Molecular epidemiologic trends of norovirus and rotavirus infection and relation with climate factors: Cheonan, Korea, 2010–2019

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Abstract Background: Viral infection outbreaks are emerging public health concerns. They often exhibit seasonal patterns that could be predicted by the application of big data and bioinformatic analyses. Purpose: The purpose of this study was to identify trends in diarrhea-causing viruses such as rotavirus (Gr.A), norovirus G-I, and norovirus G-II in Cheonan, Korea. The identified related factors of diarrhea-causing viruses may be used to predict their trend and prevent their infections. Method: A retrospective analysis of 4,009 fecal samples from June 2010 to December 2019 was carried out at Dankook University Hospital in Cheonan. Reverse transcription–PCR (RT–PCR) was employed to identify virus strains. Information about seasonal patterns of infection was extracted and compared with local weather data. Results: Out of the 4,009 fecal samples tested using multiplex RT–PCR (mRT–PCR), 985 were positive for infection with Gr.A, G-I, and G-II. Of these 985 cases, 95.3% (n = 939) were under 10 years of age. Gr.A, G-I, and G-II showed high infection rates in patients under 10 years of age. Student’s t-test showed a significant correlation between the detection rate of Gr.A and the relative humidity. The detection rate of G-II significantly correlated with wind–chill temperature. Conclusion: Climate factors differentially modulate rotavirus and norovirus infection patterns. These observations provide novel insights into the seasonal impact on the pathogenesis of Gr.A, G-I, and G-II.

Key Words: diarrhea-causing virus, climate, rotavirus, norovirus G-I, norovirus G-II, infection, temperature

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1. Introduction

Acute gastritis is the most common type of infection that affects both children and adults [1]. It exhibits various clinical symptoms such as vomiting, diarrhea, fever, and decreased diet, and some may require hospitalization [6]. The number of deaths from diarrhea viral gastroenteritis is steadily decreasing in developed countries due to improvements in hygiene standards and lifestyles worldwide, but the incidence and mortality rates in developing countries are still high [7]. Diarrhea caused by gastrointestinal infection accounts for a large proportion of mortality worldwide [5] and is a major public health problem [4]. In children under 5 years of age, severe diarrhea requires hospitalization, resulting in loss of productivity and economic loss due to hospitalization and outpatient visits for treatment [3, 8].

Pathogens that cause viral gastroenteritis with diarrhea include astrovirus (AstV), enteric adenovirus (EAdV), group A rotavirus (RotV [Gr.A]), and norovirus (NoV) [6, 7, 10]. Although there are differences that occur over time, group A rotavirus (RotV [Gr.A]) and norovirus (NoV) account for most of the occurrences [6]. Rotavirus (RotV) is the most common and major pathogen worldwide that causes severe diarrhea in infants under 5 years of age [8]. Rotavirus belongs to the Reoviridae and is classified into 10 groups of A–J, depending on the antigen that makes up the surface. It is also an important cause of diarrhea in the elderly [11]. Norovirus belongs to the family Caliciviridae [12] and include six gene groups GI–GVI based on the partial sequence of genomic RNA [9]. The genogroup of norovirus that causes human infection has three types (GI, GII, GIV), and GII accounts for a large proportion of infection by noroviruses. GII is highly likely to mutate, resulting in major genotype changes every 23 years, which is known to be associated with the epidemic [6]. Among norovirus GIIIs, genotype GI.4 is the most common genotype of norovirus enteritis [6]. Currently, norovirus disease is a major cause of acute gastritis across all age groups [12, 13] and the elderly aged 65 years or older have the highest risk of norovirus–related death, while children under 5 had the highest risk of norovirus–related medical visits [12]. Except for RotV, there is no vaccine for diarrhea–causing viruses (DV), and the prevention of infection is challenging [10].

High effects of rotavirus vaccines against rotavirus infection (vaccine efficacy, 85.98%) were identified in the United States, Latin America, and European countries. The rotavirus vaccine has reduced hospitalization rates and severe infections caused by rotavirus [14]. However, basic research on vaccines for other DV are needed and the scale of vaccination needs to be expanded. Since efforts for generating vaccines are ongoing and a vaccine is still underway, the current best clinical practice to avoid disease outbreak is early and accurate monitoring of infection.

The incidence of gastrointestinal infection is seasonal, and is associated with environmental variables, especially temperature and humidity [5]. For example, humidity in the surrounding environment can affect the survival of the virus, influencing its infection propagation [15–18]. Although it is not entirely clear how environmental factors correlate with DV, preventive medical studies are currently being conducted in many countries to determine the prevalence of diarrhea–causing viruses and their relationship to climatic factors.

