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A Clinico-Epidemiological Comparison Study of Pediatric Acute Viral Gastroenteritis at a Tertiary Care Hospital

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Background: Acute viral gastroenteritis is a common illness in young children. Rotavirus, norovirus and enteric adenovirus are the major agents for viral gastroenteritis. Their detection rates have gradually increased in Korea. Our aim was to monitor the epidemiologic characteristics of the aforementioned viruses and to determine the laboratory and clinical characteristics of pediatric patients infected with these viruses.
Methods: From December 2009 to November 2010, 685 stool specimens from patients hospitalized at Chung-Ang University Hospital were tested for the aforementioned viruses using multiplex PCR. A corresponding medical record review was retrospectively conducted.
Results: The overall prevalence rate was 44.8%, with rates of 16.3%, 1.9%, 22.7%, 3.1%, and 0.8% for rotavirus, norovirus genogroup I, norovirus genogroup II, enteric adenovirus, and astrovirus, respectively. Mixed virus infections were detected in 37 patients (5.4%). The highest incidence rates occurred in March 2010 (18.9%), in the 13-24 month age group (38.1%), and in males (53.1%). Fever and chills were most frequently observed in patients with adenovirus (44.4%) than other viruses, while diarrhea was most frequently observed in patients with rotavirus (93.7%). Leukocytosis (55.0%) and lymphocytosis (21.0%) were more common in the norovirus-infected group than other viruses-infected group.
Conclusion: Our results show different prevalence rates and clinical findings for each gastroenteritis-associated virus. To better understand the clinico-epidemiological features observed in this study, further epidemiologic and clinical investigations are needed. **(Ann Clin Microbiol 2016;19:33-38)**

Key Words: Epidemiology, Gastroenteritis, Pediatrics, Polymerase chain reaction, Viruses

INTRODUCTION

Acute gastroenteritis due to microbial infection is caused by viruses, bacteria, and protozoa. Of these, acute viral gastroenteritis is a common illness in young children. The major causal agents of acute viral gastroenteritis include rotavirus, norovirus, enteric adenovirus, and astrovirus [1]. Although improvements have been made in hygiene and lifestyle, acute viral gastroenteritis still has a great impact on young children. Symptoms of this disease include vomiting, fever, and dehydration due to continuous diarrhea. All of these symptoms can eventually lead to death if proper management does not made [2]. In South Korea, rotavirus, norovirus, enteric adenovirus, and astrovirus are the most commonly detected pathogens in patients with pediatric acute gastroenteritis. The detection rate has gradually increased [1,3,4]. Many survey reports have conducted for rotavirus, norovirus and adenovirus [5-7]. However, there is relatively little data on the laboratory and clinical differences among the major viral agents causing this disease in South Korea. The aim of this study was to monitor the prevalence, age group distribution, and gender distribution of the aforementioned viruses on a monthly basis and to determine the laboratory and clinical characteristics of patients infected with these viruses.

MATERIALS AND METHODS

1. Specimens

From December 2009 to November 2010, 685 stool speci-

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mens were obtained from patients hospitalized due to suspected acute gastroenteritis (patients who showed systemic symptoms such as fever, chill, and headache, or gastrointestinal symptoms such as vomiting and diarrhea) in the Department of Pediatrics at Chung-Ang University Hospital. Residual stool specimens after stool culture were used to perform PCR. Specimens with insufficient quantily were excluded. The specimens were tested for rotavirus, norovirus, enteric adenovirus, and astrovirus infections.

2. Identification of viruses

PCR was performed during December 2009 to November 2010. Small amount of stool (less than the size of cotton swab) was diluted in 500 μL of phosphate buffer solution, then boiled with heating block at 100°C. After boiling, the specimen was centrifuged and the supernatant was used. Viral RNA/DNA was extracted using a Viral gene-spin viral DNA/RNA gene extraction kit (iNtRON biotechnology, Seongnam, Korea). cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada). Viral nucleic acid amplification was performed using multiplex PCR with a Seeplex Diarrhea-V ACE Detection kit (Seegene, Seoul, Korea) that can detect four viruses (rotavirus, norovirus, enteric adenovirus, and astrovirus).

