

Epidemiology and Clinical Characteristics of Rotavirus and Norovirus Infections in Hospitalized Children Less Than 5 Years of Age With Acute Gastroenteritis in Tehran, Iran

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Abstract- Acute gastroenteritis is one of the most important causes of death in children in developing countries which cause by different enteropathogens, including bacteria, viruses, and parasites. Among these, most of the acute gastroenteritis in children are caused by viral infections mainly by rotavirus and norovirus. This study aimed to study the epidemiological and clinical status of acute gastroenteritis resulting from rotavirus and norovirus in children between June 2015 and June 2016 in Iran. A total of 211 stool specimens were collected from Ali Asghar Children's Hospital and Bahrami Children's Hospital in Tehran, from June 2015 to June 2016. The samples were screened by commercial enzyme immunoassay (EIA) Ridascreen kit and real time RT-PCR to detect rotavirus and norovirus genogroups I and II, respectively. The information on demographic and clinical manifestations was collected, and data analyzed using IBM SPSS statistics version 22. Overall, the detection rate of rotavirus was 25.6 %, and for norovirus infection, it was 17.5%. All norovirus positive specimens belonged to genogroup II. Higher rates of rotavirus infections were observed in children from 7 to 24 months, and higher rates of norovirus infections were detected in children from 1 to 12 months. Clinical symptoms were not different between rotavirus and norovirus case-patients. The present study not only highlights the importance of rotavirus and norovirus infections in Iran but also verifies the relevance of norovirus as the cause of severe gastroenteritis in children.

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Introduction

Acute gastroenteritis is one of the most common symptoms in many enteric infections of children less than 5 years of age, which are most vulnerable to severe acute gastroenteritis (1). A wide variety of enteropathogens, including bacteria, viruses, and parasites are known to cause gastroenteritis. Among these, approximately 70% of the episodes of acute gastroenteritis in children (2) are caused by viral infections, mainly by rotavirus and norovirus. Rotavirus, a double-stranded RNA virus and a member of the Reoviridae family, has been considered as the most

important cause of acute gastroenteritis in children less than 5 years of age. Rotavirus is responsible for approximately 40% of all hospital admissions due to gastroenteritis (3). The morbidity related to rotavirus was observed to be similar in both developed and developing countries. However, more than 80% of all mortalities associated with rotavirus infections were estimated to occur in developing countries of South Asia and sub-Saharan Africa (4). In 2013, it was estimated that 578,000 diarrheal deaths occurred in children < 5 years of age, and rotavirus be responsible for 37% (215,000) of diarrhea-related deaths (5). Over the last 30 years, achievements have been made in vaccine

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development to prevent rotavirus infections. Rotashield (Wyeth Lederle Vaccines, Philadelphia, PA) was the first rotavirus vaccine licensed but was withdrawn in 1999 by its manufacturer because of its association with intussusception during post licensure surveillance. Moreover, two globally (RotaTeq, Merck & Co and Rotarix, GSK Biologicals) and several nationally (RotaVac, ROTASIIL, Rotavin-M1, , and Lanzhou lamb RV vaccines) live oral rotavirus vaccines are licensed. In 2009, the World Health Organization (WHO) recommended including two rotavirus vaccines, Rotarix and RotaTeq in national immunization programmes worldwide (6). After the introduction of two rotavirus vaccines, the epidemiology of gastroenteritis had changed in which noroviruses were recognized as the leading causes of sporadic cases and outbreaks of gastroenteritis across all age groups worldwide. Noroviruses have been detected in 3-31% of hospitalized children and in 5-36% of outpatients, and may be responsible for the deaths of up to 200,000 children < 5 years of age annually in developing countries (7). Norovirus, previously termed 'Norwalk-like viruses,' was first identified using electron microscopy (EM) in Norwalk, Ohio, during a gastroenteritis outbreak at an elementary school (8). Noroviruses are positive sense, non-enveloped RNA viruses of the *Calciviridae* family (9), and based on a genetic characteristic of complete VP1 gene; norovirus genus is classified into seven genogroups (GI to GVII). Among norovirus genogroups, GI, GII, and GIV are responsible for disease in humans that GII has been consistently identified as the predominant genogroup in the world.

