Prevalence of Rotavirus, Adenovirus, and Astrovirus Infections among Patients with Acute Gastroenteritis in, Northern Iran

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Abstract:

Background: The aim of the study was to determine the incidence of non-bacterial acute gastroenteritis associated with diarrheal diseases in Mazandaran Province, northern Iran.

Methods: A total of 400 symptomatic cases from patients with acute gastroenteritis from Mazandaran Province in Iran were screened using EIA method for the presence of rotavirus, adenovirus and astrovirus during 2005-2006. Chi-square tests were used for testing relationships between different variables.

Results: Rotavirus, adenovirus and astrovirus were detected in 62%, 2.3%, and 3% of samples, respectively. The maximum rate of rotaviruses was detected in the <1-year-old age group, while minimum rate was found in the 10 years and older age group. Astrovirus and adenovirus were detected predominantly in the 2-5-year-old age group of children, with a prevalence of 8.3% and 3.5% respectively. All studied viral gastroenteritis peaked in the winter, and minimum rate were found in summer.

Conclusion: Our statistical analyzes indicated that viral gastroenteritis, especially Rota-viral, had the highest number of occurrences in colder seasons notably in winter and more frequently were observed among younger children.

Keywords: Gastroenteritis, Rotavirus, Adenovirus, Astrovirus, Iran

Introduction

It has been shown that acute infectious diarrhea is a major cause of morbidity in infants and young children. This represents a large burden in terms of medical and indirect costs in both developed and developing countries, (1-3). Frequently, poorly cooked clams and oysters from contaminated water, contaminated foods such as salads and cake frosting, as well as, ingestion of contaminated drinking or swimming water results outbreaks of gastroenteritis (4). According to the epidemiological studies, more than 50% of gastroenteritis cases are related to known viruses. In fact, following viral upper respiratory tract illness, viral gastroenteritis has been found to be the second most common viral clinical entity among developed and developing countries (2, 5 and 6). It has been recognized that group A rotaviruses are the major etiologic agent of gastroenteritis (1-7). Furthermore, many studies demonstrated that astroviruses adenovirus types 40 and 41, and human caliciviruses can be detected with prevalence rates ranging from 2.5 to 9%, 3 to 9%, and from 0.2 to 6.6% respectively among young children with gastroenteritis (2, 4, 8). As previousely reported, rotaviruses are the most common cause of severe childhood diarrhea worldwide (1-9); however, the illness related to rotavirus has also been observed less commonly in older children and adults. Unfortunately, rotaviral gastroenteritis may result in mortality for populations at risk such as infants and the elderly (2, 4).

The ability of adenoviruses to cause acute infection in both the gastrointestinal tract and the respiratory system, makes them quite important. It has been reported that they cause about 5% of all infectious diseases in infants and nearly 3% in children from 2-4 yr old (10). Other studies revealed that adenovirus types 40 and 41 are the sole adenoviruses that have been found associated with gastroenteritis in infants and young children, and may be the second major causative agent of gastroenteritis after rotaviruses (2, 4).

Astrovirus infection had been shown to occur in small outbreaks and primarily during the winter season of the year (11). Based on some community studies, the prevalence of astrovirus infection was estimated to be between 5 to 17%. while this figure was 2 to 16% among hospitalized children with diarrhea (12, 13). They were associated with 3-12% of diarrhea episodes in developed countries (1, 14). The detection of astrovirus infections was also reported in various developing countries of the world (14-16). Astrovirus infection in adult gastroenteritis has also been reported (14, 17). Although astrovirus infection has been associated with gastroenteritis mostly in children, adults, the elderly and immunocompromised patients (18), it is noteworthy that most surveys had concentrated on infections amongst children because they were thought to be at greater risk (14).

