Comparison of Clinical Features of Childhood Norovirus and Rotavirus Gastroenteritis in Taiwan

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Background: Viral gastroenteritis is a common acute infectious disease in infants and young children. This study compared the incidence and clinical features of childhood norovirus (NV) and rotavirus (RV) gastroenteritis in Taiwan.

Methods: Stool specimens were collected from children with acute gastroenteritis aged 6 months to 14 years who were treated at the Children's Medical Center of Taipei Veterans General Hospital between January 2004 and March 2005. The incidence, clinical manifestations, and laboratory findings of childhood NV gastroenteritis were analyzed and compared with those of patients with RV gastroenteritis. Patients with underlying diseases associated with diarrhea or those diagnosed with bacterial gastroenteritis were excluded. Stool specimens were tested for NV and RV using enzyme immunoassay (EIA). NV genogroups were determined by reverse-transcriptase polymerase chain reaction.

Results: Among the 201 patients included in this study, NV was detected in 44 (21.9%) by 1 or more tests (22 by EIA). Five of these isolates were genogroup I (11.3%), and 39 were genogroup II (88.7%). Fifty-two (25.9%) specimens had a positive EIA result for RV. Compared with NV, patients with RV gastroenteritis had a significantly higher percentage of diarrhea (94 *vs.* 69%, $p < 0.001$), fever (82 *vs.* 26.2%, $p < 0.001$), and longer hospital stay (3.81 *vs.* 2.93 days, $p = 0.048$). Laboratory studies showed significantly higher liver enzymes and C-reactive protein levels in patients with RV infection. In contrast, white blood cell counts were significantly higher in patients with NV infection.

Conclusion: Norovirus is one of the leading agents of acute gastroenteritis in children in Taiwan, and genogroup II is the predominant type. [*J Chin Med Assoc* 2008;71(11):566–570]

Key Words: gastroenteritis, genogroup, norovirus, rotavirus

Introduction

Viral gastroenteritis is a common acute infectious disease in all age groups, but especially so in infants and young children. Among these viral agents, norovirus and rotavirus account for most sporadic gastroenteritis and outbreaks. Rotaviruses are the major known etiologic agents of severe diarrhea in infants and young children in most areas of the world.[1,2]

Noroviruses, previously known as Norwalk-like viruses, are one of the most common causes of epidemic viral gastroenteritis worldwide.[3–5] They have also been identified as common causes of sporadic nonbacterial gastroenteritis in individuals of all ages.[6–8] Transmission of noroviruses occurs year-round, but the incidence of disease is higher in the winter months in temperate climates. Incubation periods are generally 24–48 hours, and secondary attack rates are high. Infection is characterized by short-lived illness of 2–3 days' duration with vomiting as a prominent symptom. The major route of person-to-person transmission is fecal-oral, and vomitus is also implicated as a vehicle of transmission.

Noroviruses are an important cause of gastroenteritis in Taiwan, and genogroup II is dominant.[9] Little is known, however, about the incidence, clinical manifestations, and laboratory findings of norovirus in Taiwan. This study aimed to investigate the characteristics of norovirus gastroenteritis and to compare them with those of rotavirus infections.

Methods

Patients under 14 years of age presenting with acute gastroenteritis (defined as acute illness associated

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with ≥3 passages of liquid or semi-liquid stool or vomiting twice or more in the preceding 24 hours) who were admitted or visited the pediatric emergency department between January 2004 and March 2005 were enrolled in this study. Those who had underlying diseases associated with diarrhea or positive stool bacterial culture were excluded. Data on the clinical manifestations, hospital stay, age, gender, monthly distribution, and laboratory results (including white blood cell [WBC] count and differential count, C-reactive protein [CRP], serum electrolytes, liver enzymes [ALT, alanine aminotransferase; AST, aspartate aminotransferase], stool analysis, and stool culture) of norovirus and rotavirus gastroenteritis were analyzed.

Stool specimens were collected and stored at –20°C before analysis. Enzyme immunoassay (EIA) was used to test for norovirus (Dako, Ely, UK) and rotavirus (Meridian, Cincinnati, OH, USA). Genogroups of norovirus were determined by reverse-transcriptase polymerase chain reaction (RT-PCR). Viral RNA was extracted from 10% (wt/vol) stool suspensions with the QIAamp viral RNA extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNA was eluted with 60 μL diethyl pyrocarbonate-treated water and stored at –80°C for further RT-PCR assays.

The primer pairs used in this study and methodology were according to a previously published paper.[10] The primer set included G1SKF (5′-CTGCCCGA-ATTYGTAAATGA-3′) and G1SKR (5′-CCAACCC-ARCCATTRTACA-3′) for GI NLV, and G2SKF (5′-CNTGGGAGGGCGATCGCAA-3′) and G2SKR (5′-CCRCCNGCATRHCCRTTRTACAT-3′) for GII NLV. This primer set was used to amplify capsid N/S domain.

