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

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Running title: Viral gastroenteritis in pediatric patients

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By reverse transcription-PCR or PCR, among 257 children with acute non-bacterial 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 in AGE caused by multiple viruses and by rotavirus alone. Majority of rotaviruses in 2004-2006 belonged to 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 the three RNA viruses (rotavirus, norovirus, and astrovirus) and one DNA virus (enteric adenovirus) (3, 9). The aim of this study was to use 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 (CGCH), Taiwan. We also genotyped the rotaviruses by DNA cloning and sequencing to understand the genotype distribution of rotavirus causing AGE in northern Taiwan.

During April 2004 to March 2006, hospitalized patients with AGE in CGCH aged from 3 months to 18 years with the manifestation of acute non-bloody 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 the 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 detection of rotavirus, norovirus, astrovirus, and enteric adenovirus were described previously (1, 10, 12). The RT-PCR reaction for RNA viruses and 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. Simultaneously we used a commercial ELISA kit for detection of rotavirus in the fecal specimens (R-Biopharm, Darmstadt, Germany). Continuous variables were analyzed by the Student’s $t$ test, and dichotomous variables by the $\chi^2$ test. $P < 0.05$ 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 of 272 patients were collected with a sampling fraction of 6.3% (272 of 4352) among total AGE patients. Fifteen were excluded by their positive results of 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, 12-36 months]) were included. Most were under five years old (226 children, 87.9%). Among the 257 with non-bacterial AGE, we identified enteric viruses in 210
(81.7%) fecal specimens by the method of RT-PCR or PCR. This included rotavirus, 78 (30.4%); norovirus, 21 (8.2%); astrovirus, 7 (2.7%); enteric adenovirus, 51 (19.8%); and mixed infection, 53 (20.6%). Overall, RT-PCR identified 123 samples positive for rotavirus (78 single rotaviral infection and 45 mixed infection). ELISA test 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 in January-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 the early spring and astrovirus infection showed no obvious seasonal predilection. In the 53 patients with mixed infection, 48 (90.6%) were co-infected by 2 viruses. Triple viral infection was found in 5 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 in AGE caused by multiple viruses, followed by rotavirus infection, while it was lowest in the norovirus infection. Statistically significant differences in the symptoms between infections caused by rotavirus or other viruses were found in terms of the frequency of vomiting, duration of vomiting, frequency of diarrhea, along with fever severity score, and the summarized disease severity score, and it is also the
case when comparing mixed infections involving rotavirus 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 in 103 samples: 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 5 cases, including 3 G2 plus G9, 1 G2 plus G3, and 1 G3 plus G9. The other 9 (8.7%) were non-typable. The seasonal distribution of different genotypes is shown in Figure 1 and G3 showed the most typical seasonal prevalence.

This study showed that RT-PCR and PCR identified the viral etiology in 81.7% of hospitalized children with AGE. As in most cases, our study demonstrated that rotavirus is the leading cause of viral gastroenteritis and the infection usually occurred in young children less than 5 years of age with a median age of 24 months (interquartile range, 9-35 months) (7). This study, in accordance with previous reports, showed a higher detection rate of rotavirus in fecal specimens by RT-PCR than by the ELISA assay (5). The seasonal distribution of rotavirus infection, i.e., a rapid increase in winter and 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 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 over emphasized.

On the other hand, a higher prevalence of enteric adenovirus and mixed viral infections were found in Taiwan, compared with 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 illness 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, which showed a similar epidemiological picture compared with previous series (8). Mixed viral infection is another important finding in this study with a significantly higher prevalence (20.6%), compared to other non-hospital-based studies (2, 11).

In this study, we identified the most prevalent four genotypes of rotaviruses as G1, G2, G3 and G9. G1 strains had been the predominant by 2000 in Taiwan, except in 1992-1993, when G2 strains were more prevalent (14). From 2001 to 2002, G1 was
the most common (51%), followed by G9 (31%) (13). Although G1 was not the most common genotype, 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 are different 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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REFERENCES


TABLE 1. Etiology and clinical manifestations of 257 pediatric patients with acute gastroenteritis

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Rotavirus</th>
<th>Norovirus</th>
<th>Astrovirus</th>
<th>Adenovirus</th>
<th>Mixed infections</th>
<th>Undetermined etiology</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n=78)</td>
<td>(n=21)</td>
<td>(n=7)</td>
<td>(n=51)</td>
<td>(n=53)</td>
<td>(n=47)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>24 (9-35)</td>
<td>24 (10.5-48)</td>
<td>21 (12-33)</td>
<td>14 (12-24)</td>
<td>24 (12-36.25)</td>
<td>24 (9-38.75)</td>
</tr>
<tr>
<td>Frequency of vomiting (times/day)</td>
<td>3 (2-5)</td>
<td>2 (1-3)</td>
<td>0 (0-1)</td>
<td>2 (1-3)</td>
<td>3 (2-3.25)</td>
<td>2 (0-3)</td>
</tr>
<tr>
<td>Duration of vomiting (days)</td>
<td>2 (2-3)</td>
<td>2 (1-2)</td>
<td>0 (0-1.75)</td>
<td>2 (0-2)</td>
<td>3 (2-4)</td>
<td>2 (0-3)</td>
</tr>
<tr>
<td>Frequency of diarrhea (times/day)</td>
<td>4 (3-8)</td>
<td>3 (2.75-5.25)</td>
<td>4 (3.25-7.25)</td>
<td>4 (3-5)</td>
<td>4 (3-6)</td>
<td>3 (2-6)</td>
</tr>
<tr>
<td>Duration of diarrhea (days)</td>
<td>4 (3-5)</td>
<td>3 (2-6.5)</td>
<td>6 (3-6)</td>
<td>4 (3-6)</td>
<td>4 (3-6)</td>
<td>4 (2-6)</td>
</tr>
<tr>
<td>Fever severity score</td>
<td>1 (1-2)</td>
<td>0 (0-1)</td>
<td>1 (0.25-2.75)</td>
<td>1 (0-1)</td>
<td>1 (0-2)</td>
<td>1 (0-2)</td>
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<tr>
<td>Disease severity score</td>
<td>11 (9-14)</td>
<td>8 (6-10)</td>
<td>10 (7.25-12.75)</td>
<td>11 (9-12)</td>
<td>12 (10-14)</td>
<td>10 (8-12)</td>
</tr>
<tr>
<td>Complications (n)</td>
<td>28</td>
<td>7</td>
<td>2</td>
<td>18</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Leukocyte count &gt; 10,000/mm³ (n)</td>
<td>30</td>
<td>8</td>
<td>3</td>
<td>25</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>CRP^b (mg/l)</td>
<td>4.5 (1-22)</td>
<td>2 (0-6.5)</td>
<td>2 (0.5-22.5)</td>
<td>6 (2-17.75)</td>
<td>2 (1-18)</td>
<td>27 (2-60.25)</td>
</tr>
<tr>
<td>Hospitalization days</td>
<td>5 (4-6)</td>
<td>6 (4-8)</td>
<td>4 (3.25-7.5)</td>
<td>5 (4-7)</td>
<td>5 (4-6.25)</td>
<td>5 (4-7)</td>
</tr>
</tbody>
</table>

^aMedian values and 25% (Q1) and 75% (Q3) quartiles are given as median (Q1-Q3).

^bCRP, C-reactive protein.
Legends

FIG. 1. Seasonal distribution of viral gastroenteritis in pediatric patients hospitalized in Chang Gung Children’s Hospital, Taiwan, 2004 to 2006 (A), and seasonal distribution of different genotypes of rotavirus in northern Taiwan, 2004 to 2006 (B).