Laboratory monitoring of gastroenteritis accompanied by diarrhea has been conducted in preference to other diseases, and is now in place in many countries [4, 5, 19]. Although there have been many investigations into the molecular mechanics of climate factors and diarrhea–causing viruses, they have been limited to short-term studies. We examined the
relationship between molecular biological mechanics and climatic factors in RotV, Nor−GI, and Nor−GII over a longer period of time from June 2010 to December 2019. The purpose of this study was to identify trends in diarrhea−causing viruses such as rotavirus (Gr.A), norovirus G−I, and norovirus G−II in Cheonan, Korea. The identified related factors of diarrhea−causing viruses may be used to predict their trend and prevent their infections.

2. Materials and methods

This was a retrospective study in which 4,009 stool specimens from June 2010 to December 2019, were tested in the Department of Laboratory Medicine, Dankook University Hospital, Cheonan, using multiplex reverse transcription PCR (mRT−PCR) targeting three species of diarrhea viruses (rotavirus [Gr.A], norovirus G−I, norovirus G−II). The samples were subjected to nucleic acid extraction within 24 h. The nomenclature and taxonomy of these viruses were selected according to the criteria of the International Committee on Taxonomy of Viruses. Data on the age and sex of the study participants were retrieved from patient records. We were unable to collect information on the incubation period of the viruses because of the retrospective nature of the study and because the time of hospital visit after the onset of the disease was different for each patient. This study was approved by the Institutional Review Board of Dankook University, Republic of Korea (approval number 2019−12−007). This study was conducted in accordance with the Declaration of Helsinki.

2.1 Real−time multiplex reverse transcription −PCR (mRT−PCR) analysis

Stool specimens were diluted with 1 mL of distilled water, and 200 μL of the diluted solution was extracted using a Minelute RNA Spin Kit (Qiagen, Germany). The extracted nucleic acid was used to synthesize cDNA with RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada) according to the manufacturer’s protocol. cDNA was used for mRT−PCR. The mRT−PCR for the three viruses was performed using the Seeplex Diarrhea−V ACE detection kit (Seegene, Korea) according to the manufacturer’s instructions with a PTC−200 PCR system (MJ Research USA). The following target genes for DV were used: VP4 for rotavirus (Gr.A); ORF2 for norovirus−GI/GII. The internal mRT−PCR control was the CesA3 gene of Arabidopsis thaliana. Distilled water was used as negative control. mRT−PCR for the three viruses was performed using Seeplex A Diarrhea−V ACE detection kit with dual specificity oligonucleotides (Seegene) and the three pathogens were detected in a single test tube.

The mRT−PCR products were electrophoresed on a 2% agarose gel with ethidium bromide for 30 min at 100−150 V. The agarose gel was rinsed with distilled water, amplification was assessed by visualizing the gel on a UV trans−illuminator, and the results were analyzed after an image was acquired. Table 1.

<table>
<thead>
<tr>
<th>Target viruses</th>
<th>Target region</th>
<th>Gene size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis (Internal control)</td>
<td>CesA3</td>
<td>1,000</td>
</tr>
<tr>
<td>Group A Rotavirus (Gr.A)</td>
<td>VP4</td>
<td>541</td>
</tr>
<tr>
<td>Norovirus G−I (G−I)</td>
<td>ORF2</td>
<td>304</td>
</tr>
<tr>
<td>Norovirus G−II (G−II)</td>
<td>ORF2</td>
<td>214</td>
</tr>
</tbody>
</table>

2.2 Climatic variables

Meteorological data of the Cheonan region were collected from June 1, 2010 to December 31, 2019, and those from the National Institute of Environmental Research (including the ASOS data for the Cheonan region) were also collected for the same period. The National Institute of Environmental Research, a research institute under the Ministry of Environment in South Korea, oversees domestic environment−related
research and education. The Cheonan region of Chungcheongnam-do, Korea has an area of 636.3 km² and a typical temperate climate due to its location at 36.47N and 127.13E. The variables examined in the study were the month, date, year, daily temperature, wind–chill temperature, average monthly temperature, relative humidity, precipitation, atmospheric pressure, age, and sex of the patients. The ASOS is a manual weather–measurement system used by the Korean Meteorological Agency, which is dedicated to observing weather factors: therefore, weather information obtained from the ASOS is recognized by the Korean Meteorological Agency. The Wind–chill temperature was calculated using the following formula provided by the Korean Meteorological administration:

\[
\text{Wind–chill temperature} = 13.12 + 0.6215 \, T - 11.37V^{0.16} + 0.3965V^{0.16} T,
\]

where \( T \) is the air temperature (°C) and \( V \) is the wind speed (km/h), measured 10 m above the ground.