RT-PCR was carried out as described previously.[10] Briefly, reverse transcription was performed in 20 μL of the reaction mixture containing 75 pmole of random hexamers (pdN6) (Pharmacia Biotech, Piscataway, NJ, USA), 4 U of AMV-XL reverse transcriptase (Life Science, Rockville, MD, USA) and 2 μL of RNA. After incubation at 42°C for 4 hours, 80 μL of the reaction mixture containing 2.5 U Taq DNA polymerase and 40 pmole of both forward and reverse primers were added.

Conditions for the PCR reactions were as follows. After an initial denaturation step at 94°C for 3 minutes, 40 amplification cycles were performed. Each cycle consisted of denaturation at 94°C for 30 seconds, primer annealing at 50°C for 30 seconds and extension reaction at 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The PCR products were separated by electrophoresis in 2% agarose gel and visualized under UV lamp after ethidium bromide staining.

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA) for Windows. χ^2 analysis was used to compare categorical data, and independent samples *t* test and Mann-Whitney U test were used to compare mean values of continuous variables. A p value < 0.05 was considered statistically significant.

Results

A total of 201 patients with acute gastroenteritis were enrolled, including 112 males and 89 females. Stool specimens were collected from all patients. Children younger than 3 years of age were at highest risk of admission because of acute gastroenteritis, followed by children 4–6 years of age (113 *vs.* 57 patients, 56.2% *vs.* 28.3%, respectively).

Norovirus was detected in a total of 44 specimens (21.9%; 28 males, 16 females) by 1 or more tests. Of these positive specimens, 22 were detected by EIA (50%). Among these cases, 5 (11.3%) were genogroup I and 39 (88.7%) were genogroup II. Fifty-two specimens (25.9%; 25 males, 27 females) were positive for rotavirus on EIA test, and dual infection with both norovirus and rotavirus was found in 2 patients.

No sex predisposition was found, and the incidences of both norovirus and rotavirus gastroenteritis were highest in children younger than 3 years of age (Table 1). Norovirus was the most common viral agent identified in November and was prevalent during late fall and winter (November to February). Rotavirus infections peaked in April, with most cases occurring between February and May, following the peak of norovirus infections (Figure 1).

The clinical manifestations and hospital stay of patients with norovirus and rotavirus gastroenteritis are shown in Table 1. Vomiting and associated respiratory symptoms were more common in norovirus infection, but the difference was not significant. In contrast, fever and diarrhea developed more frequently in rotavirus gastroenteritis, and hospital stays were also longer. Laboratory tests were analyzed, and the results are listed in Table 2. CRP and liver enzymes were significantly higher in rotavirus infection, in contrast to WBC counts in peripheral blood, which were significantly higher in norovirus infection. There were no significant differences in rates of positive stool WBC and occult blood tests between norovirus and rotavirus gastroenteritis (4% *vs.* 3% and 45% *vs.* 37%, respectively).

Table 1. Clinical manifestations and duration of norovirus and rotavirus gastroenteritis

	Norovirus ($n=42$)	Rotavirus ($n=50$)	p
Age* (yr), *n* (%)			
≤3	22 (52.4)	35 (70.0)	0.083
4–6	11 (26.2)	11 (22.0)	0.639
≥7	9 (21.4)	4 (8.0)	0.066
Positive rate in age group*, %			
≤3 yr	19.5	30.9	0.046
4–6 yr	19.3	19.3	1.000
≥7 yr	29.0	12.9	0.119
Vomiting			
n (%)*	40 (95.2)	40 (80.0)	0.163
Mean no. of days (range)†	1.69 (0–5)	1.44 (0–4)	0.28
Diarrhea			
n (%)*	29 (69.0)	47 (94.0)	<0.001
Mean no. of days (range)†	2.14 (0–12)	3.66 (0–10)	<0.001
Fever			
n (%)*	11 (26.2)	41 (82.0)	<0.001
Mean no. of days (range)†	0.79 (0–6)	1.68 (0–5)	<0.001
Hospitalization			
n (%)*	40 (95.2)	48 (96.0)	0.285
Mean no. of days (range)‡	2.93 (1–7)	3.81 (1–10)	0.048
Respiratory symptoms*, *n* (%)	13 (30.9)	7 (14.0)	0.05

χ^2 test; †Mann-Whitney U test; ‡independent samples t test.

