Low Endemic Frequency of Adenovirus, Rotavirus and Norovirus in Pediatric Diarrhea Samples from Central Iran

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Abstract

Background: Acute viral gastroenteritis is a disorder that affects children on a worldwide scale but mostly in developing countries. Adenoviruses, rotaviruses, and noroviruses are the major viral cause of childhood gastroenteritis. This study is the first to investigate the prevalence of these viruses in diarrhea samples from pediatric patients living in central Iran.

Methods: A total of 173 samples of pediatric diarrhea, from May 2015 to May 2016, was included in this descriptive cross-sectional study. The samples were analyzed using in-house developed PCR and reverse transcription (RT) -PCR methods to investigate the prevalence of adeno-, rota- and noroviruses.

Results: Out of 173 samples of pediatric diarrhea, eight were shown to contain enteric viruses (4.6%): four of these were adenovirus (2.3%), three were rotavirus (1.7%), and one represented a genogroup II norovirus (0.6%). Most of the positive samples were obtained from children under the age of seven. The most common additional clinical symptoms in pediatric patients with viral agents were fever, vomiting, and abdominal pain.

Conclusions: Adenovirus and rotavirus were rarely found as agents responsible for gastroenteritis in central Iran. Although we show that the frequency of viral gastroenteritis is low in this area compared to bacterial gastroenteritis, still longer-term monitoring of all enteropathogenic agents should provide a deeper understanding of the real endemicity and the possible occurrence of unexpected outbreaks of viral enteritis.

Background

Enteric viral infections are a major worry for public health (1). The worldwide mortality of acute diarrheal disease has decreased from 1.3 million in 2000 to about 0.6 million in 2012 because of reforms in sanitation and enhanced access to immunization programs, better healthcare, availability of clean water, and oral rehydration therapy (2). Nevertheless, the role that acute diarrheal disease plays in the morbidity and mortality of pediatric patients under five years of age is still significant, particularly in low-income populations living in areas with little or no basic infrastructure (2). Diarrhea has a notable influence on the quality of life of pediatric patients and their families (3). It has been shown that viruses account for up to 40% of all severe cases of diarrhea among children in some of the emerging economies (4). Adenoviruses, rotaviruses, and noroviruses are the leading causes of viral gastroenteritis, especially in young children (4–6). Rotavirus is known as main etiological agent of acute, severe gastroenteritis in infants worldwide, causing an estimated 527,000 deaths per annum (7). More than 65% of these deaths have been shown to have happened in just 11 countries (7). In pediatrics, rotavirus is responsible for 29 to 45% of global hospitalizations that are due to gastroenteritis. (8). In many developing countries including Iran, rotavirus vaccination has not yet been introduced.

The detection and identification of viruses are not routinely performed in hospitals and medical diagnostic laboratories in Iran, and no comprehensive studies have been conducted to determine the role

of viral agents in pediatric diarrhea in the central part of the country. Diarrhea is one of the most significant problems in public health and more detailed investigations into the causal agents can reduce financial burden of the diarrhea due to self-limiting nature of the disease (9).

Therefore, the aim of this study was to evaluate the prevalence of adenovirus, norovirus, and rotavirus infection in children in central Iran using PCR and RT PCR methods.

Methods

Sample collection

Samples of diarrhea from children (n=173) who had been referred to the Children's Educational-Therapeutic Center affiliated with the Arak University of Medical Sciences (between May 2015 and May 2016) were included in this descriptive cross-sectional study. Stool samples were collected immediately after admission and prior to therapy. Consent and questionnaire forms were supplied to the parents or guardians of participants under 16 years old. The inclusion criteria for this study were as follows: a completed consent form and a questionnaire was filled out by the patient or the patient's parents and caregivers; diarrhea (was defined as at least three liquid or loose stools in a 24-hour period accompanied by at least one of the following symptoms: abdominal pains, vomiting, fever, watery diarrhea, and dehydration) and the availability of a stool specimen.

