Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Original Article

The Emerging Importance of Norovirus as the Etiology of Pediatric Gastroenteritis in Taipei

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BACKGROUND/PURPOSE: Rotavirus is a major causative agent of pediatric gastroenteritis throughout the world. However, other viruses such as norovirus also play an important role, but have seldom been studied in Taipei, Taiwan. The aim of this study was to survey the prevalence and clinical manifestations of different types of viral gastroenteritis in Taipei, focusing especially on the disease burden of norovirus.
METHODS: Between November 2004 and April 2005, stool samples were collected from hospitalized pediatric patients with a diagnosis of acute gastroenteritis. Clinical manifestations, laboratory data, and hospitalization course of the patients were all analyzed. Stool cultures for bacteria and rotavirus antigens were performed. All samples were tested for norovirus, enteric adenovirus, and astrovirus using enzyme-linked immunosorbent assays.
RESULTS: A total of 75 stool specimens were collected during the 6-month period. Fifteen (20.0%) were positive for norovirus (3 genogroup I and 12 genogroup II). Three (4.0%) were positive for enteric adenovirus, and one (1.3%) for astrovirus. Nine (12.0%) patients had positive rotavirus antigen tests. Bacterial pathogens were found in 12 patients (16.0%), including seven cases of *Salmonella*, and five of *Campylobacter*. The patients with norovirus gastroenteritis were aged between 1.5–7.5 years old (median 20 months old). Fever was found in six patients (40.0%), and bloody, mucoid stools in three (20.0%). The mean hospitalization time was 3.3 days. None of them had complications.
CONCLUSION: During the study period, norovirus was the most common pathogen causing hospitalized pediatric gastroenteritis in our hospital. Genogroup II was the predominant type (80.0%). Clinicians in Taipei should, therefore, be aware of the emergence of norovirus infections.

KEYWORDS: child, gastroenteritis, norovirus, Norwalk-like virus, rotavirus

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Article History:
Received: Feb 3, 2009
Revised: Mar 20, 2009
Accepted: Apr 30, 2009

Introduction

Rotavirus is regarded as the most prevalent causative virus in pediatric gastroenteritis throughout the world.[1] Several studies have revealed that rotavirus is also one of the leading pathogens in Taiwan.[2–4] Other viruses such as norovirus, adenovirus, and astrovirus also play important roles, but there are only a few related studies in Northern Taiwan.[3–6] Norovirus, also known as Norwalk-like virus, is classified in a separate genus of *Caliciviridae*, and three genogroups of norovirus have been identified in humans. The transmission of norovirus is by direct person-to-person contact, or through contaminated food or water. More and more studies have reported that the prevalence of norovirus is higher than expected.[4–8] Norovirus is associated with several large outbreaks, especially at respiratory care units and nursing homes.[8–10] The aim of this study was to survey the prevalence and clinical manifestations of the different types of viral gastroenteritis in Taipei between November 2004 and April 2005. We also tried to find out the genogroups of norovirus circulating around Taipei, and the specific clinical presentations associated with norovirus infection.

Methods

Study population

A prospective study was conducted at Mackay Memorial Hospital, which is a tertiary referral hospital in Taipei, Taiwan, to analyze the pathogens involved in pediatric gastroenteritis between November 2004 and April 2005. This study was approved by the Institutional Review Board (MMH-I-S-372) of Mackay Memorial Hospital.

Stool samples for viral studies were collected from hospitalized pediatric patients with a diagnosis of acute gastroenteritis (ICD-9-CM code: 558.9). The decision to admit to hospital was based on the physician's judgment. Most of the patients had severe diarrhea with dehydration. Patients with critical conditions, underlying bowel diseases, or those on antibiotic before admission, were excluded.

Two kinds of rotavirus vaccine, RotaTeq (Merck & Co., Whitehouse station, NJ, USA) and Rotarix (GlaxoSmithKine Biologicals, Rixensart, Belgium), were not available at Mackay Memorial Hospital during the study period, but some rotavirus vaccine trials were proceeding. Those patients who had received a trial rotavirus vaccine were also excluded from the study, as were specimens of inadequate quantity, and those with mixed infections.

Laboratory analysis

After admission, cultures for stool bacteria and enteric viruses were performed. For the bacteria cultures we used rectal swabs for stool sampling, and each stool sample was cultured using the streak plate method on culture media with Hektoen enteric, *Salmonella-Shigella* and *Campylobacter* agars. If no pathogen was isolated after 72 hours, the result was deemed negative. For the viral tests, specimens were collected and stored at –20°C before analysis. Stool rotavirus was tested for using specific commercial enzyme immunoassay kits (Premier™ Rotaclone kit; Meridian Diagnostics, Cincinnati, OH, USA).[11] All samples were tested in duplicate for norovirus, enteric adenovirus, and astrovirus using the IDEIA™ NLV EIA kit (Norwalk-like virus), code no. K6043 (DakoCytomation Ltd., Ely, United Kingdom).[12] Each sample was added to microwells, and incubated simultaneously with a norovirus specific genogroup I, or genogroup II, polyclonal antibody according to the manufacturer's instructions. The presence of specifically bound enzyme-labeled antibody in the wells resulted in a color change.

