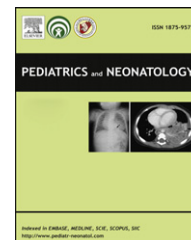


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ORIGINAL ARTICLE

Clinical Characteristics and Genotypes of Rotaviruses in a Neonatal Intensive Care Unit

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Key Words

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Background: There are few reports on the symptoms of rotavirus infections in neonates. This study aims to describe clinical signs of rotavirus infections among neonates, with a particular focus on preterm infants, and to show the distribution of genotypes in a neonatal intensive care unit (NICU). **Methods:** A prospective observational study was conducted at a regional NICU for 1 year. Stool specimens from every infant in the NICU were collected on admission, at weekly intervals, and from infants showing symptoms. Rotavirus antigens were detected by enzyme-linked immunosorbent assay (ELISA), and genotypes were confirmed by Reverse transcription-Polymerase chain reaction (RT-PCR). The infants were divided into three groups: symptomatic preterm infants with and without rotavirus-positive stools [Preterm(rota+) and Preterm(rota-), respectively] and symptomatic full- or near-term infants with rotavirus-positive stools [FT/NT(rota+)]. Demographic and outcome data were compared among these groups.

Results: A total of 702 infants were evaluated for rotaviruses and 131 infants were included in this study. The prevalence of rotavirus infections was 25.2%. Preterm(rota+) differed from Preterm(-rota-) and FT/NT(rota+) with respect to frequent feeding difficulty ($p = 0.047$ and 0.034 , respectively) and higher percentage of neutropenia ($p = 0.008$ and 0.011 , respectively). G4P[6] was the exclusive strain in both the Preterm(rota+) (97.7%) and FT/NT(rota+) (90.2%), and it was the same for nosocomial, institutional infections, and infections acquired at home.

Conclusion: Systemic illness signs such as feeding difficulty and neutropenia are specific for preterm infants with rotavirus infections. G4P[6] was exclusive, regardless of preterm birth or locations of infections. This study might be helpful in developing policies for management and prevention of rotavirus infections in NICUs.

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1. Introduction

Rotaviruses constitute a major agent that causes severe gastroenteritis in infants and young children throughout the world, resulting in approximately 2 million hospitalizations and 600,000 deaths each year.¹ In Korea, rotavirus infections are common in any seasons, particularly between late winter and spring. According to the laboratory-based surveillance from the Korean Center for Disease Control, rotavirus infection accounts for 13–15% of cases of acute diarrhea in children (www.cdc.go.kr). The rotavirus is composed of three concentric protein layers surrounding 11 segments of double-stranded RNA. Group A rotavirus, the most common group worldwide, is based on the inner layer, VP6. The outer layer is composed of two glycoproteins, VP7 and VP4, which define G and P types of rotavirus. To date, 16 G genotypes and at least 28 different P genotypes have been reported.^{2–5}

There are few reports on the symptoms associated with rotavirus infections in neonates. Previous studies have reported that diarrhea is less common in neonates than in older infants, and most neonates are asymptomatic.⁶ Recently, some studies reported neonatal rotavirus infections present with bloody mucoid stools and abdominal distension.^{7,8} Because neonates are vulnerable to infections, rotavirus may result in severe complications, such as necrotizing enterocolitis (NEC). Moreover, rotavirus spreads easily in a nursery or a neonatal intensive care unit (NICU). Therefore, it is important to understand the clinical features and genotypes in neonatal rotavirus infections. The VP4 genotype of P[6] with VP7 genotypes G1, G2, G3, G8, and G9 are known neonatal rotavirus strains, but their clinical significance is not fully understood.^{9,10} The aims of this study were to define the clinical characteristics of rotavirus infections in neonates, particularly focusing on preterm infants, and to investigate the distribution of rotavirus genotypes in a NICU.