Clinical signs and symptoms were recorded. This study was approved by the Ethics Committee of the Arak University of Medical Sciences under No. 93-176-10. DNA and RNA were either immediately extracted from the samples or after storage of the samples at -70°C.

DNA and RNA Extraction

DNA and RNA extraction were performed directly from the stool samples using the QIAamp DNA Stool Mini Kit and QIAamp Viral RNA Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The amount and purity of the extracted DNA and RNA were measured using a NanoDrop device (Thermo Fisher Scientific, Waltham. MA, USA).

PCR and RT-PCR amplification

In-house PCR and RT-PCR assays were used to identify the viral pathogens (Table 1). The assays were designed and validated at the Virology laboratory of EmamReza clinic, Arak University of Medical sciences. The analytical sensitivity, limit of detection (LOD), of the adenovirus PCR test is defined as 212 copies/ml and the LOD value for the RT-PCR assays of rota- and noroviruses was 518 and 388 copies/ml, respectively. The final volume of the PCR was 25 µl, including 12.5 µl of master mix (1X), 2 µl of DNA template (5 ng), 1 µl each of the forward and reverse primers (10 pM), 0.5 µl of Taq DNA polymerase (2.5 units), and 8 µl of double-distilled water (all purchased from Yekta Tajhiz Company, Iran). The final volume of the RT-PCR was 20 µl and included 10 µl of master mix (1X), 5 µl of RNA template (5 ng), 0.5 µl each of the forward and reverse primers (10 pM), 0.4 µl of RT-Enzyme, and 3.6 µl of RNase free water (all

purchased from Yekta Tajhiz Company, Iran). A single positive sample for each pathogen was used for sequencing (Gene Fanavaran Company, Tehran, Iran) and the resulting sequences were confirmed using basic local alignment search tool (BLAST) analysis. Positive controls of adenovirus, rotavirus, and genogroup I, II norovirus were obtained from microbiology department of Arak University of medical sciences.

Table 1. Primers used in this study

Results

Of the 173 samples of pediatric diarrhea, eight (4.6%) were positive for diarrheal viruses, four (2.3%) with adenovirus, three (1.7%) with rotavirus, and one (0.6%) with genogroup II norovirus.

Adenovirus

One (25%) male and three (75%) female patients were diagnosed for adenoviral infection. The average age of the patients was four years and eight months. The youngest of these patients was a one-year-old girl and the oldest was a seven-year-old boy. Clinical symptoms of the patients who had pediatric adenovirus included abdominal pains, vomiting, fever, watery diarrhea, dehydration (100%), and blood in the stool (75%).

Rotavirus

Of the three (1.7%) patients who were infected by rotavirus, all (100%) were male, giving a male-to-female infection ratio of 1:0. The average age of these patients was three years and six months.

Norovirus:

A one-year-old girl was infected by Genogroup II norovirus. Clinical symptoms of the pediatric norovirus patients included abdominal pains, vomiting, fever, watery diarrhea, and dehydration (100%). Coinfection with adenovirus and rotavirus was observed in a seven-year-old boy (0.5%).

Discussion

The pathophysiological role of most of the enteric viruses in gastroenteric disease remains uncertain, underlining the requirement for studying the epidemiology of these viruses (1). Viral gastroenteritis mainly occurs in winter worldwide, with transmission chiefly through the fecal-oral route (10). This report presents for the first time an estimation of the frequency of adenovirus, norovirus, and rotavirus disease in central Iran, and it shows that the prevalence for the three most important species is low in comparison to bacterial disease (11, 12). We have demonstrated before that the prevalence of campylobacteriosis and shigellosis in the same period of present study were 19.5% and 8.2%, respectively (11, 12).

