

Eight different viral agents in childhood acute gastroenteritis

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Viral gastroenteritis is the most frequent cause of acute gastroenteritis (AGE) of childhood. The aim of this study was to determine the prevalence of viral agents including astrovirus, rotavirus, adenovirus, enterovirus, norovirus, parechovirus, Aichivirus and sapovirus in children with AGE in a pediatric Turkish population.

Fecal specimens of 240 children with AGE were investigated by polymerase chain reaction, and viral agents were identified in 131 (54.6%) samples. The distribution of viral agents was as follows: 56 (42.8%) norovirus, 44 (33.6%) rotavirus, 29 (22.1%) enterovirus, 21 (16.0%) adenovirus, 21 (16.0%) parechovirus, 5 (3.8%) sapovirus and 1 (0.8%) Aichivirus. Single and multiple viral agents were detected in 38.8% and 15.8% of patients, respectively. The duration of hospitalization was longer in children with multiple viral agents than in those infected with a single viral agent ($p<0.001$). While the highest rate of rotavirus infection was detected in winter, the highest rate of norovirus was found in the summer.

In conclusion, norovirus and rotavirus are the most frequent causes of childhood AGE in our country.

Key words: adenovirus, Aichivirus, astrovirus, enterovirus, gastroenteritis, norovirus, parechovirus, polymerase chain reaction (PCR), rotavirus, sapovirus.

Acute gastroenteritis (AGE) is still one of the most common causes of morbidity and mortality in childhood in developing countries. While bacterial and parasitic pathogens are generally responsible for AGE in developing countries, viral agents display a more “democratic” aspect, infecting children in developed and developing countries alike. Worldwide, most AGE cases are due to viral infection, with rotavirus and norovirus being the most common agents¹. Although approximately 1–1.5 million AGE cases are identified in Turkey, of which 80% are in children under two years old, the true prevalence of different viral agents in childhood AGE is not well known². As studies in our country have focused mainly on the prevalence of rotavirus, we aimed to determine the prevalence of eight different viral agents in childhood AGE in a Turkish population, by means of a prospective study lasting one year.

Material and Methods

All children aged 1 month to 17 years who were diagnosed with AGE in our outpatient or emergency pediatrics clinics between January 2012 and January 2013 were included in this study. Acute gastroenteritis was defined as a decrease in the consistency of stools (loose or liquid) and/or an increase in the frequency of evacuations (typically ³3 in 24 hours), with or without fever or vomiting³. Symptoms, medical history and sociodemographic features and laboratory results were recorded. Stool samples were collected and stored at -80 °C until the procedure. In bloody stool samples, culture and microscopic examination were performed to identify any bacterial or parasitic agents.

For identification of viral agents including astrovirus, rotavirus, adenovirus, enterovirus, norovirus, parechovirus, Aichivirus and sapovirus, the multiplex PCR method (K) used by Khamrin et al.⁴ was chosen. In multiplex

PCR, multiple primer sets are included within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences (Qiagen, Hilden, Germany). The gel electrophoresis method was used to visualize those amplicons, and for the determination of the exact sizes of the amplicons, three 100 bp DNA ladders (New England BioLabs, UK) were used in each step. Pictures of the DNA bands were taken with the Gel Logic 2200 Imaging System (1708x1280 pixels, Kodak Company, NY, US), and band sizes were analyzed with both molecular imaging software and the naked eye.

The study was approved by the Ethics Committee of İnönü University Faculty of Medicine (2012/241). Informed consent was obtained from the children's parents. The study was funded by the İnönü University Scientific Study and Project Unit (BAP-2012/62).

For statistical analysis, SPSS for Windows Version 15.0 was used to perform chi-square, Mann Whitney U and Kruskal-Wallis tests. $p < 0.05$ was considered statistically significant.

Results

The mean age of the children was 26.7 ± 27.0 months (1-161 months). Among the children, 106 (44.2%) were girls and 134 (55.8%) were boys; 159 (66.3%) were under 24 months of age.