In developing countries such as Iran, only few studies have been reported to determine the incidence of viral-related acute gastroenteritis (2, 19). In countries such as Iran, etiological knowledge of acute diarrhea associated with viralrelated pathogens is very important in planning diarrhea disease control strategies, which will be useful for future vaccine development in the region. Therefore, our primary goal was to determine the incidence of non-bacterial acute gastroenteritis associated with diarrheal diseases in Mazandaran Province, northern Iran. In addition, our secondary goal was to examine the association of incidence of viral gastroenteritis in relationship to age, sex and the season distribution pattern in this population.

Materials and Methods

Samples collection

Fecal samples were collected from 400 patients admitted to Hospitals and Health Centers in Mazandaran Province, Iran, for diarrhea from Jan 2005 to Jan 2006. Specimens were transported to the Virology Laboratory of our department in a sterile specimen cup placed in a cooler and either processed immediately, or stored at -20 °C for processing later. The demographic characteristics of the patient and a clinical history for each patient was collected by a nurse or attending physician, noting the following signs and symptoms: diarrhea, vomiting, abdominal pain, dehydration, fever, and blood or mucus in stool. The majority of samples were belong to young children, while only 15 samples (3.8%) were collected from >10 years old age group. The mean and median ages were 2.9 yr (range, 1 mo to 84 yr; standard deviation, 0.45) and 18 mo, respectively.

Laboratory methods

Stool samples were screened for the presence of Rotaviruses, Astroviruses and Adenovirus by an enzyme immunoassay (EIA).

Rotavirus, Adenovirus and Astrovirus detection

Stool samples were processed to detect Rotavirus, Adenovirus and Astrovirus antigens. An enzyme immunoassay (EIA) was performed with commercial EIA kits (IDEIATM Rotavirus/ Adenovirus/Astrovirus, DakoCytomation, Denmark) according to manufacturer procedures.

Statistical methods

To estimate the relative risk of Acute Gastroenteritis attributable to viruses, we used two-dimensional tables and chi-square or fisher's exact tests. A *P*-value < 0.05 deemed significant and analysis were preformed with SPSS (Version 11.5).

Results

A total of 400 patients with diarrhea were screened for viral gastroenteritis. Their stool samples were processed for Rotavirus, Adenovirus and Astrovirus by EIA method. As indicated in Table 1 62% of studied patients were positive for rotaviruses, while astrovirus and adenoviruses were detected only in 3% and 2.3%, respectively. The proportional age distribution of patients with gastroenteritis is depicted in Table 1. Rotaviruses were the most common cause of acute gastroenteritis and occurred most frequently with 66.1% in the <1-yr old age group, while the least common rate of rotavirus infection was 46.7% among 10 yr and older age group. Rotavirus infections were identified in 64% of the male and 58.5% of the female gastroenteritis patients examined. Also as indicated in Table 1, Astrovirus was detected predominantly in the 2-5 yr old age group and the <2 yr old age group of children. In addition,

one patient in 5-10 yr old age group gave positive result for Astrovirus. The majority of enteric adenovirus detection occurred in the 2-5 yr old age groups while only a single case was positive for enteric adenovirus in the >10-yr old age group (Table 1).

The major clinical symptoms related to viral gastroenteritis in this study are shown in Table 2. Symptoms associated with rotavirus infection were mainly watery stool, vomiting, abdominal cramps, and fever. All patients with enteric adenovirus infections had symptoms associated with watery stool and abdominal cramps, while vomiting, and fever were not found in all studied cases. All patients with Astrovirus infection displayed symptoms including watery stool, vomiting, abdominal cramps, and fever.

The seasonal distribution of viral gastroenteritis in percentage of each pathogen detected is displayed in Table 3. All viral gastroenteritis peaked in the winter with minimum rate in summer.