Acute gastroenteritis mainly affects younger children less than 5 years of age. As such, management of acute viral gastroenteritis is considered among high priorities of the healthcare system in Iran. This study aimed to study the epidemiological and clinical status of acute gastroenteritis resulting from rotavirus and norovirus in young children between June 2015 and June 2016 in Iran.

Materials and Methods

Specimen collection

A total of 211 stool specimens were collected from immunocompetent children less than 5 years of age who have been hospitalized for acute gastroenteritis (defined as the symptoms of diarrhea and/or vomiting [more than 3 times per day]) at Children's Hospitals in Tehran, from June 2015 to June 2016. Specimens were transported to

the Virology Laboratory of Pasteur Institute in Iran and stored at -20°C until they were processed. Demographic data and clinical characteristics were collected by attending physicians, and the following signs and symptoms: fever, vomiting, diarrhea, abdominal cramp, respiratory symptoms, malaise, myalgia, and weight loss. The study was conducted and approved by the ethics committee of the Pasteur Institute of Iran.

Stool preparation and virus detection

A 10% (wt/vol) stool suspension was prepared in phosphate-buffered saline (PBS). Rotavirus antigen detection was performed with commercial enzyme immunoassay (EIA) Ridascreen kit (R-Biopharm GmbH, Darmstadt, Germany) according to manufacturer instructions.

For norovirus, real time RT-PCR was applied for nucleic acid detection of norovirus genogroup I and genogroup II. Viral RNA was extracted from stool suspensions using RTP DNA/ RNA Virus Mini Kit (STRATEC Molecular GmbH, Berlin, Germany) according to the manufacturer's instructions. Noroviruses genogroup I and II were detected by real time RT-PCR using primer's pair COG1F: 5'-CGY TGG ATG CGN TTY CAT GA-3'; COG1R: 5'-CTT AGA CGC CAT CAT CAT TYA C-3' and probe: Ring 1C FAM-AGA TYG CGI TCI CCT GTC CA-BHQ and primer's pair COG2F: 5'-CAR GAR BCN ATG TTY AGR TGG ATG AG-3'; COG2R: 5'-TCG ACG CCA TCT TCA TTC ACA-3' and probe: Ring 2 FAM-TGG GAG GGC GAT CGC AAT CT-BHQ for ORF1-ORF2 junction region, respectively (10,11). Real-time RT-PCR was conducted on the Corbett Research Rotor-Gene™ (Qiagen, USA) using SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer's instructions. The reactions of 50 μl volume were incubated at 50°C for 15 min, 95°C for 2 min, followed by 50 cycles of 95°C for 30 s, 55°C 30 s, 60°C for 60 s. Specimens giving Ct values of >35 were considered as negative.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 22 (SPSS Inc., Chicago, IL, USA). The data were analyzed by the multiple logistic regression test, and the results were considered statistically significant at $P < 0.05$.

Results

Epidemiology of rotavirus and norovirus

Among the 211 children with acute gastroenteritis enrolled in the study, 118 (56%) were male, and 93 (44%) were female (Table 1), resulting in a male-to-female ratio of 1.3:1. The age of the children with acute gastroenteritis was between 1 and 59 months, with a median age of 12 months. Overall, the detection rate of rotavirus was 25.6 % (54/211), and for norovirus infection, it was 17.5% (37/211). All norovirus positive specimens belonged to genogroup II. Rotavirus and norovirus were also detected in all age groups tested (<6, 7–12, 13–18, 19–24, >24 months). Higher rates of rotavirus infections were observed in children from 7 to 24 months (7-12>13-18=19-24 month age groups), and higher rates of norovirus infections were detected in children from 1 to 12 months (7-12>1-6 month age groups). The mean age of children positive for rotavirus was older than that for norovirus (13.37±7.8 months vs.

