Human Torovirus: A New Nosocomial Gastrointestinal Pathogen

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Studies were undertaken to determine if human torovirus is associated with gastroenteritis and to examine the clinical features of torovirus illness in children. The fecal excretion of torovirus in patients with gastroenteritis was compared with that in matched asymptomatic controls in a case-control study. Toroviruses were identified in 72 (35.0%) of 206 gastroenteritis cases compared with 30 (14.5%) of 206 controls (P < .001). Clinical features of torovirus gastroenteritis in 172 patients positive for torovirus were compared with those of 115 patients infected with rotavirus or astrovirus. Persons infected with torovirus were more frequently immunocompromised (43.0% vs. 15.7%) and nosocomially infected (57.6% vs. 31.3%). They also experienced less vomiting (46.4% vs. 66.7%) but had more bloody diarrhea (11.2% vs. 1.8%). An antibody response to torovirus developed mainly in older, nonimmunocompromised children (P < .01). These studies demonstrate an association between torovirus excretion and gastroenteritis in the pediatric population among immunocompromised hospitalized patients and in previously healthy patients.

Acute viral gastroenteritis in family studies was second in prevalence only to the common cold and accounted for 16% of illnesses or 1.52 cases per person per year [1]. Despite advances in management that have reduced mortality, viral gastroenteritis continues to be a major cause of hospitalization, resulting in admissions of more than 200,000 children and almost 900,000 in-patient days per year in the United States [2]. In our pediatric setting, nosocomial diarrhea occurs in up to 10.3% of room contacts [3]. Viral agents known to cause gastroenteritis include rotaviruses, astroviruses, calciviruses, and enteric adenoviruses [4–6]. However, the etiologic agent can remain undiagnosed in over half the cases of suspected infectious gastroenteritis despite advances in diagnostic technology [7].

Studies on animal gastroenteritis viruses have provided valuable insights into the discovery of new human pathogens, such as rotavirus [8]. Among the established agents of gastroenteritis in animals are the toroviruses, which include the Breda virus of cattle and Berne virus of horses [9–11]. These enveloped RNA viruses measure 100–140 nm at their largest diameter and contain a tightly coiled tubular nucleocapsid that generally assumes a donut or torus shape in the virion [9]. The particles have a fringe of peplomers on their surface that are ~10 nm long. Toroviruses were classified on the basis of the Berne virus genome sequence as members of the family Coronaviridae, which together with the family Arteriviridae are now classified in the order Nidovirales [12–14].

Torovirus-like particles were first documented in 1984 by electron microscopy of fecal specimens of persons with gastroenteritis [15]. Evidence that these toroviruses came from their immunospecific cross-reactions with the Breda virus and the ability of nucleic acid probes based on the Berne virus sequence to hybridize with RNA extracted from stool specimens positive for toroviruses by electron microscopy [16, 17]. Studies of these viruses were limited because they cannot be grown in cell culture, they lack the distinct icosahedral structure of other gastroenteritis viruses, and their peplomers are less pronounced than those of coronaviruses [16].

In our diagnostic electron microscopy laboratory, pleomorphic fringed particles that resembled particles previously designated as toroviruses were observed in a substantial number of fecal specimens from children with gastroenteritis. These were shown to be toroviruses because of a high concordance between the identification of torovirus-like particles by electron microscopy and a positive enzyme immunoassay with Breda virus reference antisera [18]. Torovirus-like particles purified from stool specimens were further shown to be toroviruses on the basis of their morphology, immunospecific interactions with Breda virus antisera, ability to elicit an immune response following infection, and the high degree of homology of the 3’ end of their genome with that of the Berne and Breda viruses [19]. These data support the hypothesis that these toroviruses are infectious agents, but better evidence is needed to establish that toroviruses actually cause gastroenteritis. We therefore conducted two epidemiologic studies to determine the role of these virus particles in gastroenteritis. The first study was a

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The investigation was approved by the Hospital for Sick Children Research Ethics Board. Informed consent was obtained from patients or their parents or guardians.

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case-control comparison of torovirus shedding in patients with gastroenteritis and in asymptomatic controls. The second compared the clinical features of symptomatic patients shedding torovirus and those shedding rotavirus or astrovirus.