3. Laboratory and clinical characteristics

The laboratory characteristics studied were WBC count, platelet count, absolute neutrophil count (ANC), lymphocyte count, and blood urea nitrogen (BUN). The clinical characteristics studied were fever (≥38°C), chills, headache, abdominal pain, vomiting (more than once), diarrhea (more than once), and oliguria. The subjective symptoms were recorded based on parents' observation. A retrospective medical record review was conducted to examine the laboratory and clinical characteristics of patients with more than one virus detected. Reference values for WBC, platelet counts, ANC, lymphocyte count, and BUN were used based on textbook criteria [8]. This study was approved by the Institutional Review Board of Chung-Ang University Hospital, Seoul, South Korea.

4. Statistical analysis

The χ^2 test was used to measure qualitative variables, and the ANOVA test was used to measure quantitative variables. All statistical analyses were carried out using the SPSS V14.0 statistical program. A P value <0.05 was considered statistically significant.

RESULTS

1. Epidemiologic features

The overall prevalence rate was 44.8%, with rates of 16.3%, 1.9%, 22.7%, 3.1%, and 0.8% for rotavirus, norovirus genogroup I, norovirus genogroup II, enteric adenovirus, and astrovirus, respectively. Mixed virus infections were detected in 37 (5.4%) of the 685 specimens. These consisted of combinations of rotavirus and norovirus genogroup II (4.1%), rotavirus

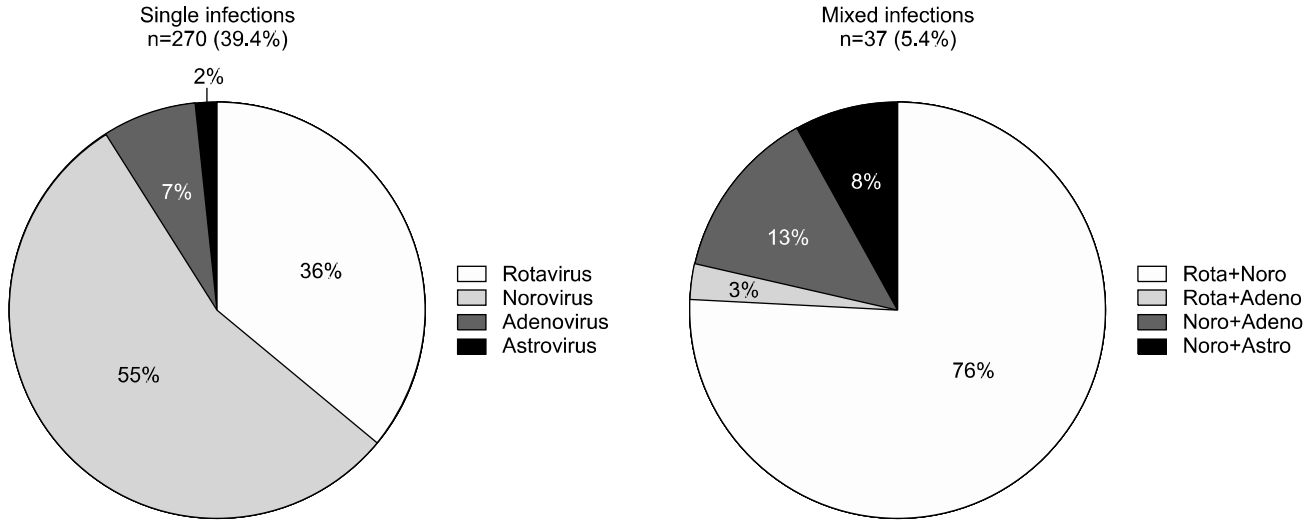

Fig. 1. Prevalence of diarrheal viruses. Distribution of positive cases from December 2009 to November 2010. Numbers in the graph are relative percentages of a certain virus.

and adenovirus (0.1%), norovirus genogroup II and adenovirus (0.7%), and norovirus genogroup II and astrovirus (0.5%) (Fig. 1). The highest incidence was found in the male group (53.1%), in March 2010 (18.9%), and in the 13-24 months age group (38.1%) (Fig. 2 and 3). Norovirus was the most prevalent virus across all ages except 25-48 months old, and from late autumn to early summer. Rotavirus was second prevalent virus during a year, and most prevalent virus in children with 25-48 months old. Adenovirus was gradually increased from July, and peak at October. Astrovirus mainly detected during early spring (Fig. 2 and 3).

