Molecular Epidemiology and Clinical Manifestations of Viral Gastroenteritis in Hospitalized Pediatric Patients in Northern Taiwan

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By reverse transcription-PCR or PCR, among 257 children with nonbacterial acute gastroenteritis (AGE), rotavirus, norovirus, astrovirus, enteric adenovirus, and multiple viruses were identified in 78 (30.4%), 21 (8.2%), 7 (2.7%), 51 (19.8%), and 53 (20.6%) patients, respectively. Higher disease severity was found for AGE caused by multiple viruses and by rotavirus alone. The majority of rotaviruses isolated from 2004 to 2006 belonged to genotypes G1 (20.4%), G2 (16.5%), G3 (27.2%), and G9 (21.4%).

Viral acute gastroenteritis (AGE) is one of the most common infectious diseases worldwide, causing significant morbidity and mortality in children (3). Four major viral pathogens associated with AGE are three RNA viruses (rotavirus, norovirus, and astrovirus) and one DNA virus (enteric adenovirus) (3, 9). The aim of this study was to use reverse transcription-PCR (RT-PCR) or PCR and a serological method to detect the major enteric viral pathogens that caused AGE in children hospitalized in Chang Gung Children's Hospital, Taiwan. We also genotyped the rotaviruses by DNA cloning and sequencing to understand the genotype distribution of rotavirus causing AGE in northern Taiwan.

Between April 2004 and March 2006, patients with AGE hospitalized in Chang Gung Children's Hospital with ages ranging from 3 months to 18 years and with manifestations of acute nonbloody diarrhea were enrolled. All stool samples were collected within 3 days of hospitalization. Medical records of these patients were reviewed retrospectively; the demographic data, detailed disease courses, findings in physical examinations, and results of laboratory testing were analyzed. Complications were defined as the occurrence of extraintestinal symptoms or laboratory data associated with AGE. The severity of AGE was evaluated as previously described (16). Fecal samples were sent to the clinical microbiology laboratory for bacterial culture of Salmonella, Shigella, and Campylobacter. Patients with positive bacterial cultures were excluded from the study. All the samples were stored at −70°C before extraction of viral nucleic acid. Extraction of viral nucleic acid was carried out with a kit (High Pure viral nucleic acid kit; Roche Diagnostics GmbH, Mannheim, Germany).

The PCR primer sets used for the detection of rotavirus, norovirus, astrovirus, and enteric adenovirus were described previously (1, 10, 12). The RT-PCR for RNA viruses and the PCR for DNA virus were performed as described earlier (1, 10, 12). The PCR products were purified, and the DNA sequences were determined by DNA autosequencer ABI 377. The sequences obtained were aligned and compared to other sequences available in the GenBank/EMBL database. Simultaneously, we used a commercial enzyme-linked immunosorbent assay (ELISA) kit for the detection of rotavirus in the fecal specimens (R-Biopharm; Darmstadt, Germany). Continuous variables were analyzed by the Student t test and dichotomous variables by the χ2 test. A P value of <0.05 was considered statistically significant. All the tests were analyzed using SAS system software version 8 for Windows.

From April 2004 to March 2006, fecal specimens from 272 patients were collected, with a sampling fraction of 6.3% (272 of 4,352) among total AGE patients. Fifteen were excluded by their positive results from stool culture for bacteria. A total of 257 viral AGE patients (145 boys and 112 girls, with a median age of 21 months [interquartile range, 12 to 36 months]) were included. Most were under 5 years old (226 children [87.9%]). Among the 257 with nonbacterial AGE, we identified enteric viruses in 210 (81.7%) fecal specimens by the method of RT-PCR or PCR. These included rotavirus (78 [30.4%]), norovirus (21 [8.2%]), astrovirus (7 [2.7%]), and enteric adenovirus (51 [19.8%]) infections and mixed infection (53 [20.6%]). Overall, RT-PCR identified 123 samples positive for rotavirus (78 single rotavirus infections and 45 mixed infections). ELISA identified 76 (29.6%) fecal samples positive for rotavirus. The seasonal distribution of viral AGE is...
shown in Fig. 1. The incidence of AGE caused by rotavirus peaked between January and March and even encompassed April and May. That by enteric adenoviruses surged from October to December and rapidly declined in early spring. Norovirus infection occurred more commonly in winter through early spring, and astrovirus infection showed no obvious seasonal predilection. In the 53 patients with mixed infections, 48 (90.6%) were coinfected by two viruses. Triple viral infection was found for five patients.

