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

Arash Arashkia¹, Behrooz Nejat¹,², Mahsa Farsi¹,², Somayeh Jalilvand³, Ali Reza Nateghian¹, Aliakbar Rahbarimanesh⁴,
Fereshteh Mohshfegh⁵, Nasir Mohajel¹, Zabihollah Shoja¹

¹ Department of Virology, Pasteur Institute of Iran, Tehran, Iran
² Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran
³ Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
⁴ Ali-Asghar Children's Hospital, Iran University of Medical Sciences, Tehran, Iran
⁵ Department of Pediatric Infectious Diseases, Bahrami Children's Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received: 28 Apr. 2019; Accepted: 28 Oct. 2019

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) Ridiscreen 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.

Keywords: Rotavirus; Norovirus; Acute gastroenteritis; Clinical characteristics

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
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 leading causes of sporadic cases and outbreaks of gastroenteritis across all age groups worldwide. 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 GCC GAT CGC AAT CT–BHQ for ORF1–ORF2 junction region, respectively (10,11). Real-time RT-PCR was conducted on the Corbett Research Rotor-GeneTM (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 for 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.

| Gender | Overall (n=211) | Rotavirus (n=54) | Norovirus (n=37) |
|--------|----------------|-----------------|-----------------|
| Male   | 118 (56)       | 27 (50)         | 23 (62)         |
| Female | 93 (44)        | 27 (50)         | 14 (38)         |
| Age (months) |
| 1-6    | 36 (17)        | 6 (11)          | 12 (32)         |
| 7-12   | 119 (57)       | 31 (57)         | 20 (54)         |
| 13-18  | 15 (7)         | 7 (13)          | 1 (3)           |
| 19-24  | 30 (14)        | 7 (13)          | 3 (8)           |
| 25-59  | 11 (5)         | 3 (6)           | 1 (3)           |
| Clinical manifestations |
| Fever  | 177 (84)       | 46 (85)         | 30 (81)         |
| Vomiting (>3 episodes/day) | 142 (67) | 39 (72) | 28 (76) |
| Diarrhea (>3 episodes/day) | 200 (95) | 51 (94) | 36 (97) |
| Abdominal Cramp | 168 (80) | 44 (82) | 25 (68) |
| Respiratory symptoms |
| Malaise | 70 (33) | 27 (50) | 15 (41) |
| Myalgia | 24 (11) | 9 (17) | 6 (16) |
| Weight loss | 27 (13) | 8 (15) | 1 (3) |

| 95% CI for odds ratio (OR) | Lower | OR | Upper | 95% CI for odds ratio (OR) | Lower | OR | Upper |
|---------------------------|-------|----|-------|---------------------------|-------|----|-------|
| Male                       | 0.68  | 1.3| 2.49  | 0.37                      | 0.79  | 1.69|
| 1-6                        | 0.15  | 0.78| 4     | 0.50                      | 4.66  | 42.92|
| 7-12                       | 0.26  | 1.06| 4.4   | 0.24                      | 2.05  | 17.74|
| 13-18                      | 0.42  | 2.33| 12.9  | 0.05                      | 1     | 19.36|
| 19-24                      | 0.16  | 0.81| 4.06  | 0.93                      | 1.05  | 11.82|
| 25-59                      | 0.16  | 1   | 1     | 0.8                      | 1.86  | 4.31|
| Fever                      | 0.44  | 1.082| 2.65  | 0.31                      | 0.80  | 2.1 |
| Vomiting (>3 episodes/day) | 0.77  | 1.56| 3.14  | 0.8                      | 1.86  | 4.31|
| Diarrhea (>3 episodes/day) | 0.31  | 1.21| 4.76  | 0.31                      | 2.571 | 21.26|
| Abdominal Cramp            | 0.4   | 0.93| 2.14  | 0.19                      | 0.44  | 1.01|
| Respiratory symptoms       | 1.14  | 2.76*| 6.7   | 0.81                      | 2.12  | 5.54|
| Malaise                    | 0.15  | 0.4  | 1.08  | 0.13                      | 0.42  | 1.26|
| Myalgia                    | 0.41  | 1.01| 2.5   | 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 prevalent 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 counties (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 tented 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.

Acknowledgment

This work was supported by Pasteur Institute of Iran; under Grant numbers 783 and 881.

References

1. Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;381:1405-16.
2. Webb A, Starr M. Acute gastroenteritis in children. Aust Fam Physician 2005;34:227-31.
3. Ramani S, Kang G. Viruses causing childhood diarrhoea in the developing world. Curr Opin Infect Dis 2009;22:477-82.
4. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et al. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 2009;200:9-15.
5. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD, World Health Organization-Coordinated Global Rotavirus Surveillance N. Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013. Clin Infect Dis 2016;62:96-105.
6. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years.

Acta Medica Iranica, Vol. 57, No. 11 (2019) 643
Epidemiology of rotavirus and norovirus

before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 2012;12:136-41.