2. Clinical features

Because the number of astrovirus infections was too small to determine significant clinical differences, the clinical features for only the three other viruses were determined. Among infections caused by a single viral agent, fever was observed in patients with adenovirus (94.4%), rotavirus (64.8%) and norovirus (62.0%) infections; chills were observed in patients with adenovirus (44.4%), norovirus (5.2%), and rotavirus (5.2%) infections; headache was observed in patients with adenovirus (5.5%), rotavirus (1.0%) and norovirus (0.0%) infections; diarrhea was observed with rotavirus (93.7%), adenovirus (88.9%), and norovirus infections (65.6%). The differences were statistically significant. Interestingly, the incidence of diarrhea was higher in the rotavirus-infected group (93.7%) than in the norovirus-infected group. However, leukocytosis (55.0%) and lymphocytosis (21.0%) were more common in the norovirus-infected group (Table 1). There was no significant clinical difference detected between the mono- and co-infected groups.

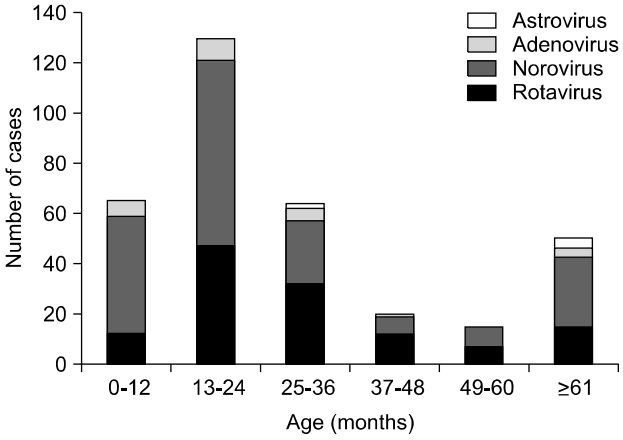

Fig. 2. Distribution of acute gastroenteritis viral agents by age groups.

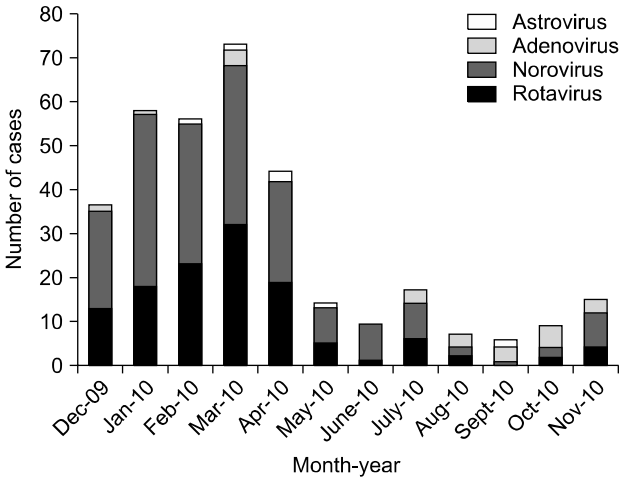

Fig. 3. Distribution of acute gastroenteritis viral agents by months.

DISCUSSION

Since the 1940s, viruses have been suspected as important causes of gastroenteritis, although the etiology has remained unknown in most cases. In 1972, Kapikian et al. [9] first identified a virus (Norwalk virus) as a cause of gastroenteritis based on its presence in feces after a diarrhea outbreak. One year later, Bishop et al. observed rotavirus in the duodenal mucosa of children with gastroenteritis [10]. In 1975, astroviruses and enteric adenovirus were identified in the feces of children with acute diarrhea [11].