Anthropometric measurements showed that 7 (2.9%) and 9 (3.8%) were below the third percentile for height and weight, respectively. While 141 children (58.8%) were hospitalized for AGE, the others (41.3%) were evaluated and managed at the outpatient clinic.

By month, the most referrals for AGE were seen in July (14.2%), and the fewest in April (5.4%) ($p = 0.097$). By season, the most referrals were observed in summer (31.3%), followed by winter (27.5%), fall (21.3%) and spring (20%).

Children were evaluated with respect to the duration and severity of the diarrhea. The mean duration of diarrhea was 3.6 ± 2.3 days (1-11 days). Duration was 3 days or less in 61.3%, and 7 days or more in 33.7% of the children. The mean number of bowel movements reported per day was 5.3 ± 2.6 . When hospitalized children and outpatients were compared, it was observed that the

mean duration of diarrhea was longer and the number of bowel movements was higher in hospitalized cases ($p < 0.001$ and 0.009 , respectively) (Table I). Vomiting accompanied diarrhea in 195 children (81.2%), with a mean duration of 2.9 ± 2.1 days (1-10 days). Again, when hospitalized and outpatient cases were compared, it was observed that while the mean duration of vomiting was higher in hospitalized cases ($p = 0.023$), the mean number of daily vomiting episodes was not different ($p = 0.877$) (Table I). Fever accompanied diarrhea in 127 children (52.9%), with a median temperature of 38.5°C ($37\text{-}40^\circ\text{C}$ axillary). No difference was found between hospitalized and outpatient cases in that respect ($p = 0.852$). A combination of diarrhea, vomiting and fever was observed in 101 children (42.1%). None of the patients died due to any complications of AGE.

Twenty children (8.3%) had a history of rotavirus vaccination. The source of water at home was recorded: well water was being used by 9 families (3.8%), tap water by 138 (57.5%), table water by 43 (17.9%) and boiled tap water by 50 (20.8%). Parents of 59 children (24.6%) reported that there was another family member suffering from AGE at the same time.

Viral agents were identified in 131 children (54.6%). Norovirus was detected in 23.3% of the cases, rotavirus in 18.3%, enterovirus in 12.1%, parechovirus in 8.8%, adenovirus in 8.8%, sapovirus in 2.1% and Aichivirus in 0.4%. Astrovirus was not detected in any children. Among those cases with an identifiable viral agent, norovirus was on the top of the list, appearing in 42.8% of cases, followed by rotavirus (33.6%), enterovirus (22.1%), parechovirus (16%), adenovirus (16%), sapovirus (3.8%), and Aichivirus (0.4%).

A number of features of children with and without viral AGE are shown in Table II.

The educational levels of the mothers and fathers and the socioeconomic levels of the families did not differ between the virus-positive and -negative cases ($p = 0.225$, $p = 0.926$ and $p = 0.395$, respectively).

The month-by-month distribution of the viral agents is shown in Figure 1. While rotavirus, adenovirus, norovirus and enterovirus appeared to be causes of AGE year-round,

sapovirus, parechovirus and Aichivirus were more prevalent in the second half of the year. A single agent was identified in 93 stool samples (71%); multiple viral agents were identified in 39 (29%). Most of the latter (26%) had 2 different viral agents; two (1.5%) had 3, and two (1.5%) had 4 different viral agents. The hospitalization rate was 100% for those who had multiple viral agents and 53.8% for those with a single agent ($p<0.001$). Among cases with a single agent, the hospitalization rate was highest (69.2%) for those with enterovirus and lowest (46.2%) for those with adenovirus. The duration of diarrhea in norovirus infection was found to be higher in those over 2 years of age than in those under 2 years ($p=0.013$).

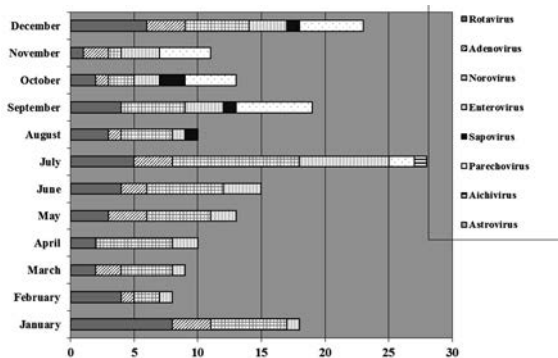


Fig. 1. Distribution of viral agents throughout the year.