Table	1: Distribution	of viral agen	ts in acute	gastroenteritis	patients by age	in Mazandaran	Province ^a

Rotavirus		Astrovirus		Adenovirus		ND ^c		Total	
n	%	n	%	n	%	n	%	Total	
82	66.1	2	1.6	0	0	38	30.3	124	
95	65.5	2	1.4	2	1.4	46	31.7	145	
48	57.1	7	8.3	6	7.1	23	27.5	84	
16	50	1	3.1	0	0	15	46.9	32	
7	46.7	0	0	1	6.7	7	46.6	15	
248	62	12	3	9	2.3	129	32.2	400	
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a- Northen Iran b- Age in year c- Not Determined

 Table 2: Distribution of clinical symptoms associated by viral acute gastroenteritis patients (%)

		Diarrheic patients with following symptoms (%)						
Pathogens N	o. of cases	Watery/loose stool	Vomiting	Abdominal cramp	Fever			
Rotavirus	248	99	62	64	56			
Astrovirus	12	100	100	100	100			
Adenovirus	9	100	67	100	77			

Table 3: Distribution of vira	l agents in a	cute gastroenteritis	patients by season	in Mazandaran	province
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Saasan	Rotavirus		Astrovirus		Adenovirus		ND ^b		Tatal
Season	n	%	n	%	n	%	n	%	Total
Spring	12	34.3	0	0	0	0	23	65.7	35
Summer	10	37	1	3.7	1	3.7	15	55.6	27
Autumn	63	62.4	1	1	1	1	36	35.6	101
Winter	163	68.8	10	4.2	7	3	55	23.2	237
total	248	62	12	3	9	2.3	129	32.2	400

a- Northen Iran b Not Determined

Discussion

Diarrheal diseases are causes of major public health problems in developed and developing countries (1-3). An understanding of the relative contribution of viral gastroenteritis is essential for implementation of appropriate public health measures in controlling these diseases. This study showed that rotaviruses were an important etiologic agent of acute gastroenteritis throughout the year among patients with diarrhea in Mazandaran Province, and were detected in 62% of patients with acute gastroenteritis. Previous studies in developed and developing countries have similarly shown that rotavirus is an important pathogen, responsible for 55% of the gastroenteritis in Australia (20), 79% in Germany (21), 42% in Indonesia (2), 14.1% in Saudi Arabia (22), 32.5% in Northern Jordan (23), 55.9% in China (24) and 61% in France (1). In the present study a 61.1% infection rate was found among children aged <1 yr. This finding is in agreement with previous studies done in Tehran, Iran (19), which showed a 70% rate of rotavirus in diarrheic patients who were 2 yr of age or less. Another study in Bahrain showed that rotavirus was detected most frequently among the age group of 6-11 mon (25). In Central Africa, rotaviruses were found most frequently among children less than 1year-old (26). No significant difference in the detection rate between male and female patients in the current study was noted. This is in contrast to the findings in China and Bahrain, where the majority of rotavirus infections occurred in males (24, 25). The major clinical symptoms associated with rotavirus infection in this study showed a high percentage associated with watery stool (99%), vomiting (62%), abdominal cramps (64%) and fever (56%). This finding was similar to studies conducted in Indonesia, Thailand and Egypt (27, 28). There was statistical correlation between the detection rate of rotavirus and season in this study; findings indicated that 68.8% of acute gastroenteritis patients in winter were infected by rotavirus. In

the other hand, 78% of Rotaviral gastroenteritis incidence was occurred during winter, while only 2% of this infection was found among diarrheal patients in summer. This pattern is usually observed in temperate climate regions and it is not applicable to all climate conditions. However, a study in Egyptian children indicated that most (90%) of rotavirus diarrheal incidences occurred during the warmer months of July-November (27). In another study, it was mentioned that the seasonal nature of rotaviral gastroenteritis was not universal and in countries within 10° of the equator infection occurred year-round (29). Astroviruses have been increasingly identified as important agents of acute gastroenteritis in children (30-33) and elderly (34). Outbreaks of astrovirus gastroenteritis have been reported for young adults, including military recruits (35), students, teachers (36), and children (37). The present study allowed us to explore the age-related prevalence, the seasonality of infection and the medical significance of astrovirus infection from patients admitted for diarrhea. Our findings reveled that about 3% of acute gastroenteritis patients were infected by Astroviruses and maximum rate (8.3%) of infection was observed among the age group of 2-5 yr, while we never find any astrovirus infection among >10-years-old age groups. The prevalence of astrovirus infection in this study was strikingly age-related and primarily occurred among children in the first years of life. In the other hand, according to our results, the proportional distribution of astrovirus infection was as follows: children <1 yr of age 16.7% (2/12); children between 1 and 2 yr old 16.7% (2/12); between 2 and 5 yr old 58.3% (7/12); between 5 and 10yr old 8.3% (1/12) and those older or equal 10 yr 0% (0/12). Indeed, almost 92% of astrovirus infections was occurred among <5 yr old age children. The seasonal pattern of astrovirus incidence documented in our study is consistent with a majority of other studies describe a predilection of astrovirus for winter or the rainy season in populations living also in temperate regions (38, 39). However, reports exist