10.9±7.7 months). The most common symptoms of children with acute gastroenteritis were diarrhea (95%), followed by fever (84%), abdominal cramp (80%), vomiting (67%), malaise (33%), respiratory symptoms (23%), weight loss (13%), and myalgia (11%) (Table 1). Clinical symptoms were not different between rotavirus and norovirus case-patients. The majority of children with rotavirus and norovirus infections experienced classic symptoms, including diarrhea, fever, vomiting, and abdominal cramp. Children with rotavirus and norovirus infections significantly showed malaise manifestations when compared to children without virus infection ($P<0.05$). Moreover, in children with rotavirus infection showed significantly fewer respiratory syndromes compared to children without virus infection ($P<0.05$) (Table 1).

Table 1. Demographic information and clinical characteristics of rotavirus and Norovirus infection in children less than 5 years of age.

| | | Overall (n=211) | Rotavirus (n=54) | 95% CI for odds ratio | | | Norovirus (n=37) | 95% CI for odds ratio | | |
|------------------------------------|--|--------------------|---------------------|-----------------------|-------|-------|---------------------|-----------------------|-------|-------|
| | | | | Lower | OR | Upper | | Lower | OR | Upper |
| Gender | Male | 118 (56) | 27 (50) | 0.68 | 1.3 | 2.49 | 23 (62) | 0.37 | 0.79 | 1.69 |
| | Female | 93 (44) | 27 (50) | | | | 14 (38) | | | |
| Age (months) | 1-6 | 36 (17) | 6 (11) | 0.15 | 0.78 | 4 | 12 (32) | 0.50 | 4.66 | 42.92 |
| | 7-12 | 119 (57) | 31 (57) | 0.26 | 1.06 | 4.4 | 20 (54) | 0.24 | 2.05 | 17.74 |
| | 13-18 | 15 (7) | 7 (13) | 0.42 | 2.33 | 12.9 | 1 (3) | 0.05 | 1 | 19.36 |
| | 19-24 | 30 (14) | 7 (13) | 0.16 | 0.81 | 4.06 | 3 (8) | 0.93 | 1.05 | 11.82 |
| | 25-59 | 11 (5) | 3 (6) | | | | 1 (3) | | | |
| Clinical manifestations | Fever | 177 (84) | 46 (85) | 0.44 | 1.082 | 2.65 | 30 (81) | 0.31 | 0.80 | 2.1 |
| | Vomiting (>3 episodes/day) | 142 (67) | 39 (72) | 0.77 | 1.56 | 3.14 | 28 (76) | 0.8 | 1.86 | 4.31 |
| | Diarrhea (>3 episodes/day) | 200 (95) | 51 (94) | 0.31 | 1.21 | 4.76 | 36 (97) | 0.31 | 2.571 | 21.26 |
| | Abdominal Cramp | 168 (80) | 44 (82) | 0.4 | 0.93 | 2.14 | 25 (68) | 0.19 | 0.44 | 1.01 |
| | Respiratory symptoms | 48 (23) | 7 (13) | 1.14 | 2.76* | 6.7 | 6 (16) | 0.81 | 2.12 | 5.54 |
| | Malaise | 70 (33) | 27 (50) | 0.15 | 0.3* | 0.6 | 15 (41) | 0.2 | 0.44* | 0.97 |
| | Myalgia | 24 (11) | 9 (17) | 0.15 | 0.4 | 1.08 | 6 (16) | 0.13 | 0.42 | 1.26 |
| | Weight loss | 27 (13) | 8 (15) | 0.41 | 1.01 | 2.5 | 1 (3) | 0.81 | 6.35 | 49.31 |

All variables were nominal.