2. Methods

2.1. Patients and stool samples

A prospective observational study was carried out at a NICU at a university medical center from July 1, 2007 to June 30, 2008. We kept the following policies for rotavirus infections during the study period: (1) rotavirus screening: stool specimens were collected from every neonate in the NICU on admission and then at weekly intervals; and (2) testing for symptomatic infants: all infants with gastrointestinal symptoms or clinically suspected sepsis were evaluated for the rotavirus. For detection of rotavirus antigen, all stool specimens were diluted 10-fold with phosphate buffered saline (pH 7.4) and clarified by centrifugation $10,000 \times g$ for 10 minutes. The supernatants were tested for group A rotavirus antigen by Enzyme-linked immunosorbent assay (ELISA) with VP6 group-specific antibodies (Dako Diagnostics, Cambridgeshire, UK). To determine the distribution of rotavirus genotypes, rotavirus double-stranded RNA was extracted using Trizol reagent (Life Technologies, Grand Island, NY, USA), and then Reverse transcription-Polymerase

chain reaction (RT-PCR) of the VP7 gene was performed using primers specific for genotypes G1–G6, G8–G10, and G11.^{11–13} RT-PCR of VP7 and VP4 genes were conducted according to previously described methods.¹⁴ With this method, infants with rotavirus-positive stools were selected for this study. In addition, 37 symptomatic preterm infants with rotavirus-negative stools were chosen as controls. The infants with systemic illness such as respiratory disease, congenital malformation, congestive heart disease, culture proven sepsis, and urinary tract infection were excluded. As these diseases could also induce systemic symptoms such as fever and feeding difficulty, they can thus act as compounding factors. Finally, all included infants were divided into three groups: symptomatic preterm infants (a gestational age < 35 weeks) with rotavirus-positive stools [Preterm(rota+)], symptomatic preterm infants with rotavirus-negative stools [Preterm(rota-)], and symptomatic full- or near-term infants (gestational age ≥ 35) with rotavirus positive stools [FT/NT(rota+)] (Figure 1). This study protocol was approved by the hospital's Institutional Review Board, and written informed consent was obtained from parents or legal caregivers.

2.2. Clinical outcome measures

The primary aim was to find clinical characteristics of rotavirus infections in preterm infants. Clinical variables were compared between Preterm(rota+) and Preterm(rota-), and also between Preterm(rota+) and FT/NT(rota+). Two nurses and one doctor who were unaware of the study examined the patients and recorded clinical symptoms. Fever was defined as a rectal temperature of $\geq 38.0^\circ\text{C}$. Diarrhea was defined as a twofold or greater increase in the frequency of watery or looser than normal stool within a 24-hour period. Vomiting was also defined as a twofold or greater increase in the frequency. Feeding difficulty included poor oral intake defined by less than half of the feeding volume in a day, compared to the previous day, and a twofold or more increase in desaturation events (oxygen saturation below 90%) during feeding that resulted in interruption of feeding. The definition of abdominal distension was based on an increased abdominal girth ≥ 2.0 cm compared to baseline. Frank bloody stool and lethargy were determined by the medical staff. Apnea was defined as cessation of breathing for longer than 20 seconds or any duration if accompanied by cyanosis and bradycardia under circumstances with no specific stimulation, such as feeding or sampling of infants. Blood samples were obtained from symptomatic infants for a complete blood count, electrolytes, C-reactive protein, and blood cultures. Neutropenia was defined as an absolute neutrophil count $< 1000/\text{mm}^3$, and thrombocytopenia as a platelet count $< 100,000/\text{mm}^3$. Metabolic acidosis was diagnosed by a HCO_3 concentration less than 18 mmol/L.

The secondary aim was to demonstrate the distribution of rotavirus genotypes by group and location where the infections occurred. 'Nosocomial infections' were defined if symptoms occurred in hospitalized infants or occurred more than 48 hours after admission. The cut-off was 48 hours, to discriminate the location of infections. 'Infections