Methods

Patients

The studies were conducted between 1 October 1993 and 30 September 1995 in patients admitted to the Hospital for Sick Children, a 410-bed primary, secondary, and tertiary care center. In this setting, fecal specimens are submitted for diagnostic virology for all patients admitted with symptoms of gastroenteritis or who develop these symptoms while hospitalized. Specimens from ~1500 patients are examined each year. In the first study, the frequency of torovirus identification in patients with gastrointestinal symptoms (cases) was compared with that of asymptomatic controls. Once this was accomplished, a second study was undertaken to establish a more complete microbiologic diagnosis, examine the serologic response, and describe the progress of the infection.

Study 1

Stool specimens from patients with gastroenteritis were examined for viruses by negative contrast electron microscopy [19, 20]. Case-patients were selected from among this symptomatic group using a table of random numbers. For each case, a control patient was selected and matched by ward and date of admission within 1 week of the case. These matching criteria were used to ensure that the two populations were comparable with respect to underlying illness and season and duration of hospitalization. Controls were not symptomatic for gastroenteritis and had experienced no changes in stool frequency or consistency on the day of selection. Patients were excluded from enrollment if they had an underlying gastro-

Table 1. Characteristics of case-control study patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diarrhea patients (n = 206)</th>
<th>Asymptomatic controls (n = 206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean, range)</td>
<td>3.3 (0.02–18.2)</td>
<td>3.2 (0.01–17.6)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>133</td>
<td>122</td>
</tr>
<tr>
<td>Female</td>
<td>73</td>
<td>84</td>
</tr>
<tr>
<td>% immunocompromised (no./total)</td>
<td>19.9 (41/206)</td>
<td>16.0 (33/206)</td>
</tr>
<tr>
<td>% torovirus-positive (no./total)</td>
<td>35.0 (72/206)</td>
<td>14.5 (30/206)</td>
</tr>
</tbody>
</table>

* P<.001, χ² test.
Table 2. Torovirus-positive stool specimens in immunocompromised and nonimmunocompromised patients (study 1).

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 206)</td>
<td>(n = 206)</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>19/41 (46.3)%</td>
<td>3/33 (9.0)%</td>
</tr>
<tr>
<td>Nonimmunocompromised</td>
<td>53/165 (32.1)%</td>
<td>27/173 (15.6)%</td>
</tr>
</tbody>
</table>

NOTE. Data are no./total (%).

* P < .005, χ² test.

* P < .001, χ² test.

intestinal disorder (e.g., Crohn’s disease). Stool specimens from controls were collected within 72 h of enrollment of the case specimen and examined for viruses by electron microscopy; the electron microscopist was blinded to patient clinical status. All stool specimens were collected in a standard stool specimen container (25 mL) and processed within 48 h. Control patients were reassessed on day 4 after enrollment to determine if they developed gastrointestinal symptoms subsequent to enrollment. Case patients were excluded from the analysis when no matching control was available or when a stool specimen was not obtained from the matched asymptomatic control.

Study 2

At a different time from that of study 1, patients whose stool specimens were positive for torovirus, rotavirus, or astrovirus by electron microscopy were enrolled in study 2. Information on clinical presentation and course throughout hospitalization was collected from patients or parents by questionnaire. After discharge, each patient was followed with a visit from the study nurse. In torovirus-positive patients, stool specimens were examined daily by electron microscopy during the hospitalization and at follow-up. Acute sera from these patients were collected at the time of diagnosis, and convalescent sera were obtained 2–6 weeks after enrollment.

Definitions

Diarrhea was defined as ≥3 loose stools in a 24-h period. Nosocomial infection was defined as onset of gastrointestinal symptoms on or after day 3 of admission [3]. Immunosuppressed patients included those with the following conditions: severe combined immunodeficiency, leukemia, other malignancies treated by chemotherapy, solid organ or bone marrow transplant recipients, human immunodeficiency virus infection or AIDS, and those receiving large doses of immunosuppressive therapy for any cause. Seroconversion was defined as a rise in antibody titer to torovirus of ≥4-fold by hemagglutination-inhibition (HAI) assay.