Table 1. Comparison of laboratory findings and clinical characteristics for each viral agent

	Rotavirus (n=96)	Norovirus (n=153)	Adenovirus (n=18)
Leukocytosis*	42 (43.8%)	84 (55.0%)	8 (44.4%)
Thrombocytosis	15 (15.6%)	28 (18.3%)	4 (22.2%)
Neutrophilia	20 (20.8%)	34 (22.2%)	4 (22.2%)
Lymphocytosis*	12 (12.5%)	32 (21.0%)	4 (22.2%)
Elevated CRP	76 (79.1%)	98 (64.0%)	15 (83.3%)
Elevated BUN	32 (33.3%)	53 (34.6%)	2 (11.1%)
Fever†	67 (69.8%)	95 (62.0%)	17 (94.4%)
Chill†	5 (5.2%)	8 (5.2%)	8 (44.4%)
Headache†	1 (1.0%)	0 (0%)	1 (5.5%)
Abdominal pain	25 (26.0%)	31 (20.2%)	8 (44.4%)
Nausea	35 (36.4%)	44 (28.7%)	8 (44.4%)
Vomiting	86 (89.6%)	119 (77.8%)	16 (88.9%)
Diarrhea*†	90 (93.7%)	100 (65.4%)	16 (88.9%)
Oliguria	6 (6.2%)	5 (3.3%)	1 (5.5%)

*Difference between rotavirus and norovirus, †Statistically difference ($P<0.05$) among the three viral agents.
Abbreviations: CRP, C-reactive protein; BUN, blood urea nitrogen.

Most survey reports have focused on common single virus infections. However, few studies have compared the clinical characteristics of the four common types of gastroenteritis-causing viruses. According to a study which surveyed the causes of viral gastroenteritis in Korea from 2000 to 2006, the total prevalence was 18.5%. Of these cases, rotaviral infections were responsible for 66.0% of cases, and noroviral infections were responsible for 21.9% of cases [12]. Also, a neonate outbreak has been reported as caused by norovirus [13]. According to an epidemiologic study of noroviral infection from 2007 to 2010, the prevalence of noroviral infection was highest in children younger than 3 years of age (14.6%) [14]. An acute gastroenteritis study performed in Seoul, Korea during 2004 to 2007 showed that the prevalence of viral gastroenteritis was 28.3% in 2004 and 42.2% in 2007. These data suggest that cases of viral gastroenteritis were increasing. Rotavirus prevalence was the highest in 2004 and 2005, while norovirus prevalence was the highest in 2006 and 2007. Both of these viruses are important causative viral agents [3]. In our study, the overall prevalence of viral gastroenteritis was 44.8%, a finding that agrees with previous studies. However, the prevalence of norovirus (55.0%) was higher than that of rotavirus (36.3%). This difference is likely due to the methodological differences in their detection and vaccination for rotavirus. There are limited data available on simultaneous infections in gastroenteritis, and the rate of such infections varies widely in the literature [1,15,16]. One study performed in Gyeonggi province, South Korea reported an overall mixed infection rate of 6.2%, with rotavirus-norovirus (44.4%) and rotavirus-astrovirus (40%) as the most common viral co-infections [1]. The present study found that the incidence and nature of mixed infections (5.4%) (with rotavirus-norovirus mixed infection as the most prevalent) were similar to those reported in other studies. Clinical symptoms were not significantly different when single and mixed infections were compared. A larger sample size should be used to further evaluate the prevalence of and clinical differences between single and mixed enteric virus infections.

In general, acute viral gastroenteritis in pediatric patients is more prevalent in males than females [17,18]. The present study supports this trend, as the male group (53.1%) had a higher prevalence than the female group (46.9%). Viral incidence increased through winter (December: 12.6%), peaked during winter-spring (February: 16.3%, March: 18.9%), and decreased from late spring to autumn. The incidence rate was highest in infants younger than three years (76.5%). These distributions were similar to those found in other studies performed in Korea [1,4]. Our data showed the detection rate of norovirus was higher than rotavirus during March and April, 2009. According to datas from Korea Centers for Disease Control and Prevention (KCDC), the detection rate of norovirus was about 4% higher than that of rotavirus in February 2010. In March 2010, the detection rate of rotavirus was about 3% higher than that of norovirus. In other words, the detection rate of norovirus at early spring was relatively higher than other years. In this study, the detection rate of norovirus was highest from December 2009 to April 2010, that was different in general. However, it was similar distribution with the data from KCDC.