Bloody diarrhea was detected in one patient with rotavirus (3.7%), two with norovirus (6.7%) and one with enterovirus infection (7.7%) ($p=0.626$). Rotavirus infection was detected in two children with a history of rotavirus vaccination.

Discussion

It is very well known that AGE occurs mostly in young children, with higher morbidity and mortality rates. The mean age of our patients was 26.7 ± 2 months; 66.2% were under 2 years old. The high rate of hospitalization in our study (58.8%) was thought to be due to the referral of more severe cases to our hospital, which is a tertiary medical center.

A multiplex PCR method, which was able to differentiate multiple types of viruses based on different amplicon sizes, was used for simultaneous detection of eight AGE-causing viruses in a single reaction tube⁴. Although a reduction in sensitivity due to competition for reagents when multiple templates are amplified in a single reaction might be expected, the overall detection rates for viruses causing diarrhea have been found not to differ between conventional and novel multiplex PCR methods⁴.

Viral etiology was detected in 54.6% of children.

Table I. Characteristics of Symptoms in Children with AGE

Mean	Hospitalized patients	Outpatients	P value
Duration of diarrhea (days)	4.2 ± 2.4	2.8 ± 2.0	<0.001
Bowel movements	5.6 ± 2.7	4.8 ± 2.5	0.009
Duration of vomiting (days)	3.2 ± 2.2	2.5 ± 1.8	0.023
Number of vomiting episodes	4.2 ± 2.3	4.2 ± 2.4	0.877

Table II. Some Features of Children with or without Viral AGE

	Viral agent-positive	Viral agent-negative	P
Female/male	57/74	49/60	0.823
Mean age (months)	26.9 ± 27.9	26.5 ± 28.0	0.981
Fever	68 (51.9%)	59 (54.1%)	0.732
Bloody diarrhea	4 (3.1%)	3 (2.8%)	0.890
Mean duration of diarrhea (days)	3.4 ± 2.3	3.8 ± 2.4	0.120
Mean number of bowel movements	5.4 ± 2.7	5.2 ± 2.5	0.391
Mean duration of vomiting (days)	2.7 ± 2.0	3.2 ± 2.1	0.081
Mean number of vomiting episodes	4.0 ± 2.3	4.6 ± 2.3	0.033

In other studies from our country, viral AGE frequency was reported to be between 18% and 44%⁵⁻⁷. However, none of the studies cited investigated more than three viral agents. In other countries, frequency was found to be between 32% and 45%⁸⁻¹⁰.

The most common agent in our study was norovirus (42.3% of viral agents), which was followed by rotavirus (33.6%) and enterovirus (22.1%). Astrovirus was not identified in any of the cases. To the best of our knowledge, none of the studies conducted in our country so far have investigated those eight viral agents together in AGE. In the study by Özdemir et al.⁵, the most common agent was reported to be rotavirus (72.6%), followed by adenovirus (23.6%) and astrovirus (3.7%). Another study from our country with a high number of cases (941) revealed a lower rate of viral etiology (18.1%), with rotavirus and adenovirus being the two most common viruses⁸. Interestingly, Akihara et al.¹⁰ reported that they did not detect rotavirus in any of their cases; instead, adenovirus was the most common viral agent, followed by astrovirus and sapovirus. In contrast, another Japanese study, by Nakanishi et al.¹¹, reported rotavirus to be the most common viral agent, followed by norovirus.

Hospitalization rates did not differ between the various viral etiologies; however, patients with multiple viral infections had a greater hospitalization rate ($p < 0.001$). This result suggests that multiple viral agents cause a more severe clinical manifestation.