*: $P<0.05$

Discussion

Acute gastroenteritis is one of the most important causes of death in children in developing countries. Rotavirus and norovirus are two main common causes of viral gastroenteritis worldwide. Rotavirus is the major cause of severe gastroenteritis in children less than 5 years of age in both developed and developing countries, while norovirus causes disease across all age groups. In the pre-vaccine era, rotavirus remained the most predominant viral agent of acute gastroenteritis, according to previous reports in the world. The

incidence of rotavirus gastroenteritis has declined slightly over time from 42.5% in 2000 to 37.3% in 2013 (5), suggesting a fall of the burden of rotavirus disease globally following vaccine introduction. Since the introduction of the rotavirus vaccine is considered a high priority for countries like Iran, it is very important to provide a clear picture of the prevalence and epidemiology of rotavirus infection. In the present study, rotavirus accounted for 25.6% of all the children with acute gastroenteritis, which is close to the results of earlier studies from Iran (range, 15.3%–67.6%) (12) as well as to the reports of rotavirus infection in some other

countries (13-15). Noroviruses are also considered as an important cause for hospitalization of children worldwide, with nearly the same prevalence and clinical impact of rotaviruses (16). However, the prevalence of norovirus tended to be relatively higher in cases of acute gastroenteritis in outpatient (20-24%) compared with inpatient settings (17%). Furthermore, the prevalence was found to be higher in low-mortality developing (19%) as well as developed countries (20%) compared with high mortality developing countries (14%) (17). This finding should not be interpreted as norovirus causing a smaller burden in these settings. Thus, low prevalence in low-income settings might suggest a more prominent role for other pathogens that are largely controlled through water and sanitation improvements in high-income settings. In Iran, norovirus infection was detected in 17.5% of children less than 5 years of age with acute gastroenteritis, which is close to the reported data from low-mortality developing and developed countries (17-19). However, the prevalence of norovirus in children less than 5 years of age in the present study was higher than previously reported from Iran (4-12.5%) (19-21). This discrepancy may be related to many factors such as variations in study design, epidemic season and methods applied. In addition, a gradient of increasing prevalence from the present study to the previous reports from Iran, might suggest a pattern similar to the one reported for low-mortality countries.

All norovirus strains detected in the present study belonged to genogroup II. It has also been documented by several molecular epidemiological studies that GII genogroup of noroviruses tended to have a wider circulation than GI genogroup in Iran (20,22) and other regions in the world (23-25).

Rotavirus and norovirus infections were detected in all age groups. The rate of virus detection was higher in the 7-12 month age group than other age groups and declined after 24 months, which was similar to other reports (26,27). Our findings indicate that rotavirus and norovirus viral infections generally occur in early childhood, indicating the susceptibility of children to rotavirus and norovirus in their early childhood. Infections might also result in protective immunity against re-infection after early childhood.

In the present study, rotavirus and norovirus infections manifested clinical characterizations, including diarrhea, fever, vomiting, and abdominal cramp at a higher rate, as shown in many other studies (28-30). No apparent and essential difference in clinical findings was found between patients with rotavirus and norovirus gastroenteritis. However, malaise and lack of

respiratory syndromes may be found mainly in rotavirus and norovirus gastroenteritis. The health challenges of rotavirus and norovirus infections appear to be underappreciated in Iran and many other countries, as both rotaviruses and noroviruses are not routinely covered by stool specimen's analysis for children suffering from gastroenteritis.

In conclusion, the present study not only highlights the importance of rotavirus and norovirus infections in Iran but also verifies the relevance of norovirus as the cause of severe gastroenteritis in children. Infections mainly occurred in children under 24 months of age. The common clinical symptoms in children with rotavirus and norovirus infections were diarrhea, vomiting, fever, and abdominal cramp, which makes it difficult to differentiate norovirus from rotavirus based on clinical signs. Therefore, introducing routine rotavirus and norovirus testing for hospitalized children with gastroenteritis as well as establishing a National Virus Reference Laboratory to analyze both the burden and the molecular epidemiology of viral intestinal infections appears to be necessary for childhood population in Iran.

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