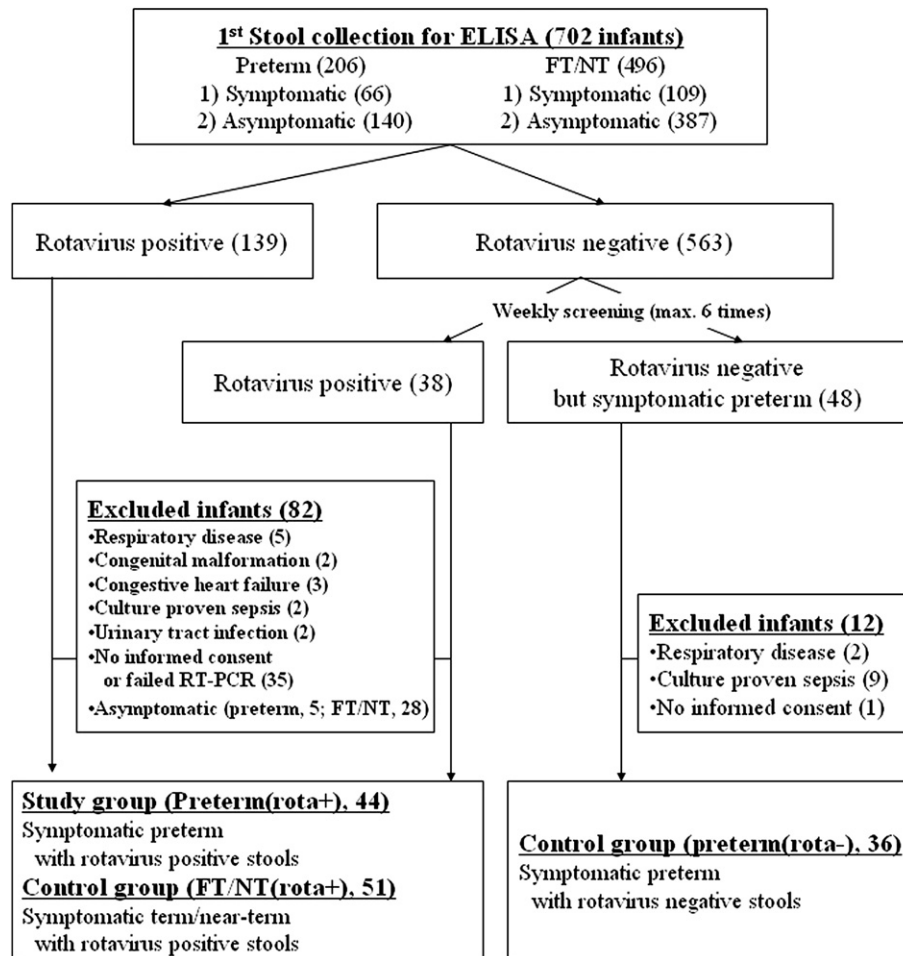


Figure 1 Study population.

acquired at home' were considered if symptoms developed at home. 'Institutional infections' referred to infections that occurred at postnatal care centers. Postnatal care centers included commercial places that serve caregivers for mothers and newborn infants after discharge from delivery hospitals. In these places, some assistants took care of many neonates in a room similar to a nursery.

2.3. Statistical analysis

Demographic features and laboratory findings were analyzed by the Student *t* test and the Fisher exact test. Continuous data are expressed as the mean \pm standard deviation or median (25–75th percentile). Multiple logistic regression analysis was performed to find the specific clinical symptoms in Preterm(rota+). The significant symptoms are graphed with *p* values and odds ratio with 95% confidence intervals.

3. Results

3.1. Incidence and demographic features

The study population is shown in Figure 1. Out of 850 infants, 702 were evaluated for rotaviruses. Of these

infants, 177 infants (25.2%) were confirmed to have rotavirus infections. The percentage of asymptomatic infections was 18.6% (33 of 177 infants). Table 1 shows the demographic data. Nosocomial infections accounted for 93.2% in the Preterm(rota+) group [$p < 0.001$ compared with the FT/NT(rota+) group]. In the FT/NT(rota+) group, institutional infections (35.3%) and infections acquired at home (25.5%) were more common than in the Preterm(rota+) group ($p < 0.001$ and $p = 0.015$, respectively). Symptoms of rotavirus developed at an older age in the Preterm(rota+) group compared with those in the FT/NT(rota+) group (26.1 ± 17.1 vs. 12.9 ± 8.5 , $p < 0.001$).