which describe higher astrovirus prevalence during spring and summer months than during the cold months of the year (40-42). The reason of the different seasonal astrovirus pattern is unclear.

Enteric adenoviruses were found in 9/400 (2.3%) patients with acute diarrhea. Previous studies in developing countries such as in China, Saudi Arabia, Singapore and Indonesia showed that enteric adenovirus was found in 2.5%, 5.3%, 3% and 4% of diarrheal patients respectively (2, 22, 24, 43). The fact that enteric adenoviruses produced symptoms that were milder than symptoms in patients with rotavirus infection may have resulted in under-reporting, in that the number of children with adenovirus infections seeking hospital attention may have been few in number. In the present study, the majority of enteric adenovirus infections were detected in children from 0 to 5 vr old. This finding is in agreement with a study conducted in Saudi Arabia (22). Enteric adenovirus infection in this study appeared to have notable seasonal distribution; in which 7 out of 9 (77.8%) adenoviral gastroenteritis were found in winter. The incidence of enteric viral pathogens among patients with gastroenteritis in Mazandaran Province, Iran, is estimated in this study. Data from this study show that for acute gastroenteritis, 67.3% were associated with infection with studied enteric viruses. Knowledge of the etiology of acute diarrhea associated mainly with viral pathogens is relevant for planning diarrhea disease control strategies in Iran, which will be useful for future vaccine development in the region.

Ethical Consideration

All Ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc) have been considered carefully.

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References

- 1. Bon F, Fascia P, Dauvergne M, Tenenbaum D, Planson H, Petion AM, et al. (1999). Prevalence of Group A Rotavirus, Human Calicivirus, Astrovirus, and Adenovirus Type 40 and 41 Infections among Children with Acute Gastroenteritis in Dijon, France. J Clin Microbiol, 37(9): 3055-58.
- 2. Subekti D, Lesmana M, Tjaniadi P, Safari N, Frazier E, Simanjuntak C, et al. (2002). Incidence of Norwalk-like viruses, rotavirus and adenovirus infection in patients with acute gastroenteritis in Jakarta, Indonesia. *FEMS Immunol Med Microbiol*, 33: 27-33.
- 3. Bern C, Martines J, deZoysa I, Glass RI (1992). The magnitude of the global problem of diarrheal disease: a ten-year update. *Bull WHO* 70: 705-714.
- Blacklow NR, Greenberg HB (1991). Medical progress: Viral gastroenteritis. New Engl J Med, 325: 252-63.
- Black RE (2001). Diarrheal diseases. In: *In-fectious Disease Epidemiology*, Eds, Nelson KE, Williams CM, Graham NMH, Aspen Publication, Gaithersburg, pp. 497-517.
- Kapikian AZ, Kim HW, Wyatt RG, Cline WL, Arrobio JO, Brandt CD, et al. (1976). Human reovirus-like agent as the major pathogen associated with `winter' gastroenteritis in hospitalized infants and young children. *New Engl Med*, 294: 965-72.
- Toshiyuki Mikami, Toyoko Nakagomi, Rika Tsutsui, Kazuko Ishikawa, Yohko Onodera, kokichi Arisawa, Osamu Nakagomi (2004). An outbreak of gastroenteritis during school trip caused by serotype G2 group A rotavirus. *J Med Virol*, 73: 460-64.
- B. Garthright WE, Archer DL, Kvenberg JE (1998). Estimates of incidence and costs of intestinal infectious diseases in the United States. *Public Health Rep*, 103: 107-15.