Laboratory Tests

Stool specimens. These were prepared for diagnostic electron microscopy as described previously [19, 20]. In brief, a 10%–20% suspension of specimen was prepared in a 1% solution of ammonium acetate, applied to a polyvinyl carbon–coated 400-mesh electron microscope grid, and stained with a 2% solution of sodium phosphotungstate at pH 7.0. The specimen was examined under an electron microscope (Phillips EM300) at ×50,000 magnification. Specimens were considered positive for torovirus if they contained spherical or kidney-shaped particles (100–140 nm in diameter) with a characteristic fringe of peplomers ~10 nm long as shown in figure 1 [19]. Other viral pathogens, including rotaviruses, adenoviruses, astroviruses, and Norwalk virus-like agents, were identified by their morphologic features [20, 21]. All specimens were examined by an experienced electron microscopist blinded to specimen source. For patients participating in study 2, stool samples were also submitted for bacterial isolation [22] and tested for Escherichia coli O157:H7 [23], examined for ova and parasites [24], and tested for Clostridium difficile cytotoxin [25].

Serology. Antibody to torovirus was quantitated in acute and convalescent sera by HAI assay as described previously [19] by use of a purified torovirus preparation from a single patient. The test was done without knowledge of the clinical and demographic features of the cases. The HAI assay used a 0.5% suspension of rabbit erythrocytes and 4 hemagglutination units of virus per well. In the analysis of the results of these tests, age and immune status (immunocompromised vs. immunocompetent) were included in a logistic regression model to predict for seroconversion.

Statistical analysis. We used McNemar’s χ² test to compare concordance of matched pairs of gastroenteritis patients and asymptomatic controls described in study 1. Relative odds were calculated by dividing the number of cases with torovirus times the number of controls without torovirus by the number of controls with torovirus times the number of cases without torovirus. Unpaired Student’s t tests for continuous measures and χ² analysis or Fisher’s exact tests were used to compare clinical findings between patients positive for torovirus and patients positive for astrovirus or rotavirus. Descriptive statistics were used to express duration of symptoms and shedding of virus. Geometric means and 95% confidence intervals were used to describe serologic responses [26].

Results

Study 1: torovirus excretion in gastroenteritis patients and asymptomatic controls. Two hundred and six case-control
Table 3. Demographic features of torovirus and rotavirus/astrovirus cases (study 2).

<table>
<thead>
<tr>
<th>Demographic feature</th>
<th>Torovirus cases (n = 172)</th>
<th>Rotavirus/astrovirus cases (n = 115)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.0</td>
<td>2.0</td>
<td>.006</td>
</tr>
<tr>
<td>Range</td>
<td>0.02–18.6</td>
<td>0.05–16.5</td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>91</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>81</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>102</td>
<td>76</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>70</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>% nosocomial infections</td>
<td>57.6 (99/172)</td>
<td>31.3 (36/115)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean days in hospital before illness</td>
<td>19.4</td>
<td>10.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% immunocompetent (no./total)</td>
<td>43.0 (74/172)</td>
<td>15.7 (18/115)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Data are % (no./total), except low duration. NS = not significant.

pairs were included in this analysis. Of the initial cases selected, 176 were excluded for one or more of the following reasons: underlying gastrointestinal disorder (5), lack of a control patient hospitalized at a comparable time (106), or lack of a stool specimen from the control patient (65).

Demographic characteristics were similar for cases and controls as summarized in table 1. Among the 206 cases, a virus was identified in 88: torovirus, 72; rotavirus, 8; and other viruses, including astrovirus, Norwalk-like viruses, and adenovirus, 8. In contrast, a virus was detected in 33 of the 206 matched asymptomatic patients: 30 had torovirus, 1 had rotavirus, and 2 had other viruses. Significantly more cases (35.0%) than controls (14.5%) were positive for torovirus (P < .001). Since a substantial number of the patients were immunocompromised, cases and controls of this subset were independently analyzed for excretion of torovirus (table 2). In this subset, significantly more cases than controls were again positive for torovirus. This was also true among the immunocompetent patients. When controls were reassessed, 23 controls had developed gastroenteritis within 4 days of study enrollment. Of these, 10 were positive for torovirus. When these 10 controls were excluded, only 20 (10.2%) of 196 controls were positive for torovirus.

