Prevalence and Genetic Diversity of Norovirus in Acute Gastroenteritis Cases in the Southwest Province of Turkey

Sevin Kırdar¹, Tülin Başara², İmran Kurt Ömürlü³

¹Department of Medical Microbiology, Faculty of Medicine, Aydın Adnan Menderes University, Aydın, Turkey
²Laboratory of Medical Microbiology Training and Research Hospital, Aydın Adnan Menderes University, Aydın, Turkey
³Department of Biostatistics, Faculty of Medicine, Aydın Adnan Menderes University, Aydın, Turkey

INTRODUCTION

Enteric viruses, such as enteric adenovirus, rotavirus, norovirus, astrovirus, and sapovirus are the most common etiologic agents of childhood viral gastroenteritis.¹ Norovirus is also one of the most frequent causes of acute gastroenteritis (AGE) outbreaks in semi-closed settings, such as schools, hospitals, nursing homes, worship places, cruise ships, and military facilities.

Moreover, norovirus has emerged as the most frequent cause of AGE in children in countries with public vaccination programs including rotavirus vaccine.² Norovirus may lead to severe AGE in developing countries, with an estimated 70,000–210,000 deaths in children annually.³

Noroviruses belong to the family Caliciviridae. Norovirus generally show high genetic variability and are classified into 10 genogroups (GI to GX) based on the capsid (VP1) protein. They can be further divided into 48 capsid genotypes based on VP1 and 60 P-types.⁴

Robust cell culture or small animal model is unavailable; however, successful replication of human norovirus in human intestinal enteroids has been reported.⁵ Norovirus can be detected by laboratory assays, such as enzyme-linked immunoassay (ELISA) and immunochromatographic assays, that detect the viral antigen, and by molecular tests, such as real-time reverse transcription-polymerase chain reaction (rRT-PCR), which is accepted as the gold standard for norovirus diagnosis.³

This study aimed to determine the presence of norovirus by ELISA and rRT-PCR methods in AGE cases in Aydın, Turkey. Additionally, positive samples were genotyped to understand which genotypes have currently circulated in our hospital.

MATERIAL AND METHODS

This retrospective descriptive study conducted between September 2017 and May 2019. Stool samples were collected from patients with acute gastroenteritis symptoms from Aydın Adnan Menderes University Hospital from September 2017 to May 2019. The samples were tested using the commercial Third Generation Ridascreen norovirus ELISA and rRT-PCR. Positive samples were genotyped by sequencing of conventional positive RT-PCR products followed by phylogenetic analysis.

Aims: Noroviruses may cause both epidemic and sporadic acute gastroenteritis globally. Thus, this study evaluated the prevalence of norovirus in stool samples of hospitalized patients with acute gastroenteritis in Aydın, Turkey using enzyme-linked immunomassay (ELISA) and real-time reverse transcription-polymerase chain reaction (rRT-PCR) and genotyped positive samples to detect which genotypes have currently circulated.

Methods: This retrospective descriptive study collected 92 stool samples from patients with acute gastroenteritis symptoms from Aydın Adnan Menderes University Hospital from September 2017 to May 2019. The samples were tested using the commercial Third Generation Ridascreen norovirus ELISA and rRT-PCR. Positive samples were genotyped by sequencing of conventional positive RT-PCR products followed by phylogenetic analysis.

Results: Of the 92 samples, 5 (5.4%) using ELISA and 12 (13%) using rRT-PCR tested positive for norovirus. All positive samples were genogroup II (GII). Two norovirus positive samples were genotyped successfully using DNA sequencing of the nested conventional PCR products. One sample (GII/Hu/TR/2019/Aydin25) could be categorized as GII.3 and the other (GII/Hu/TR/2019/Aydin20) as GII.13.

Conclusion: rRT-PCR testing of stool samples is more sensitive than Ridascreen ELISA. Data from our study provide protocols for how to study norovirus epidemiology.
RESULTS

During the study period, fecal specimens from 92 patients with AGE were collected, of which 55 (59.8%) were males and 37 (40.2%) were females. The age of patients ranged from 0 to 83 years with a median age of 2 years.

Of the 92 samples, 5 (5.4%) using ELISA and 12 (13%) using rRT-PCR tested positive for norovirus. Five patients with an ELISA positive result were all males, whereas 10 were males and 2 females in the 12 patients with positive real-time RT-PCR. A statistically significant difference was found between norovirus positivity by ELISA and age groups ($P = 0.002$); however, positivity by rRT-PCR between age groups was not statistically significant ($P = 0.182$) (Table 1). Samples from the 0–5 years age group most frequently tested positive for norovirus; however, no significant difference was found in the positivity rate between the 0–2 years and 3–5 years age group for either the samples tested by ELISA or by rRT-PCR ($P = 1.000$ for both tests, data not shown). Overall, 12 samples tested positive for GI, of which two (GI/Hu/TR/2019/Aydin20 and GI/Hu/TR/2019/Aydin25) samples were successfully sequenced. Using BLAST, 1 sequence (GI/Hu/TR/2019/Aydin25) had the highest sequence identity with GI.3 viruses, whereas 20 was identical to GI.13 in sequence GI/Hu/TR/2019/Aydin (Figure 1). The following accession numbers were assigned by GenBank (GI/Hu/TR/2019/Aydin20) MT815529 (VP1), MW392526 (RdRp); GI/Hu/TR/2019/Aydin25 MT815530 (VP1), MW392525 (RdRp).