Because none of the viral agents were markedly more prevalent in patients of a particular age group or sex, or from families of a particular educational or socioeconomic level, and no specific clinical finding was more common in any type of viral AGE, prediction of viral etiology according to these parameters seems impossible. Bloody diarrhea likewise did not exclude viral etiology, although identification of a viral agent in bloody diarrhea does not necessarily mean it is the cause of the diarrhea. In our series of 7 children with bloody diarrhea, 2 had norovirus, 1 had rotavirus and 1 had enterovirus in their stool samples.

After rotavirus vaccination programs, a relative increase in norovirus infection has been observed. Our results, revealing a higher

prevalence of norovirus than rotavirus, which had previously been reported as the most common agent in childhood AGE in our country, might be the reflection of rotavirus vaccination, though the latter is not included in our national vaccination schedule. A few studies on the prevalence of norovirus in our country between 2008 and 2011 showed that 14-33% of children with AGE were positive for norovirus¹²⁻¹⁵. Similar results have been reported in other countries (17-33%)^{16,17}. We observed that norovirus infection was not dependent on the season, although it was most common in summer and least common in fall (35.7% vs. 14.3%). Seasonal variation has been reported in some studies, with norovirus occurring predominantly in winter or summer^{12,18,19}.

Rotavirus is one of the most common and most focused-on viral agents in our country. The fact that one-third to one-half of the viral agents causing AGE are rotavirus (our figure is 33.6%)²⁰, and the fact that this virus may result in a severe clinical presentation in infancy, led to production of a successful vaccine. Although rotavirus vaccination is not part of our national vaccination scheme, some families have their children vaccinated for it. Previously, rotavirus was identified in 8-40% of children with AGE in our country^{5,6,21-25}. Among the children in this study with rotavirus infection, 75% were younger than 2 years old, a finding consistent with that of some other studies^{23,26,27}. It is known as a winter viral agent^{25,27} in countries with temperate climates, such as ours^{5,6,21,24}. A high rate of hospitalization is another feature of rotavirus AGE²⁹. Previous studies from our country revealed that approximately one-third of patients were hospitalized^{6,24}; our rate was much higher (48.1%).

The prevalence of adenovirus in AGE has been reported to be between 3% and 17%³⁰⁻³³. In Turkey, a similar frequency has been reported: between 5% and 14%³⁴⁻³⁷. We could not find any seasonal prevalence in adenovirus AGE. Astrovirus is not very common in AGE; the reported prevalence is between 2% and 10%^{17,38,39}. One study in our country showed 1.7% (6/363) of patients with AGE as being infected with astrovirus⁷. Astrovirus could not be identified in any of our stool samples.

No previously existing study from our country

had investigated sapovirus in AGE. We found a 2% prevalence, which is consistent with the results of studies in Japan: between 1.7% and 5%^{10,11,40}. We found fall to be the predominant time of occurrence, although this virus has been reported to occur sporadically throughout the year¹¹. Parechovirus, the prevalence of which in Turkey has been unknown, affects both the gastrointestinal and respiratory systems. Of our cases, 8.8% had parechovirus AGE, a rate very similar to that found by Pham et al. in Sri Lanka (8.3%)⁴¹. In Thai children, prevalence has been reported as 14.6%⁴². It is known as a summer–fall viral agent⁴³; we also detected parechovirus most frequently in the fall.

Enterovirus is a year-round, age-independent causative agent for AGE⁴⁴. We found a 12% prevalence of enterovirus occurring between July and September. Only one patient with Aichivirus was identified in our study. No previous report existed regarding Aichivirus AGE in our country, but a study from Paris indicated the prevalence as 1.6%⁴⁵.

AGE caused by multiple viral agents was detected in 14% of our patients. Most of them were infected with two different viral agents (14%), but three or four agents were identified in a small number of patients (1.6%). Özdemir et al.⁷ found that 9.6% and 0.6% of their patients were infected with two and three viral agents, respectively, the most common combination being rotavirus and adenovirus. Infection with more than one viral agent was reported in 12.8% of children in a study in Japan⁴.

In conclusion, this is the first study in our country to investigate eight different viral agents in childhood AGE. Most of our patients had norovirus or rotavirus infections; this emphasizes the importance of screening for these two agents in AGE cases.

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