Detection of 'Norwalk-like viruses' in Vellore, southern India

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Results and Discussion

The number of samples received by the laboratories and the pathogens identified beside NLVs were similar to previous years, with 130 (28.5%) positive for bacterial pathogens, 76 (16.7%) positive for parasites and 102 (22.4%) positive for rotavirus, mainly in children (Table 1). Bacterial enteropathogens included Campylobacter, Vibrio cholerae O1 and O139, Shigella spp., Salmonella spp., diarrhoeagenic Escherichia coli, Aeromonas and non-agglutinating vibrios. Of the parasites identified in 76 samples, Cryptosporidium and Giardia were the most commonly seen. Other parasites included Entamoeba histolytica, Enterobius vermicularis, Dientamoeba fragilis and Trichuris trichura. In 12 (15%) patients, NLVs were detected by EIA alone (n = 3), RT-PCR alone (n = 1) or both tests (n = 8) (Table 2). The 1 EIA-negative/PCR-positive sample was found to contain a GRV-like strain following sequence analysis. However, only 1 of 4 samples positive in the G1 EIA was positive by PCR; following amplification sequencing this strain was found to be similar to Southamptom virus. The inability to amplify viral RNA from the other G1 EIA-positive specimens may suggest the presence of novel genotypes not recognized by the primers used in this study; alternatively, the EIA is false positive, although only 1 of 3 samples which were EIA positive/PCR negative could be considered a low positive, with a value near the cut-off point of the test.

All patients excreting NLVs had diarrhoea of <3 days' duration with up to 8 stools a day. Nausea and vomiting, characteristically associated with NLV infection, were reported in all patients, further investigation revealed that 3 patients (study numbers 22, 25 and 33, Table 2) belonged to a single family; they were staying in a hotel and were eating their meals in a restaurant also patronized by another patient (study number 25). All had GRV detected by either RT-PCR, EIA or both tests.

Although NLVs were detected in only 3 of 17 children (defined as subjects aged <15 years) in this study, it is likely that the true incidence of infection is higher, since only samples where no other pathogen was found were included in the NLV testing. In this setting, asymptomatic carriage of enteric pathogens and infection by multiple putative enteric pathogens has been documented, with 60% of children with acute gastroenteritis having multiple enteric pathogens (Matthews et al., 1996). While it is likely that multiple infections due to caliciviruses and other enteric pathogens do occur, it is difficult to assign a causal role to an organism in the presence of more than 1 pathogen. In this study, 15% of samples available for testing, which did not contain any other pathogen, were positive for caliciviruses. At a minimum, even if the remaining 68 stool samples from

Materials and Methods

The study involved examination of 456 faecal samples submitted to laboratories of the Christian Medical College and Hospital between November 1998 and January 1999. The hospital is a tertiary care centre, which serves a rural population of 100 000 and an urban population of 300 000. Sixty-four samples were from inpatients in the hospital. The samples were tested for bacterial enteropathogens by culture, for rotavirus by latex agglutination, and for parasites by direct examination and modified acid-fast staining. Of 148 specimens, 80 (57 from children, 63 from adults) negative for all aforementioned pathogens had sufficient material for testing in EIAs for G1 NLVs and the genogroup 2 NLV, Grimsby virus (GKV). The G1 assay was based on a monoclonal antibody that is broadly reactive to all G1 NLVs whereas the GRV EIA is based on polyclonal antisera to recombinant Grimsby VLPs and is type specific (Hale et al., 1999, 2000). All samples were also subjected to RT-PCR using broadly reactive primers specific for the viral RNA-dependent RNA polymerase with subsequent sequencing of amplicons (Green et al., 1995).
Acknowledgements

Ando, T., Monroe, S. S., Gentsch, J. R., Jin, Q., Lewis, D. C. & Estes, M. K., Atmar, R. L. & Hardy, M. E. (1997). Norwalk The use of these diagnostic tests in larger investigations will allow more detailed studies of the epidemiology and impact of NLV infections in India and other developing countries.

