza Fiaccadori F, *et al*. 2017. Norovirus in feces and nasopharyngeal swab of children with and without acute gastroenteritis symptoms: first report of GI.5 in Brazil and GI.3 in nasopharyngeal swab. *J. Clin. Virol.* **87:** 60-66.
10. Novikova NA, Al'tova EE, Noskova NV, Epifanova NV, Tamoikina NA. 1991. The detection of rotavirus RNA in nasopharyngeal smears by molecular hybridization. *Zh. Mikrobiol. Epidemiol. Immunobiol.* **1991:** 23-25.
11. Okimoto S, Hyodo S, Yamamoto M, Nakamura K, Kobayashi M. 2011. Association of viral isolates from stool samples with intussusception in children. *Int. J. Infect. Dis.* **15:** e641-e645.
12. Hayase Y, Tobita K, Sato H. 2002. Detection of type B influenza virus genes from biopsied gastric mucosa. *J. Gastroenterol.* **37:** 101-105.
13. Avila MM, Carballal G, Rovaletti H, Ebekian B, Cusminsky M, Weissenbacher M. 1989. Viral etiology in acute lower respiratory infections in children from a closed community. *Am. Rev. Respir. Dis.* **140:** 634-637.
14. Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, *et al*., and Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. 2005. Avian influenza A (H5N1) infection in humans. *N. Engl. J. Med.* **353:** 1374-1385.
15. Yao L, Korteweg C, Hsueh W, Gu J. 2008. Avian influenza receptor expression in H5N1-infected and noninfected human tissues. *FASEB J.* **22:** 733-740.
16. Sata T, Roth J, Zuber C, Stamm B, Heitz PU. 1991. Expression of alpha 2,6-linked sialic acid residues in neoplastic but not in normal human colonic mucosa. A lectin-gold cytochemical study with *Sambucus nigra* and *Maackia amurensis* lectins. *Am. J. Pathol.* **139:** 1435-1448.
17. Whiley DM, Sloots TP. 2005. Comparison of three in-house multiplex PCR assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using real-time and conventional detection methodologies. *Pathology.* **37:** 364-370.
18. Kosters K, Reischl U, Schmetz J, Riffelmann M, Wirsing von Konig CH. 2002. Real-time LightCycler PCR for detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. *J. Clin. Microbiol.* **40:** 1719-1722.