In similar studies in Iran, adenoviral prevalence in pediatric diarrheal stool samples was reported to be 2.3% in northern Iran and 9% in Shiraz (13, 14). Adenoviral prevalence has been shown to be 1.5% in Thailand and in a range of 14% in Turkey (15, 16). In the current study, the average age of the patients infected with adenovirus was four years and eight months. This result contrasts with a report from Shiraz of an average age of infection of eighteen months (14). In similar studies in Mashhad, the age range was twelve to twenty-four months (75%), and in Egypt, the age range was one to two years (17, 18). In this study, the infection ratio of males to females was 0.3:1, which was different from the ratios found in other adenoviral prevalence studies (19), but similar to the ratios found in other adenoviral prevalence studies (20). In current study, watery diarrhea, abdominal pains, fever, vomiting, and dehydration were the most common particular symptoms in pediatric patients with adenoviral infections, similar to the results of a previous study (13). The PCR test for diagnosis of adenovirus viral infections is considered as the gold standard (21). There are some problems in organizing the diagnosis of adenovirus infections, including the absence of a sensitive and rapid diagnostic method for use in public health laboratories and hospitals, causing the prevalence of these viral infections to be underestimated (21).

In this study, the prevalence of rotavirus in pediatric patients with diarrhea was 1.7% (3 cases). In similar studies in Iran, rotaviral prevalence in pediatric diarrheal stool samples was reported to be 35% in Ahvaz and 42% in Shiraz (14, 22). In other countries, rotaviral prevalence was shown to be 6.1% in Japan and 21% in France (8, 15). The difference between the results may be due to the duration of the studies, age of patients, different seasons of study, and the methods used for detecting rotavirus, (23). This study was conducted during the hot and cold seasons of the year. Outbreaks of rotaviral gastroenteritis vary with the seasons (14). Seventy eight percent of rotaviral gastroenteritis occurred during winter, while only 2% of this infection was seen among diarrheal patients in summer. This pattern is generally seen in temperate climate areas, but it is not applicable to all climate conditions. A report in Egyptian pediatrics demonstrated that most (90%) rotavirus diarrheal incidences happened during the warmer months of July–November (13). In another study, it was mentioned that the seasonal nature of rotaviral gastroenteritis was not global, and in countries within 10º of the equator, infections happened year-round (13). In similar studies, the peak of rotaviral infections in Iran (Tehran) and Latin America were found to be in autumn and winter (24, 25). Rotavirus outbreaks have been previously reported from different geographical places in Europe and the USA (26). In Europe, rotavirus is widespread during January–March (27). In the USA, studies have shown a seasonal pattern for rotavirus prevalence that starts in the southwest in November and reaches the northeast in April or May (26). However, in tropical countries like Malaysia, a seasonal pattern for rotavirus prevalence was not seen (27). Annual seasonality of viral enteritis depends on climate globally. A higher prevalence of rotavirus-caused diseases is detected in colder temperatures, and a relatively low humidity and dry weather were detected in several reports (10). The higher prevalence in these studies may depend on families staying indoors in cold weather, leading to an enhancement in contact transmission, as the dry conditions encourage aerosol formation of virus-laden particles from patients' feces (10). Improvements in sanitation and the availability of clean water have not reduced the number of rotavirus diarrheal cases (28).

In this study, the average age of the patients infected with rotavirus was three years and six months. This result contrasts with the reported average age of infection in Isfahan of eighteen months (29). Most of the rotavirus infections analyzed in our study occurred in children under seven years of age. In similar a study in Isfahan, the age range was between six and twelve months (29). This result differed from those of other studies done in Iran (Isfahan, Shahrekord), Egypt, and Kuwait, where the higher frequency of rotavirus was among infants less than twelve months (23). In this study, the infection ratio of males to females was 1:0, which was different from the ratios found in other rotavirus prevalence studies in Iran (Zabol), Yemen, and Hanoi (20, 23). In this study, watery stool, vomiting, abdominal pain, and fever were the most common particular symptoms in pediatric patients with rotavirus infections. In similar studies, the most common clinical symptoms in pediatric patients' diarrheal stool samples were reported to be watery stool, vomiting, abdominal cramps, and fever in Northern Iran (13). Since the frequency of rotavirus is low in the present study, there is no need for vaccination in the central region of Iran.