3.2. Primary outcome

Figure 2 shows the results of the multiple logistic regression analysis of various clinical symptoms between the Preterm(rota+) and Preterm(rota-) groups, and also between the Preterm(rota+) and FT/NT(rota-) groups. Feeding difficulty was the best clinical symptom for identifying infants in the Preterm(rota+) group [$p = 0.047$ compared with the Preterm(rota-) group and $p = 0.034$ compared with the FT/NT(rota+) group]. Lethargy was a significant symptom in the Preterm(rota-) group compared with the Preterm(rota+) group, but it did not differ from the FT/NT(rota+) group. In the latter, vomiting and fever were

Table 1 Demographic features and laboratory findings in the Preterm(rota+) group compared with the Preterm(rota-) and FT/NT(rota+) groups.

Variables	Preterm(rota+)* (n = 44)	Preterm(rota-)† (n = 36)	FT/NT(rota+)‡ (n = 51)
Gestational age (wk) [§]	31.4 ± 2.14	30.4 ± 2.42	37.9 ± 2.29
Birth weight (kg) [§]	1.65 ± 0.48	1.50 ± 0.42	2.94 ± 0.63
Age at onset of illness (d) [§]	26.1 ± 17.1	22.2 ± 7.92	12.9 ± 8.5
Boys	26 (59.1)	20 (55.6)	24 (47.1)
Cesarean delivery	29 (65.9)	25 (69.4)	26 (51.0)
Admission from outside [§]	3 (6.8)	4 (11.1)	45 (88.2)
Nosocomial infections [§]	41 (93.2)	—	20 (39.2)
Institutional infections [§]	0	—	18 (35.3)
Infections acquired at home [§]	3 (6.8)	—	13 (25.5)
Breast milk feeding	21 (47.7)	16 (44.4)	25 (49.0)
Neutropenia ^{,§}	8 (18.2)	1 (2.8)	1 (2.0)
Thrombocytopenia	2 (4.5)	0	0
Metabolic acidosis [§]	9 (20.5)	6 (16.7)	26 (51.0)
C-reactive protein (nmol/L) [¶]	0.48 (0.10–23.81)	0.19 (0.10–7.81)	0.67 (0.10–7.33)

Data are presented as mean ± standard deviation, n (%), or median.

[§] $p < 0.05$ in the Preterm(rota+) group compared with the FT/NT(rota+) group.

^{||} $p < 0.05$ in the Preterm(rota+) group compared with the Preterm(rota-) group.

* Preterm(rota+): symptomatic preterm infants (gestational age < 35 weeks) with rotavirus-positive stools.

† Preterm(rota-): symptomatic preterm infants with rotavirus-negative stools.

‡ FT/NT(rota+): symptomatic full- or near-term infants (gestational age ≥ 35 weeks) with rotavirus positive stools.

¶ Values are expressed by median (25–75th percentile).

more common than in the Preterm(rota+) group. Only one infant in the Preterm(rota+) group had fever. Peak body temperature showed no significant difference when the infants had fever (38.24 in the FT/NT group and 38.20 in the Preterm group). Stool frequencies seemed to be more frequent in the FT/NT(rota+) group [7.63 ± 3.70 times

a day in the FT/NT(rota+) group and 5.83 ± 1.47 in the Preterm(rota+) group], but no significant difference was found. Apnea was observed more frequently in the Preterm(rota-) group (22.2%) compared with the other groups; however, there was no significant difference found. Only two infants in the Preterm(rota+) group and one infant in