Symptoms of pediatric acute viral gastroenteritis include abdominal pain, nausea, vomiting, diarrhea, fever, myalgia, and headache. In this study, systemic symptoms (fever, chills and headache) were dominant in cases of adenovirus infection. This can be explained by higher C-reactive protein values and combined respiratory infections that are associated with adenoviral gastroenteritis [19]. More than half of the cases occurred in children under 24 months of age. These children were considered not capable of providing reliable information about systemic symptoms. Therefore, symptoms were collected based on parents' observations. A low number of cases presented chills and headache. More cases are needed to evaluate the significance of these symptoms. Similar to what was found in previous studies, diarrhea was significantly associated with rotavirus (93.7%), adenovirus (88.9%), and norovirus (65.6%) ($P<0.05$) [1,3]. Among the two most common viral agents, diarrhea was observed more frequently in rotaviral infections (93.6%) than in noroviral infections (65.6%) ($P<0.05$). This result corresponds with previous studies that have demonstrated the similar symptoms of noroviral and rotaviral gastroenteritis, and that noroviral gastroenteritis has a milder course [20-22]. Leukocytosis (55.0%) and lymphocytosis (21.0%) were more frequently observed in the norovirus-infected group than in the rotavirus-infected group, again matching findings from a previous study [23]. For noroviral gastroenteritis cases, the clinical symptoms were milder than the laboratory findings.

Our study has limitations. This study was performed during a year only, and conducted in a single institution. In addition, the subjective symptoms were mostly collected based on parents' observation, therefore the reliability was not high.

In conclusion, pediatric acute viral gastroenteritis was predominant in males, in children younger than three years, and in the winter and early spring. Symptom differences among the vi-

ral agents were noted. Diarrhea was most frequently observed in rotaviral gastroenteritis, and systemic symptoms were predominantly observed in adenoviral gastroenteritis. For noroviral gastroenteritis cases, clinical symptoms were milder than laboratory findings. Continuous specimen collection and analysis are needed to better understand the clinico-epidemiological features observed in this study. In addition, further research is required regarding the epidemiologic characteristics of enteric viral infections.

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=국문초록=

단일 의료기관에서의 소아 급성 바이러스성 장염에 대한 임상 역학적 분석

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배경: 급성 바이러스성 위장관염은 소아에서 흔한 질환으로 그 중 rotavirus, norovirus, 그리고 enteric adenovirus에 의한 경우가 대부분을 차지한다. 한국에서도 이러한 바이러스들에 의한 급성 위장관염이 많이 보고되어 있고, 바이러스 검출률 역시 점차 증가하고 있는 추세이다. 본 연구에서는 소아 위장염 환자에서 검출된 바이러스의 역학적 특성과 감염 환자의 임상적 특성을 분석하였다.

방법: 2009년 12월부터 2010년 11월까지 장염 관련 바이러스검사가 의뢰된 685개의 대변 검체를 대상으로 다중 중합효소연쇄반응으로 바이러스를 검출하였고, 후향적으로 임상기록지를 분석하였다.

결과: 전체 양성률은 44.8%였고 5.4% (37명)에서 중복 감염이었으며, rotavirus, norovirus genogroup I, norovirus genogroup II, enteric adenovirus 및 astrovirus가 각각 16.3%, 1.9%, 22.7%, 3.1% 및 0.8%의 양성률을 보였다. 바이러스는 2010년 3월(18.9%), 13-24개월의 환아(38.1%), 그리고 남아(53.1%)에서 높은 빈도로 검출되었다. 설사는 rotavirus 검출 환자에서 가장 흔하게 관찰된(93.7%) 반면, 오한은 adenovirus 검출 환자에서 가장 흔하게 나타났다(44.4%). 백혈구증가와 림프구증가는 norovirus 감염 환자에서 55.0%와 21.0%로 흔하였다.

결론: 본 연구는 각 바이러스에 따라 검출 빈도와 임상 소견이 다름을 보여 주었고, 추후 이러한 임상 역학적 특성을 확인하기 위한 지속적인 관찰이 필요하다고 생각되었다. [Ann Clin Microbiol 2016;19:33-38]

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