Clinical comparison

Clinical manifestations, laboratory data, and hospitalization courses were analyzed. Mucoid, or blood-tinged stools were recorded as positive in character. Complete blood counts, C-reactive protein (CRP) levels, and electrolytes were compared between the different pathogens. In addition, the age of admission was divided into four groups: < 6 months old; 6–24 months old; 25–60 months old; and > 60 months old. The prevalence of the different pathogens within each age group was then compared.

The clinical presentations of the most common pathogens, i.e. norovirus and rotavirus, were then subjected to detailed comparison.

Statistical analysis

To compare the clinical manifestations and laboratory tests for the different pathogens, we used SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). A χ^2 test was used for categorical data, and an independent-samples *t* test was used for continuous parameters. A p value < 0.05 was considered statistically significant.

Results

Two hundred pediatric patients were admitted with a diagnosis of gastroenteritis during the 6-month study period. A total of 75 stool specimens were collected according to our design, and the sampling fraction was 37.5%. Among the 75 patients enrolled, 43 (57.3%) were male and 32 (42.7%) were female. Twenty-eight patients (37.3%) had positive viral test results, and 12 (16.0%) had bacterial pathogens (Table 1). Three patients with mixed infections (norovirus plus *Salmonella*, norovirus plus *Campylobacter*, and rotavirus plus *Shigella* and *Campylobacter*) were excluded. Viruses were more common than bacteria, and norovirus was the leading pathogen (15 patients, 20.0%), followed by rotavirus (9 patients, 12.0%). Adenovirus and astrovirus were rare. Twelve norovirus cases were classified as genogroup II and the other three as genogroup I. Twelve patients (16.0%) had bacteria isolated from their stools. Seven (9.3%) contained *Salmonella* and five (6.7%) contained *Campylobacter*. No patients had positive blood culture results.

Most of the patients infected were under 2 years of age (22/39; 56.4%). There was no significant difference in the age distribution between patients with norovirus and rotavirus gastroenteritis (independent *t* test; $p=0.573$) (Table 2).

Generally, the patients with norovirus gastroenteritis had milder symptoms than those with other infections. When compared with rotavirus infections, the norovirus infected patients had a lower percentage of fever (6/15 *vs.* 9/9; $p=0.003$), vomiting (3/15 *vs.* 6/9; $p=0.022$), and shorter hospital stays (3.33 *vs.* 6.67 days; $p=0.006$). Lower leukocyte counts and CRP levels were also noted, but

Table 1. Pathogens of pediatric gastroenteritis patients ($n=75$)[a]

Pathogen	Number of patient
Virus	28 (37.3)
Norovirus	15 (20.0)
Rotavirus	9 (12.0)
Adenovirus	3 (4.0)
Astrovirus	1 (1.3)
Bacteria	12 (16.0)
Salmonella spp.	7 (9.3)
Campylobacter	5 (6.7)
Total	40 (53.3)

[a]Data presented as *n* (%).

Table 2. Age distribution of pediatric gastroenteritis patients with different pathogens[a]

Parameters	Norovirus ($n=15$)	Rotavirus ($n=9$)	Adenovirus ($n=3$)	Bacteria ($n=12$)	p[b]
Age (mo)	20 (1.5–90)	48 (2–84)	13 (12–20)	43 (9–96)	
Mean age (mo)	37.6±27.9	31.2±24.0	15.0±4.4	44.8±32.2	
Age distribution (mo)					
<6	1 (6.7)	1 (11.1)	–	–	
6–24	7 (46.7)	4 (44.4)	3 (100)	6 (50.0)	
25–60	4 (26.7)	3 (33.3)	–	2 (16.7)	
>60	3 (20.0)	1 (11.1)	–	4 (33.3)	
Fever	6 (40.0)	9 (100)	2 (66.7)	11 (91.7)	0.003
Vomiting	3 (20.0)	6 (66.7)	2 (66.7)	3 (25.0)	0.022
Bloody mucoid stool	3 (20.0)	3 (33.3)	1 (33.3)	6 (50.0)	0.465
Hospital stay (d)	3.33±1.50	6.67±3.87	5.67±2.08	5.58±2.07	0.006
WBC (10^9/L)	9.027±3.369	11.400±4.800	16.667±4.336	8.358±2.309	0.168
CRP (mg/dL)	3.43±2.99	5.12±7.96	3.21±3.84	4.09±3.77	0.464

[a]Data presented as median (range), mean±standard deviation, or *n* (%); [b]comparison between norovirus and rotavirus. WBC = White blood cell; CRP = C-reactive protein.

the differences were not statistically significant. There were no deaths or serious complications in this series.