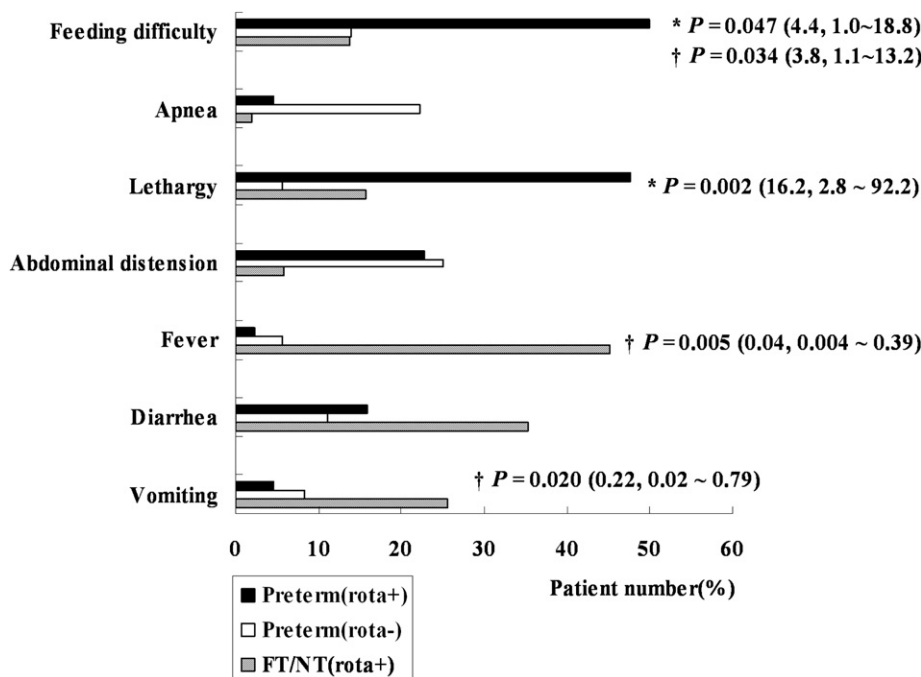


Figure 2 Comparison of clinical symptoms by multiple logistic regression analysis. Statistically significant symptoms ($p < 0.05$) in the Preterm(rota+) group compared with the Preterm(rota-) and FT/NT(rota+) groups are indicated by * and †, respectively; p values with odds ratio and 95% confidence intervals are also expressed.

the FT/NT(rota+) group showed apnea. Abdominal distension had a tendency to be found in preterm infants regardless of the presence of rotavirus infections. Frank bloody stool was observed in two infants from the Preterm(rota+) group, two infants from the Preterm(rota-) group, and one infant from the FT/NT(rota+) group.

The frequency of neutropenia was higher in the Preterm(rota+) group compared with the others [$p = 0.008$ vs. the Preterm(rota-) group and $p = 0.011$ vs. the FT/NT(rota+) group]. By contrast, metabolic acidosis ($\text{HCO}_3^- < 18$ mmol/L) was more common in the FT/NT(rota+) group than in the Preterm(rota+) group ($p = 0.002$).

3.3. Secondary outcome

G4P[6] was the predominant strain (89/95 infants, 93.7%) in this study. Table 2 shows the percentage of G4P[6] strain according to the locations of the infections. All infants except for six had the G4P[6] strain. Other strains found included G3P[8] in one preterm and two full-term infants, G1P[8] in two full-term infants, and P[6] with an undefined G genotype in one full-term infant. Stool specimen from the last infant could not be amplified by RT-PCR using G genotype-specific primers. Although infants with rotavirus infections acquired at home had relatively lower percentages of the G4P[6] strain (10 out of 13 infants, 76.9%) in the FT/NT(rota+) group, G4P[6] was the only strain that was prevalent at all locations.

4. Discussion

The results of this study identified the clinical characteristics of rotavirus infections in neonates, particularly in

preterm infants, and demonstrated that the rotavirus strain G4P[6] was the predominant strain in a NICU, regardless of the gestational age at birth or the presence of nosocomial infections.

The incidence of rotavirus infections found in this study was similar to long-term incidence (1.7–50%) observed in other NICUs.⁷ However, asymptomatic infants were less common than in previous studies.^{8,15} These findings are reliable because this study performed rotavirus screening for all hospitalized infants at regular weekly intervals. Preterm infants showed higher rates of nosocomial infections. Most preterm infants in our NICU had been hospitalized since birth, thus the proportion of nosocomial infections must have been higher than in term infants.