Norovirus was detected throughout the year, but its prevalence was highest in the winter and spring (Table 2). No statistically significant difference was found between norovirus positivity and seasons ($P = 0.192$). Co-infections with rotavirus or adenovirus were detected in 9 cases.

### TABLE 1. Detection of Norovirus by ELISA and Real-time RT-PCR According to Age Groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>ELISA results (n,%)</th>
<th>PCR results (n,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>0–5</td>
<td>3 (3.3)</td>
<td>68 (73.9)</td>
</tr>
<tr>
<td>6–17</td>
<td>-</td>
<td>16 (17.4)</td>
</tr>
<tr>
<td>≥18</td>
<td>2 (2.2)</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (5.4)</td>
<td>87 (94.6)</td>
</tr>
</tbody>
</table>

* $P < 0.002$; ** $P = 0.182$

### TABLE 2. Seasonal Distribution of Real-time RT-PCR Results for Norovirus

<table>
<thead>
<tr>
<th>Season</th>
<th>PCR results (n,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Winter</td>
<td>22 (75.9)</td>
</tr>
<tr>
<td>Spring</td>
<td>46 (92%)</td>
</tr>
<tr>
<td>Summer</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Autumn</td>
<td>7 (87.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>80 (87%)</td>
</tr>
</tbody>
</table>
DISCUSSION

The present study determined the prevalence and genetic diversity of norovirus that is detected in hospitalized patients with AGE in Aydin, Turkey. The Ridascreen ELISA had a sensitivity of 42% and a specificity of 100% compared to the rRT-PCR. All rRT-PCR positive samples (100%) were positive for GII. The two samples that were successfully amplified by conventional RT-PCR could be genotyped as GII.3 and GII.13.

Based on a systematic review, norovirus causes AGE in approximately 20%-24% of patients in community or outpatient clinics and 17% of hospitalized patients worldwide. The first norovirus outbreak was reported from central Anatolia in 2008 in Turkey. Several water and foodborne norovirus outbreaks have been reported since then. Altindis et al. reported norovirus in 17% of sporadic AGE in Turkey.
Previous studies used ELISA and revealed the rate of norovirus ranged from 9.7% to 26%,12-14 Our study revealed 5 (5.4%) stool samples that were positive for norovirus using ELISA, which was similar to a recent report from Canakkale.12 Previous norovirus prevalence determined by rRT-PCR ranged from 15.1% to 33.4%.7,11-13,15,16 Using rRT-PCR, a similar positivity rate was found in our study.11-13 The sensitivity rate of the ELISA test was similar to other studies, which reported the sensitivity rates for Rida screen norovirus kit that range from 31.6% to 65.3%, respectively.17 The ELISA kit showed a lower sensitivity than reported by Dimitriadis et al.18 in Australia (71%), Aksuet al.12 in Turkey (60%), and Zhang et al.19 in China (96%), which was similar to a study conducted by Rovida et al.20 Most studies, including ours, a >90% ELISA specificity.7,12,16 Due to its high sensitivity and specificity, rRT-PCR is considered the gold standard for norovirus detection.

Globally, GII noroviruses are associated with 90%-100% of all infections.21,16,21 The limited studies from Turkey that performed genotyping primarily detected GI.4, GI.6, GI.16, and GI.21 viruses.15,16-18 To the best of our knowledge, this is the first report on detected GI.3 and GI.13 genotypes of norovirus infections in Turkey.

Co-infections with two or more enteric pathogens are common, and the increasing use of molecular techniques has revealed even higher rates.22 Our study detected co-infections in 9 (9.8%) norovirus rRT-PCR-positive samples. The most common co-infections were with rotavirus (n = 8) and adenovirus (n = 1).

Our study has several limitations. The number of participants with AGE was small and we could only successfully sequence 2 samples, which indicates that the used primers were suboptimal for robust genotyping.

In summary, our study detected and genotyped noroviruses in Aydın, Turkey with tourism and increased social interactions between the Aegean part of Turkey and Europe, thereby increasing the risk of norovirus transmission. Our pilot data will increase the knowledge of the importance of norovirus infections in Turkey.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Review Committee of the Aydın Adnan Menderes University (No:2017/1229) and supported by Aydın Adnan Menderes University Research Fund (Project number: 17058).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Consent for Publication: Written informed consent was obtained from the patients.


Conflict of Interest: No conflict of interest was declared by the authors.

Funding: The authors declared that this study received no financial support.

REFERENCES

1. Alp Avci G, Akbabac M. Incidence of Rotavirus, enteric adenovirus and Norovirus in children with acute gastroenteritis under five years. TMCD. 2018;48:264-272. [Crossref]
3. Yoon SH, Kim HR, Ahn JG. Diagnostic accuracy of immunochromatographic tests for the detection of Norovirus in stool specimens: A systematic review and meta-analysis. Microbiol Spectr. 2021;9:e0046721. [Crossref]
6. Kim SH, Cheon DS, Kim JH, et al. Outbreaks of gastroenteritis that occurred during school excursions in Korea were associated with several waterborne strains of Norovirus. J Clin Microbiol. 2005;43:4836-4839. [Crossref]
17. Liu L, Moore MD. A survey of analytical techniques for noroviruses. Foods. 2020;9:318. [Crossref]