The clinical manifestations and severity evaluation of viral AGE in pediatric patients are shown in Table 1. We found that the total disease severity score was highest for AGE caused by multiple viruses, followed by rotavirus infection, while it was lowest for norovirus infection. Statistically significant differences in the symptoms between infections caused by rotavirus and those caused by other viruses were found in terms of the frequency of vomiting, duration of vomiting, and frequency of diarrhea, along with the fever severity score and the summarized disease severity score; this was also the case when mixed infections involving rotavirus were compared to infections caused by other viruses (all \( P < 0.05 \)). Complications occurred in 78 (37.1%) of the 210 patients in this study. The most common complication was electrolyte imbalance (hyponatremia, hypokalemia, or hypochloremia) (40 [19.0%]), followed by hypoglycemia (<80 mg/dl) (36 [17.1%]).

The following rotavirus genotypes were identified for 103 samples (numbers [percentages] are indicated in parentheses): G1 (21 [20.4%]), G2 (17 [16.5%]), G3 (28 [27.2%]), G4 (1 [1%]), G9 (22 [21.4%]), and mixed types (5 [4.9%]). Mixed types were identified in five cases, including three of G2 plus G9, one of G2 plus G3, and one of G3 plus G9. The other nine (8.7%) were nontypeable. The seasonal distribution of differ-
ent genotypes is shown in Fig. 1, and G3 showed the most typical seasonal prevalence. This study showed that RT-PCR and PCR identified the viral etiology for 81.7% of hospitalized children with AGE. As in most cases, our study demonstrated that rotavirus is the leading cause of viral gastroenteritis, and in our study the infection usually occurred in young children of less than 5 years of age, with a median age of 24 months (interquartile range, 9 to 35 months) (7). This study, in accordance with previous reports, showed a detection rate for rotavirus in fecal specimens by RT-PCR higher than that by ELISA (5). The seasonal distribution of rotavirus infection, i.e., a rapid increase in winter and a peak in spring, was also found in this study (4). The detailed evaluation of disease severity by major parameters in this study indicated that either rotavirus alone or mixed infection including rotavirus caused significantly more vomiting and diarrhea, higher body temperature, and a higher disease severity score than other enteric viruses. The clear evidence of severe AGE caused by rotavirus strongly suggests that an adequate prevention and control of rotavirus infection cannot be overemphasized. On the other hand, the prevalence of enteric adenovirus and mixed viral infections found in Taiwan was higher than that found by previous reports from other areas of the world (9, 11).

Norovirus had been identified as an etiological agent for AGE in humans of all ages, and the illness is generally mild and self-limited (6). We have found a similar spectrum of illnesses and seasonal distribution (more common in the colder season) associated with norovirus infection (15). Astrovirus is the least common cause of AGE in this study, and it showed an epidemiological picture similar to what was found with a previous study (8). The prevalence of mixed viral infection is another important finding from this study, and we have shown a prevalence significantly higher (20.6%) than that found by other non-hospital-based studies (2, 11).

In this study, we identified the four most prevalent genotypes of rotaviruses as G1, G2, G3, and G9. G1 strains were predominant by 2000 in Taiwan, except for 1992 and 1993, when G2 strains were more prevalent (14). In 2001 and 2002, G1 was the most common (51%), followed by G9 (31%) (13). Although G1 was not the most common genotype in this study, it still took an important position; on the other hand, G9 was again recognized as one of the major genotypes to cause AGE.

In summary, the disease burden and severity of AGE in children differ from virus to virus. By molecular methods, a better etiological identification and genotype analysis can be achieved. These methods are a useful tool for a more comprehensive investigation into the clinical manifestations and disease burden and severity associated with each virus.

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