Previous studies reported that rotavirus infections in neonates presented differently than in children, who usually present with diarrhea.^{6,16} The results of this study were consistent with these findings. Although vomiting was more common in the FT/NT(rota+) group than in the Preterm(rota+) group, gastrointestinal symptoms such as diarrhea and vomiting developed less frequently than in older children, as reported previously.^{6,16} In contrast to this study, some studies found that gastrointestinal symptoms including frequent or bloody stools were major signs of rotavirus infections in preterm infants.^{7,17} This study aimed to identify specific clinical signs in preterm infants with rotavirus infections. In this regard, preterm infants with rotavirus infections were compared not only to preterm infants without rotavirus infections but also to FT/NT infants with rotavirus infections. Finally, we found that feeding difficulty was the clinical symptom that most efficiently identified preterm infants with rotavirus infections. Neutropenia was another important finding of rotavirus infections in preterm infants. Generally, feeding difficulty and neutropenia indicate signs of systemic illness in neonates.¹⁸ These findings suggested that rotavirus infections in preterm infants caused more systemic symptoms and differed from rotavirus infections in full-term or older infants, mainly presenting as fever or gastrointestinal symptoms.

The prognosis of rotavirus infections was good in this study. Previous reports suggested that rotavirus was associated with NEC; however, only one of the infants in this study progressed to NEC. All infants had a short course of illness with rotavirus infections. The mean duration of recovery from symptoms was less than 2 days, and all infants with rotavirus infections recovered within 5 days (data not shown).

In this study, FT/NT infants had fever as the major symptom of rotavirus infections. Infants presenting with a temperature $\geq 38^\circ\text{C}$ were presumed to have sepsis and, in these cases, additional laboratory tests were performed.¹⁸ The results of this study suggest that rotavirus should be considered as one of the differential diagnoses in FT/NT infants with fever. Metabolic acidosis often developed as a result of rotavirus infections in these infants. As most of the preterm infants were in the hospital before becoming ill, dehydration occurred less frequently than in the FT/NT infants.

The G4P[6] rotavirus strain was the dominant strain found in both preterm and FT/NT infants. Moreover, this strain did not differ among nosocomial, institutional

Table 2 The proportions of rotavirus strains among each location of infection source in the Preterm(rota+) and FT/NT(rota+) groups.

Location of infection source	Preterm(rota+)* (n = 44)	FT/NT(rota+)† (n = 51)
Nosocomial infections	41	20
G4P[6]	40 (97.6)	19 (95.0)
G3P[8]	1 (2.4)	1 (5.0)
Institutional infections	0	18
G4P[6]	–	17 (94.4)
G1P[8]	–	1 (5.6)
Infections acquired at home	3	13
G4P[6]	3 (100)	10 (76.9)
G3P[8]	0	1 (7.7)
G1P[8]	0	1 (7.7)
P[6] with undefined G genotype	0	1 (7.7)

Data are presented as n or n (%).

* Preterm(rota+): symptomatic preterm infants (gestational age < 35 weeks) with rotavirus-positive stools.

† FT/NT(rota+): symptomatic full- or near-term infants (gestational age ≥ 35) with rotavirus positive stools.

infections, or infections acquired at home. Previous studies have reported that the rotavirus strains in neonatal nurseries were distinct from those found in the community; single strain can circulate in neonatal nurseries for long periods.^{19,20} For this reason, most studies on neonatal rotavirus have focused on nosocomial infections.^{7,9,20} However, this study included not only nosocomial infections but also infections acquired at home and institutional infections. Rotavirus infections acquired at home accounted for 25.5% of the FT/NT(rota+) group. It is surprising that a single strain, G4P[6], was predominant even in rotavirus infections acquired at home. Based on this finding, it is possible that rotavirus infections in the NICU can be influenced by exposure from the community, similar to other pediatric wards. Rotavirus vaccines (Rotarix and RotaTeq) are available since 2007 in Korea. If widespread vaccination can lower the incidence of rotavirus infections in the community, it can also affect NICU outbreak. Further study is needed regarding the vaccination status and the epidemiology including genotypic diversity.

In conclusion, the results of this study show that systemic illness signs such as feeding difficulty and neutropenia are specific clinical signs for preterm infants with rotavirus infections. The rotavirus G4P[6] was the most prevalent strain found in the NICU in this study. Moreover, G4P[6] was also the most prevalent strain in nosocomial, institutional infections, and infections acquired at home. This information can be helpful in the diagnosis and development of policies for rotavirus infections in the NICU.

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