This study was designed to determine whether there was an association between torovirus excretion and gastroenteritis. Accordingly, the patient and control specimens were examined only for viruses with the understanding that other microbial pathogens would be tested for in study 2. To obtain an insight as to whether the asymptomatic patients may have had bacterial infections not diagnosed in study 1, a retrospective analysis was performed on a subset of asymptomatic patients whose stool specimens were submitted for bacteriologic testing as part of the routine investigation outside the study protocol. This analysis revealed no bacterial pathogens in stool specimens from 28 cases for whom bacteriologic testing was done.

Study 2: clinical features of torovirus-positive gastroenteritis patients. Between 1 October 1993 and 30 September 1995, clinical features were analyzed for 172 torovirus-, 102 rotavirus-, and 13 astrovirus-infected gastroenteritis patients. The monthly frequency distribution of viruses identified by electron microscopy in 1993 is shown in figure 2. While rotavirus infections were most frequent during the winter and spring, the incidence of torovirus infections remained relatively constant throughout the year. As shown in table 3, torovirus-positive patients were older, developed nosocomial infections more often, and were more frequently immunocompromised than were patients infected with rotavirus or astrovirus.

The clinical manifestations of torovirus were similar to those of rotavirus or astrovirus except that children infected with torovirus had less vomiting and more bloody diarrhea (table 4). Of the 19 torovirus-positive patients presenting with bloody diarrhea, 9 were immunocompetent, and 1 had a concomitant C. jejuni infection. Nine additional torovirus-positive patients developed bloody diarrhea within 2–15 days after study enrollment and had no other pathogen in the stool sample. Among those with rotavirus or astrovirus, only 2 presented with bloody diarrhea; another 6 developed bloody diarrhea after enrollment, 2 of whom were positive for C. jejuni.

Bacterial pathogens were detected in stool specimens from both groups of patients. Among those infected with torovirus, Yersinia enterocolitica was isolated from 1 and C. jejuni from 2. C. difficile cytotoxin was detected in 7 patients in a subset of 81 tested in the rotavirus-astrovirus group and in 8 of a subset of 128 torovirus-positive patients. One torovirus-positive patient (of 64 tested) had Giardia lamblia cysts, and 1 rotavirus-positive patient (of 61 tested) had Dientamoeba fragilis present in stool specimens.

The average duration of symptoms was similar, although rotavirus-positive patients required parenteral hydration for a longer period (6.4 vs. 4.8 days). Stool specimens collected on a follow-up visit 2–6 months after study enrollment from 168 patients infected with torovirus showed that 14 were shedding the virus.

When immunocompromised patients were compared with previously healthy torovirus-positive patients, they were significantly older and more likely to have acquired the infection in the hospital (78% vs. 42%) (table 5). Three immunocompr-
immunocompetent patients. The geometric mean titers of torovirus antibodies in acute and convalescent sera were also significantly lower for immunocompromised patients (17.61) than for immunocompetent patients (33.97).

Discussion

These studies provide strong evidence for a causative role for torovirus in gastroenteritis. First, the relative odds of torovirus among gastroenteritis cases was 3.1-fold that for controls. However, if the 10 control patients who became symptomatic within 4 days of sampling are excluded, the relative odds become 4.7, which approaches those described with enteric adenovirus and astrovirus [4, 27]. Secondly, over half of the torovirus-positive patients were the lower frequency of vomiting and high frequency of nosocomial diarrhea among such patients [35]. Among symptomatic controls, torovirus was detected at a lower rate in immunocompromised patients than in immunocompetent patients (9.0% vs. 15.6%; table 2), whereas among children with gastroenteritis, it was more common among immunocompromised than in immunocompetent patients. This further suggests that the recovery of torovirus by the immunocompromised is related to the illness rather than to a proclivity of immunocompromised patients to carry this organism. Furthermore, this virus was a common cause of diarrhea in hospitalized patients who were previously healthy. An outbreak of gastrointestinal illness in a neonatal intensive care unit due to particles similar to toroviruses (coronavirus-like particles) has been described [36].

Two clinical features that distinguish torovirus-infected patients are the lower frequency of vomiting and high frequency of convalescent sera. In paired sera from 88 patients, 51 demonstrated seroconversion with a ≥4-fold increase in antibody titer (table 6). Of the 44 patients ≤2 years old, half (22) experienced seroconversion, whereas of the 44 patients ≥2 years old, 29 (66%) seroconverted. Of the 30 immunocompromised patients, 11 seroconverted, and of the latter 11, 10 (91%) were older than 2 years. Of the 58 immunocompromised patients, 40 experienced seroconversion, whereas of the 44 patients infected by rotavirus or astrovirus, established gastroenteritis viruses. The association of torovirus with acute and persistent diarrhea was recently reported in a smaller cohort of children in an urban Brazilian slum [30]. In that study, 33 children had acute diarrhea and 41 had persistent diarrhea. There were 17 controls.