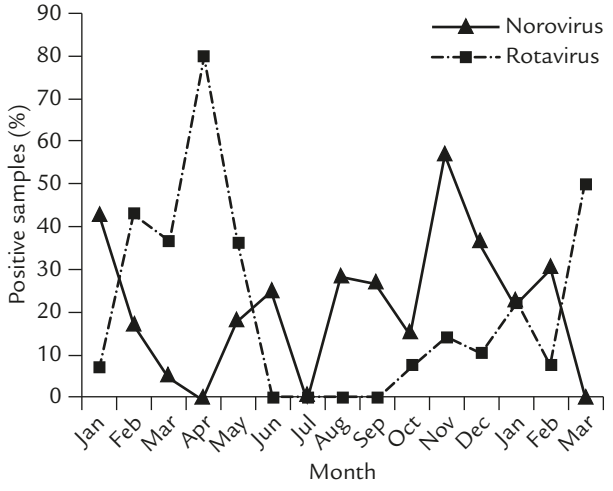

Figure 1. Temporal distribution of cases of childhood norovirus and rotavirus gastroenteritis.

Discussion

This study demonstrated that norovirus is a significant cause of childhood gastroenteritis in Taiwan, present in 10.9% of all specimens tested by EIA. This level is similar to that found in previous studies,[2,11–13] in which noroviruses were found in 6.5–18% of all cases of gastroenteritis. The incidence doubled, however, when EIA was used in combination with the more sensitive method of RT-PCR. Among acute gastroenteritis caused by norovirus, genogroup II is predominant worldwide.[5,7,10,14,15] In our study, it accounted for 88.7% of norovirus infections.

Rotavirus is the leading cause of acute gastroenteritis, and the incidence of rotavirus gastroenteritis was higher than for norovirus gastroenteritis in this study. Studies in Europe demonstrated that rotavirus was responsible for 31–80% of pediatric nosocomial diarrhea.[16,17]

Norovirus infection occurs year-round but with a peak in late fall and winter. In this study, the temporal peak distribution of norovirus infections (November to February) preceded that of rotavirus infections (February to May), which is consistent with the results of other studies.[14,15,18–20]

Rotavirus is the most common cause of acute diarrhea in infants and children less than 3 years old, whereas norovirus typically affects children and adults.[21] In this study, rotavirus and norovirus infections were most prevalent in children less than 3 years of age. Compared to Froggatt et al[14] who found the peak incidence of norovirus infection among children aged

Table 2. Laboratory findings of norovirus and rotavirus gastroenteritis

	Norovirus ($n = 42$)		Rotavirus ($n = 50$)		p
	Samples, n	Mean ± SD	Samples, n	Mean ± SD	
ALT* (U/L)	34	27.3 ± 23.5	44	32.3 ± 13.7	0.002
AST† (U/L)	25	32.9 ± 14.4	37	47.0 ± 17.9	0.002
CRP* (mg/dL)	37	0.7 ± 1.9	49	0.9 ± 1.1	0.026
WBC† (cells/μL)	41	14,107 ± 7,066	49	9,396 ± 3,343	< 0.001

**Mann-Whitney U test; †independent samples t test. ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein; WBC = white blood cell.*

1–2 years of age (13.2%), we found that the proportion of norovirus-positive cases was highest in children older than 7 years of age, with a positive rate of 29.0%.

Vomiting is more common than diarrhea in children with norovirus infection, whereas in adults, the opposite pattern is observed.[22] Delay in gastric emptying may be responsible for the nausea and vomiting associated with this agent.[23] Norovirus gastroenteritis is usually a mild illness and characteristically lasts about 24–48 hours in volunteer challenge studies.[24,25] In this study, fever and diarrhea presented in most patients with rotavirus gastroenteritis, and the clinical course was significantly longer than that of norovirus, as reflected by length of hospital stay.

Viremia after rotavirus infection was proposed to be associated with extraintestinal involvement.[26,27] In this study, liver enzymes were normal in most cases but were higher in rotavirus than in norovirus infection (reference values: ALT, 0–40 U/L; AST, 5–45 U/L). The average CRP level in both groups was mildly elevated and was higher in rotavirus infection (reference value, 0–0.5 mg/dL). In acute nonbacterial gastroenteritis, the stools are characteristically loose and watery and leukocytes are not typically present. In our study, leukocytes in stools were found in 3% and 4% of patients with rotavirus and norovirus gastroenteritis, respectively, with no significant difference. The high percentage of positive occult blood test in stools was contributed to by mucosal damage from acute diarrheal disease.

Several important pathogens of human viral gastroenteritis, including astroviruses, enteric adenoviruses and other human caliciviruses were not examined in this study. They may contribute to the high percentage of "other" viral gastroenteritis. More conservative primers might increase the detection rate of norovirus and can be used in the future.

In conclusion, both norovirus and rotavirus are common agents in nonbacterial gastroenteritis. This study clearly establishes the importance of norovirus as a cause of acute childhood gastroenteritis in Taiwan and genogroup II as the predominant type. Noroviruses were found to be prevalent in the winter season and rotaviruses in spring. The clinical manifestations and laboratory findings may also be helpful in differentiating norovirus from rotavirus infection.

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