Discussion

Rotavirus remains the most prevalent viral pathogen in Taiwan according to several studies.[2,3,5,13] Surprisingly, the prevalence of norovirus is greater than that of rotavirus in our study. Table 3 compares the prevalence of norovirus reported by various investigators in Taiwan, Thailand and Japan. Our findings are similar to levels reported in other studies such as those by Veeravigrom et al in Thailand (22.7%),[14] and Colomba et al in Italy (18.6%).[15] Chen et al reported the prevalence of norovirus to be as high as 29.3%, but half of the patients had mixed infections,[3] so the prevalence of an isolated norovirus infection was only 14.6%. We excluded mixed infections from our study. A survey in 1975 showed that 24% of Taiwanese children between 6–12 years old were positive for anti-Norwalk antibody.[16] The prevalence of norovirus has been shown to change over time, and in different areas.[5,10] Our study only represents the prevalence in pediatric hospitalized patients in the Taipei between late 2004 and early 2005. Nonetheless, it shows the important role of norovirus in pediatric gastroenteritis.

There are several genogroups of norovirus. Genogroups I and II are the most common in human gastroenteritis, although this may vary in different areas, years, seasons, and outbreaks.[17,18] In this study, genogroup II was the predominant type, as was reported in another study done in Northern Taiwan during a similar period to our study; that series had a similar norovirus prevanence of 21.9%.[4] However, another study performed one year earlier in Northern Taiwan showed the prevalence of norovirus to be low (11.7%), and genogroup I was the dominant type.[17] The predominant genogroup in Osaka, Japan, during five winters (1996–2000) was genogroup II.[18]

The commercial enzyme-linked immunoassay (EIA) kit used for norovirus detection in this study, IDEIA NLV, contains several strains: genogroup I (Desert Shield, Norwalk, Southampton, Chiba), and genogroup II (Camberwell, three different Toronto/Mexico strains, Snowy Mountain, two different Hawaiian strains, and three Japanese isolates). The sensitivity and specificity of our kit was 55.5% and 98.3%, respectively.[21] The diagnostic EIA method has

Table 3. Prevalence and predominant genogroup of norovirus infection

Study	Present study	Chen et al[5]	Wu et al[4]	Liu et al[17]	Chen et al[3]	Veeravigrom et al[14]	Iritani et al[18]
Country	Taiwan	Taiwan	Taiwan	Taiwan	Taiwan	Thailand	Japan
Study period	Nov 2004 to Apr 2005	Apr 2004 to Mar 2006	Jan 2004 to Mar 2005	Jan 2004 to Mar 2004	Oct 2003 to Sep 2004	Nov 2002 to Oct 2003	Apr 1996 to Mar 2000
Sample size	75	272	201	94	82	101	669
Prevalence (%)	20.0	8.2	21.9	11.7	14.6/29.3[a]	22.7	15.7
Predominant genogroup	Type II (80.0%)	–	Type II (88.7%)	Type I (81.8%)	–	–	Type II (88.7%)

[a]Prevalence of single norovirus infection was 14.6%, while prevalence of single norovirus and mixed infection was 29.3%.

promising specificity, but unsatisfactory sensitivity, which may result in a lower detection rate. Some new strains have been noted in other outbreaks of norovirus gastroenteritis but they are, as yet, undetectable by these commercial EIA kits. Electron microscopy and reverse transcription-polymerase chain reaction are regarded as the diagnostic gold standards, but their clinical applications are relatively limited. The EIA method is more suitable for preliminary screening.[22]

Generally speaking, the severity of clinical manifestations of norovirus infection is relatively milder than other infections,[4,23] as also shown in our data. The most common symptoms are fever, vomiting and diarrhea. Severe norovirus infections may occur in infants, elders, or immunocompromised patients. Benign seizures have also been noted in patients with norovirus infections.[24] No seizures, or other complications, developed in our study population.

Norovirus may be the major non-bacterial pathogen in outbreaks of gastroenteritis,[19,25] and both children and adults are susceptible,[9] and re-infection may occur.[26] Because the severity of the clinical symptoms is milder than for other bacterial- and rotavirus-mediated types of enteritis, subclinical infection is easily neglected. Therefore, outbreaks follow. Over the period of our study, there was no cluster of infection noted in our study population. The spread of norovirus is rapid, and the main transmission pathway is via contaminated food and person-to-person contact.[19] Early detection reminds us of the importance of decontamination measures, rather than the unnecessary use of antibiotics. In our study, we saw no evidence of a cluster of infections.

Both rotavirus and norovirus are common in cold seasons, and norovirus usually hits its peak earlier.[2,3,27] Our study covered the period between late fall, winter, and early spring, and rotavirus is considered to be the most prevalent virus in these seasons. Therefore, the higher prevalence of norovirus relative to rotavirus indicates a real disease burden of norovirus.

There are several limitations of our study. Our study was conducted in only one institution, and only sampled over a 6-month period. Since norovirus infection tends to be mild, and the serious gastroenteritis patients tends to be handled by the specialists within gastroenterology or infectious diseases, the high prevalence of norovirus infection may have been underestimated.

Rotavirus vaccines were not available in Taiwan during the study period. Since rotavirus vaccines are used in Taiwan now, the prevalence of norovirus gastroenteritis may increase in the future.[28]

In conclusion, norovirus was determined to be the most common viral pathogen in Taipei between winter 2004 and spring 2005, in children hospitalized with gastroenteritis. The emergence of norovirus infections in Taipei demands the awareness and attention of clinicians worldwide.

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