Torovirus appears to be a common agent of gastroenteritis at our institution. Particles resembling toroviruses have been reported in stools of gastroenteritis patients, although in some cases they were designated “coronavirus-like particles” [15, 31–33]. During a 2-year period at our hospital, 20% of stool samples from gastroenteritis patients were positive for torovirus, more than double those positive for rotavirus (7%) and for astrovirus and Norwalk-like viruses (2%). Toroviruses were present all year and had seasonal distribution similar to that described for corona-like viruses [33]. These findings compare favorably with our recent report on the role of bovine torovirus in diarrhea of calves [34]. In that study, toroviruses were the predominant viral agent associated with diarrhea.

Table 5. Comparison of immunocompromised and nonimmunocompromised torovirus cases.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Nonimmunocompromised (n = 98)</th>
<th>Immunocompromised (n = 74)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>72</td>
<td>19</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>≥2</td>
<td>26</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Gender (no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>% nosocomial infections (no./total)</td>
<td>4.18 (41/98)</td>
<td>78.3 (58/74)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% shedding torovirus in stool ≥8 days</td>
<td>43.9 (18/41)</td>
<td>62.5 (35/56)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. NS = not significant.

Table 6. Geometric mean titers of torovirus antibodies in acute and convalescent sera and occurrence of seroconversion in immunocompromised and nonimmunocompromised patients (study 2).

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Seroconversion</th>
<th>Titer (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acute</td>
</tr>
<tr>
<td>Immunocompromised (n = 30)</td>
<td>11</td>
<td>6.5 (5.66–7.46)</td>
</tr>
<tr>
<td>Nonimmunocompromised (n = 58)</td>
<td>40</td>
<td>7.62 (6.48–7.60)</td>
</tr>
</tbody>
</table>

* P<.01.
of bloody diarrhea. Descriptions of illness in patients with enteric infection by coronavirus-like particles have indicated that diarrhea is the most prominent symptom, and patients often experienced frank or occult blood in stool [15, 32, 36]. A fatal case of gastroenteritis associated with coronavirus-like particles has also been reported [37]. Bloody diarrhea is generally not a feature of viral gastroenteritis and is more commonly associated with bacterial infections such as those caused by Campylobacter species or enterohemorrhagic E. coli [38]. Although low platelet counts in immunocompromised patients could predispose such patients to bloody diarrhea, almost half of the patients with this symptom were immunocompetent and did not have thrombocytopenia, suggesting that this symptom cannot be explained wholly by host factors. Studies of torovirus infections in calves (Breda virus) indicate that these viruses infect differentiating epithelial cells in the crypts of the intestinal villi, especially in the large intestine [10, 11]. Investigations of human torovirus infections are needed to compare the pathology of torovirus with that of other gastroenteritis viruses.

Because this study was of hospitalized patients, the importance of torovirus infection in the community is not known. While over half of the torovirus-positive gastroenteritis patients were considered to have a nosocomial infection, a substantial number were admitted for treatment of gastroenteritis. This suggests that torovirus causes gastroenteritis in the community. The Brazilian study identified torovirus in the community but other pathogens were also found, including enteraggregative E. coli [30]. In our study, bacterial coinfections were predominantly with cytotoxin-producing C. difficile in both torovirus- and rotavirus-positive patients. Other microbial infections, namely Y. enterocolitica, G. lambia, and D. fragilis, occurred singly and of the 2 C. jejuni isolates from torovirus-positive patients, 1 child had bloody diarrhea. These findings are consistent with our observation that nonviral microbial pathogens are likely to have been rare in the case and control specimens analyzed in study 1.

Our results support the hypothesis that human torovirus is an important cause of gastroenteritis. Further epidemiologic studies to determine its frequency in the community and to identify mechanisms of transmission of this organism are indicated as are further studies to explain the pathophysiology of illness due to this agent.

Acknowledgments

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