In this study, the prevalence of norovirus was 0.5% (1 case). In similar studies in Iran, noroviral prevalence in pediatric diarrheal stool samples was reported to be 0.6% in Tabriz and 8.8% in Tehran (24, 30). Noroviral prevalence was shown to be 5.8% in China and 14.8% in Japan (31, 32). The worldwide reported prevalence of norovirus among fecal samples from pediatric patients with acute sporadic gastroenteritis has a broad range, depending on whether patients have been hospitalized or not, the ages of the children, and the methodology that has been used (10). Norovirus infection is characterized by nausea, vomiting abdominal cramps and diarrhea without blood (33). In this study, abdominal pains, vomiting, fever, watery diarrhea, and dehydration were the most common particular symptoms in patient with norovirus infections, almost similar to previously reported study. There are reports indicating that vomiting in a common symptom of rotavirus infection (34, 35). However, in Burkina Faso, fever was viewed to be the most prevalent symptom (36). Some patients might only experience diarrhea or vomiting. Body aches and low-grade fever might also be correlated with the infection (37).

In this study, coinfection with adenovirus and rotavirus was observed in a seven year old boy (0.5%). In similar studies, adenovirus-rotavirus co-infection prevalence in pediatric diarrheal stool samples was reported to be 1.3% in Italy and 8% in Turkey (16, 38). Adenovirus and rotavirus coinfections have been found between 1.2% – 8.2% in some other reports (10). Eight percent coinfection with different rotavirus and norovirus was reported in China in 2011 (39).

In the report, most commonly reported symptoms were diarrhea, fever, vomiting, abdominal pain in virus-infected patients. These findings are in accordance with those conducted in South of Iran, Venezuela, Spain, Hungary and in Brasil were found fever and lymphocytosis to be significantly higher in adenovirus-rotavirus co-infected patients (40, 41)

Clinically, it would be difficult to separate viral gastroenteritis from gastroenteritis caused by other microorganism particularly, bacterial gastroenteritis. Molecular methods raised the overall diagnostic efficacy by 10%, and by 2.5% each for adenovirus and rotavirus (10). Reports which detected adenovirus using PCR have commonly reported higher frequencies (42).

Conclusion

Adenovirus, norovirus and rotavirus were rarely found as viral agents responsible for gastroenteritis among children in central Iran. Although the frequency of viral gastroenteritis has been low in this area compared to bacterial gastroenteritis (11, 12), long-term monitoring of all enteropathogenic agents should be continued in order to screen for the unexpected occurrence of outbreaks and for getting better visibility on the precise seasonality of viral and bacterial diarrhea. Doing so will also help to better understand the precise epidemiology of this serious disease and to help improve appropriate medical diagnosis and management of diarrhea in pediatric patients.

Abbreviations

PCR:polymerase chain reaction, RT-PCR:reverse transcription polymerase chain reaction, DNA:Deoxyribonucleic acid, RNA:ribonucleic acid, BLAST:basic local alignment search tool, USA:United States.

Declarations

Ethics approval and consent to participate

This study received ethical approval from the Arak University of Medical Sciences (Number: 2116). Informed consent was obtained from a parent and/or guardian for participants under 16 years old. A signed consent form was obtained from each patient. There was no access to any information that enabled authors to identify individual patients.

Consent for publication

Not applicable.

Availability of data and materials

All data pertaining to this study are within the manuscript. The datasets analyzed and/or used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors stipulate that they have no conflict of interest in regard to this study.

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Authors' contributions

EGR conceptualized and designed the study. EA were involved in the data collection, generation, and performed data analysis, MM supervised the lab works and wrote the first draft, AvB performed data analysis and writing of the paper. All authors have read and approved this version of the manuscript.

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Disclosure

AvB is an employee of bioMerieux, a company designing, developing and selling infectious disease tests. The company had no influence on the design and execution, either of the study or in the writing of the manuscript. The authors report no other conflicts of interest in this work.

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Table

Due to technical limitations, Table 1 is only available for download from the Supplementary Files section.

Supplementary Files

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