- 9. De Zoysa I, Feachem RG (1985). Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. *Bull WHO* 63: 569-83.
- Pacini DL, Collier AM, Henderson FM (1987). Adenovirus infections and respiratory illnesses in children in group day care. *J Infect Dis.* 156: 920-26.
- Matsui MS, Greenberg HB (2007). Astroviruses. In: *Fields Virology*. Eds, Knipe DM., Howley PM. 4th ed. Lippincott Williams & Wilkins, USA, pp. 875-76.
- Oliver AR, Phillips AD (1988). An electron microscopical investigation of faecal small round viruses. *J Med Virol*, 24(2): 211-18.
- 13. Walter JE, Mitchell DK (2000). Role of astrovirus in childhood diarrhoea. *Curr Opin Pediat*, 12: 275-79.
- 14. Bhattacharya R, Sahoo GC, Nayak MK, Ghosh S, Dutta P, Bhattacharya MK, et al. (2006). Molecular epidemiology of human astrovirus infections in Kolkata, India, *Infect Genet Evol*, 6: 425-35.
- 15. Liu C, Grillner L, Jonsson K, Linde A, Shen K, Lindell AT, et al. (2006). Identification of viral agents associated with diarrhea in young children during a winter season in Beijing, China. *J Clin Virol*, 35(1): 69-72.
- Phan TG, Okame M, Nguyen TA, Maneekarn N, Nishio O, Okitsu S, Ushijima H (2004). Human astrovirus, norovirus (GI, GII) and Sapovirus infections in Pakistani children with diarrhoea. *J Med Virol*, 73(2): 256-61.
- 17. Pager CT, Steele AD (2002). Astrovirus-associated diarrhea in South African adults. *Clin Infect Dis*, 35 (11): 1452-53.
- Belliot G, Laveran H, Monroe SS (1997). Detection and genetic differentiation of human astroviruses: phylogenetic grouping varies by coding region. *Arch Virol*, 142: 1323-34.
- 19. Zarnani AH, Modarres SH, Jadaliv F, Sabahi F, et al. (2004). Role of rotaviruses in children with acute diarrhea in Tehran, Iran. *J Cline Virol*, 29: 189 -93.

- Barnes GL, Uren E, Stevens KB, Bishop RF (1998). Etiology of acute gastroenteritis in hospitalized children in Melbourne, Australia, from April 1980 to March 1993. *J Cli Microbiol*, 36(1): 133-38.
- 21. Oh DY, Gaedicke G, Schreier E (2003). Viral agent of acute gastroenteritis in German children: prevalence and Molecular diversity. *J Med Virol*, 71: 82-93.
- Akhter J, Burdette JM, Qadri SMH, Myint SH (1999). Etiology of gastroenteritis at a major referral centre in Saudi Arabia. *J Int Med Res*, 22: 47-54.
- 23. Youssef M, Shurman A, Bougnoux ME, Rawashdeh M, Bretagne S, Strockbine N (2000). Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. *FEMS Immunol Med Microbil*, 28: 257-63.
- Qiao H, Nilsson M, Abreu ER, Hedlund KO, Johansen K (1999). Viral diarrhea in children in Beijing, China. J Med Virol, 57: 390-96.
- 25. Dutta, SR, Khalfan SA, Baig BA, Philipose L, Fulaytil R (1990). Epidemiology of Rotavirus diarrhoea in children under 5 years in Bahrain. *Int J Epidemiol*, 19: 722-27.
- 26. Georges MC, Wachsmuth IK, Meunier DMV, Nebout N, Didier F, Siopathis MR, Georges AJ (1984). Parasitic, bacterial, and viral enteric pathogens associated with diarrhea in Central African Republic. *J Clin Microbiol*, 19: 571-75.
- 27. Naficy AB, Abu-Elyazeed R, Holmes JL, Rao MR, Savarino SJ, Kim Y, et al. (1999). Epidemiology of rotavirus diarrhea in egyptian children and implications for disease control. *Am J Epidemiol*, 150: 770-77.
- 28. Hendrick MK, Cuevas LE, Hart CA (1995). Rotavirus diarrhea in Thai infants and children. *Ann Trop Paediatr*, 15: 147-52.
- LaBaron CW, Lew J, Glass RI, Weber JM, Ruiz-Palacios GM (1990). Rotavirus Study Group, Annual rotavirus epidemic patterns in North America. J Am Med Assoc, 264: 983-88.