7. Patel MM, Widdowson M-A, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 2008;14:1224-31.

8. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 1972;10:1075-81.

9. Green KY. Caliciviridae: the noroviruses. In: Howley PM, ed. Fields Virology. Philadelphia, PA, USA: Lippincott Williams & Wilkins 2013:582-608.

10. Rolfe K, Parmar S, Mururi D, Wreghtt T, Jalal H, Zhang H, et al. An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genotyping. J Clin Virol 2007;39:318-21.

11. Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushima S, Hoshino FB, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 2003;41:1548-57.

12. Shojai Z, Jalilvand S, Mokhtari-Azad T, Nategh R. Epidemiology of cocirculating human rotaviruses in Iran. Pediatr Infect Dis J 2013;32:178-81.

13. John BM, Devgan A, Mitra B. Prevalence of rotavirus infection in children below two years presenting with diarrhea. Med J Armed Forces India 2014;70:116-9.

14. Yang SY, Hwang KP, Wu FT, Wu HS, Hsiung CA, Chang WC, et al. Epidemiology and clinical peculiarities of norovirus and rotavirus infection in hospitalized young children with acute diarrhea in Taiwan, 2009. J Microbiol Immunol Infect 2010;43:506-14.

15. Chen CJ, Wu FT, Huang YC, Chang WC, Wu HS, Wu CY, et al. Clinical and Epidemiologic Features of Severe Viral Gastroenteritis in Children: A 3-Year Surveillance, Multicentered Study in Taiwan With Partial Rotavirus Immunization. Medicine (Baltimore) 2015;94:e1372.

16. Koopmans M. Progress in understanding norovirus epidemiology. Curr Opin Infect Dis 2008;21:544-52.

17. Ahmed SM, Hall AJ, Robinson AE, Verhoeef L, Premkumar P, Parashar UD, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis 2014;14:725-30.

18. Boga JA, Melon S, Nicieza I, De Diego I, Villar M, Parra F, et al. Etiology of sporadic cases of pediatric acute gastroenteritis in asturias, Spain, and genotyping and characterization of norovirus strains involved. J Clin Microbiol 2004;42:2668-74.

19. Shojai Z, Jalilvand S, Mollaei-Kandelous Y, Validi M. Epidemiology of viral gastroenteritis in Iran. Pediatr Infect Dis J 2014;33:218-20.

20. Romani S, Mohhebi SR, Hosseini SM, Azimzadeh P, Vahedi M, Derakhshan F, et al. Prevalence of norovirus infection in children and adults with acute gastroenteritis, Tehran, Iran, 2008-2009. Food Environ Virol 2012;4:1-5.

21. Roodarsi SR, Bitaijan F, Gachkar L, Jadali F, Adabian S, Nia RSS, et al. Detection of Noroviruses Isolated From Children With Acute Gastroenteritis by Rt-PCR in Iran. Arch Pediatr Infect Dis 2013;1:57-60.

22. Fazeli Z, Baghaie N, Khavarinejad RA, Khoramdel M, Sigaroodi A, Nadji SA. Hospital based study of prevalence and genotyping of Noroviruses and Sapoviruses isolated from children with acute gastroenteritis referred to Masih Daneshvari hospital. Gastroenterol Hepatol Bed Bench 2010;3.

23. Fretz R, Herrmann L, Christen A, Svoboda P, Dubuis O, Viollier E, et al. Frequency of Norovirus in stool samples from patients with gastrointestinal symptoms in Switzerland. Eur J Clin Microbiol Infect Dis 2005;24:214-6.

24. Tran TH, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GI/II. 4 variants. J Clin Virol 2013;56:93-185.

25. Wollants E, De Coster S, Van Ranst M, Maes P. A decade of norovirus genotypic diversity in Belgium. Infect Genet Evol 2015;30:37-44.

26. Shiota K, Kambhampati A, Hall AJ, Lopman BA. Global age distribution of pediatric norovirus cases. Vaccine 2015;33:4065-8.

27. Steele AD, Madhi SA, Cunliffe NA, Vesikari T, Phua KB, Lim FS, et al. Incidence of rotavirus gastroenteritis by age in African, Asian and European children: Relevance for timing of rotavirus vaccination. Hum Vaccin Immunother 2016;12:2406-12.

28. Subekti D, Lesmana M, Tjaniadi P, Safari N, Frazier E, Simanjuntak C, et al. Incidence of Norwalk-like viruses, rotavirus and adenovirus infection in patients with acute gastroenteritis in Jakarta, Indonesia. FEMS Immunol Med Microbiol 2002;33:27-33.

29. Narkeviucyte I, Tamusauskaite I. Peculiarities of norovirus and rotavirus infections in hospitalised young children. J Pediatr Gastroenterol Nutr 2008;46:289-92.

30. Cheng AC, McDonald JR, Thielman NM. Infectious diarrhea in developed and developing countries. J Clin Gastroenterol 2005;39:757-73.