References

Table 1. Outcome of investigation of 456 faecal specimens of patients with acute gastroenteritis in Vellore (India) in 1998/99

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number (%)</th>
<th>0–5</th>
<th>5–15</th>
<th>&gt;15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>130 (28.5)</td>
<td>34 (19.3)</td>
<td>27 (28.1)</td>
<td>69 (37.5)</td>
</tr>
<tr>
<td>Parasitic</td>
<td>76 (16.7)</td>
<td>23 (13.1)</td>
<td>29 (21.1)</td>
<td>24 (14.1)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>102 (22.4)</td>
<td>75 (41.5)</td>
<td>22 (22.0)</td>
<td>7 (3.8)</td>
</tr>
<tr>
<td>Negative for the above</td>
<td>148* (32.5)</td>
<td>46 (26.1)</td>
<td>21 (21.6)</td>
<td>81 (44.0)</td>
</tr>
<tr>
<td>Total</td>
<td>456 (100.0)</td>
<td>176 (100.0)</td>
<td>96 (100.0)</td>
<td>184 (100.0)</td>
</tr>
</tbody>
</table>

*80 of 148 samples had sufficient material for further testing for 'Norwalk-like viruses' – see Table 2.

Table 2. Clinical and laboratory features of 12 patients in Vellore (India) with 'Norwalk-like viruses' infection

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age in years/sex</th>
<th>No. days diarrhea</th>
<th>No. of stools/day</th>
<th>Other symptoms</th>
<th>EIA</th>
<th>PCR</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30/M</td>
<td>1</td>
<td>5</td>
<td>Nausea, vomiting</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>12</td>
<td>35/M</td>
<td>3</td>
<td>6</td>
<td>None</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>22</td>
<td>34/M</td>
<td>2</td>
<td>6</td>
<td>Nausea, vomiting</td>
<td>-</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>25</td>
<td>34/F</td>
<td>2</td>
<td>6</td>
<td>Nausea</td>
<td>GRV</td>
<td>+</td>
<td>Not done</td>
</tr>
<tr>
<td>33</td>
<td>12/M</td>
<td>2</td>
<td>6</td>
<td>Nausea, vomiting</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>43</td>
<td>58/M</td>
<td>3</td>
<td>6</td>
<td>None</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>45</td>
<td>52/F</td>
<td>3</td>
<td>4</td>
<td>None</td>
<td>G1</td>
<td>+</td>
<td>SOV</td>
</tr>
<tr>
<td>55</td>
<td>29/M</td>
<td>3</td>
<td>8</td>
<td>Nausea, vomiting</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>60</td>
<td>2/M</td>
<td>2</td>
<td>7</td>
<td>Dehydration</td>
<td>G1</td>
<td>-</td>
<td>Not done</td>
</tr>
<tr>
<td>64</td>
<td>21/M</td>
<td>2</td>
<td>6</td>
<td>None</td>
<td>GRV</td>
<td>+</td>
<td>GRV</td>
</tr>
<tr>
<td>75</td>
<td>14/F</td>
<td>3</td>
<td>5</td>
<td>Nausea, vomiting</td>
<td>G1</td>
<td>-</td>
<td>Not done</td>
</tr>
</tbody>
</table>

EIA, enzyme immunoassay; PCR, polymerase chain reaction; M, male; F, female; GRV, Grimsby virus; G1, genogroup 1; SOV, Southampton virus.

non-bacterial, non-parasitic, non-rotavirus gastroenteritis, for which sufficient material was not available for further testing, had been negative, 8.1% of all such gastroenteritis can be ascribed to caliciviruses, i.e., a higher percentage than previously estimated (SINGH et al., 1989; MATHEWS et al., 1996).

In this study, we have compared detection of antigen by EIAs with viral RNA detection by PCR and found that the combination of 2 EIAs identified 11 of 12 infections. The use of RT-PCR allows detection and comparison of genetic diversity among NLVs, but is expensive and labour intensive, when compared to EIA. While antigen-detection EIAs are not yet available for all NLVs, this is an affordable diagnostic format useful for screening large numbers of samples. Our results indicate that NLVs are prevalent in South India, and can cause diarrhoea in both children and adults. During 1998–99, GRV-like strains appeared to have predominated; it is possible that 4 of the strains detected came from the same restaurant, although food samples were not obtained for testing. The use of these diagnostic tests in larger investigations will allow more detailed studies of the epidemiology and impact of NLV infections in India and other developing countries.

Acknowledgements

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References


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