- Dalton RM, Roman ER, Negredo AA, Wilhelmi ID, Glass RI, Sanchez-Fauquier A (2002). Astrovirus acute gastroenteritis among children in Madrid, Spain. *Pediatr Infect Dis J*, 21: 1038-41.
- 31. Guix S, Caballero S, Villena C, Bartolome R, Latorre C, Rabella N, et al. (2002). Molecular epidemiology of astrovirus infection in Barcelona, Spain. J Clin Microbiol, 40: 133-39.
- Medina SM, Gutierrez MF, Liprandi F, Ludert JE (2000). Identification and type distribution of astroviruses among children with gastroenteritis in Colombia and Venezuela. J Clin Microbiol, 38: 3481-83.
- 33. Schnagl RD, Belfrage K, Farrington R, Hutchinson K, Lewis V, Erlich J, Morey F (2002). Incidence of human astrovirus in central Australia (1995 to 1998) and comparison of deduced serotypes detected from 1981 to 1998. J Clin Microbiol, 40: 4114-20.
- 34. Gray JJ, Wreghitt TG, Cubitt WD, Elliot PR (1987). An outbreak of gastroenteritis in a home for the elderly associated with astrovirus type 1 and human calicivirus. J Med Virol, 23: 377-81.
- 35. Belliot G, Laveran H, Monroe SS (1997). Outbreak of gastroenteritis in military recruits associated with serotype 3 astrovirus infection. *J Med Virol*, 51: 101-106.
- 36. Oishi I, Yamazaki K, Kimoto T, Minekawa Y, Utagawa E, Yamazaki S, et al (1994). A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. J Infect Dis, 170: 439-43.
- 37. Mitchell DK, Monroe SS, Jiang X, Matson DO, Glass RI, Pickering LK (1995). Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase-polymerase chain reaction. J Infect Dis, 172: 1437-44.
- 38. Cruz JR, Bartlett AV, Herrmann JE, Cáceres P, Blacklow NR, Cano F (1992).

Astrovirus associated diarrhea among Guatemalan ambulatory rural children. *J Clin Microbiol*, 30: 1140-44.

- 39. Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Logan L, et al. (1998). Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. *J Infect Dis*, 178: 334-39.
- 40. Giordano MO, Martinez LC, Isa MB, Paez Rearte M, Nates SV (2004). Childhood astrovirus-associated diarrhea in the ambulatory setting in a Public Hospital in Cordoba city, Argentina. *Rev Inst Med Trop Sao Paulo*, 46(2): 93-96.
- 41. Guerrero ML, Noel JS, Mitchell DK, Calva JJ, Morrow AL, Martínez J, et al. (1998). A prospective study of astrovirus diarrhea of infancy in Mexico City. *Pediat Infect Dis J*, 17: 723-27.
- 42. Noel J, Cubitt D (1994). Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981-93. *Epidem Infect*, 113: 153-59.
- 43. Mendis L, Kumarasinghe G, Chow C, Liew HY, Ramachandran NP, Yayawardane K, et al. (1995). Bacteria, viruses, yeasts and protozoans associated with diarrheal disease in Singapore. *Pathology*, 27: 48-52.