### 2.3 Statistical analysis

SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to perform descriptive statistical analysis, frequency analysis, Student's t-test, and binomial logistic–regression analysis to investigate the relationship of meteorological data and particulate matter concentrations with Gr.A, G–I, and G–II infection rates. Continuous data were presented as the mean, whereas categorical data were presented as the frequency, percentage, and interquartile range (IQR), where appropriate. For all analyses, a two–tailed \( p\)-value <0.05 indicated a statistically significant difference.

### 3. Results

#### 3.1 Overall statistics

In this study, the fecal samples of 4,009 patients who visited Dankook University Hospital for diarrhea between June 2010 and December 2019 were tested. Of the total 4,009 patients, 2,294 were men (57.22%) and 1,715 were women (42.78%). Of the 4,009 samples taken during the study, 985 (24.57%) tested positive for Gr.A, G–I, and G–II. The detection rates for Gr.A, G–I, and G–II were 24.0% (\( n = 551/2,294 \)) for males and 37.7% (\( n = 434/1,715 \)) for females. The detection rate of Gr.A was 10.0% (\( n = 229/2,294 \)) for males and 11.1% (\( n = 190/1,715 \)) for females. The detection rate of G–I was 0.5% (\( n = 11/2,294 \)) for males and 0.3% (\( n = 5/1,715 \)) for females. The detection rate of G–II was 13.6% (\( n = 311/2,294 \)) for males and 13.9% (\( n = 229/1,715 \)) for females (Table 2). G–II was the most frequently detected virus with 27.49% (\( n = 550/4,009 \)) followed by Gr.A with 21.06% (\( n = 419/4,009 \)).

#### 3.2 Statistics by age

Gr.A had the highest detection rate of 97.6% (\( n = 409/419 \)) in patients under the age of ten, and the second–highest detection rate of 1.4% (\( n = 6/419 \)) in patients between ages 10–20 (Table 2). G–I had the highest detection rate of 87.5% (\( n = 14/16 \)) in patients under the age of ten, and the second–highest detection rate was 12.5% (\( n = 2/16 \)) for patients between 10–20 years of age (Table 2). G–II had the highest detection rate of 93.8% (\( n = 516/550 \)) in patients under ten years of age, and the second–highest detection rate was 4.2% (\( n = 23/550 \)) in patients between 10–20 years of age (Table 2). Distinct infection patterns were observed in different age groups.

#### 3.3 Statistics by year

During the study period (2010–2019), the overall detection rate of Gr.A, G–I, and G–II was the lowest at 12.82% (\( n = 66/515 \)) in 2019 and the highest at 35.1% (\( n = 126/359 \)) in 2011. Gr.A had detection rates of 8.9%, 7.1% and 9.4% in 2011,
Table 2. Age/Sex demographics of the patient

<table>
<thead>
<tr>
<th>Age</th>
<th>Rotavirus (Gr.A)</th>
<th>Norovirus G-I</th>
<th>Norovirus G-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>409 (97.6%)</td>
<td>14 (87.5%)</td>
<td>516 (93.8%)</td>
</tr>
<tr>
<td>10~19</td>
<td>6 (1.4%)</td>
<td>2 (12.5%)</td>
<td>23 (4.2%)</td>
</tr>
<tr>
<td>20~29</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>30~39</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>40~49</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>50~59</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>60~69</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
<td>3 (0.5%)</td>
</tr>
<tr>
<td>70~80</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>80~89</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>90+</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Rotavirus (Gr.A)</th>
<th>Norovirus G-I</th>
<th>Norovirus G-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>229 (54.7%)</td>
<td>5 (68.8%)</td>
<td>239 (56.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>190 (45.3%)</td>
<td>11 (31.2%)</td>
<td>311 (43.5%)</td>
</tr>
</tbody>
</table>

2014 and 2015 respectively. G–II had detection rates of 11.5%, 11.7% and 11.1% in 2012, 2016, and 2017 respectively. G–I had detection rates of 0.7%, 0.6% and 0.8% in 2010, 2011, and 2016 respectively (Fig. 1). Infections exhibited seasonal variations, with peaks in winter.

3.4 Statistics by month

The detection rate of Gr.A was the highest at 23.4% (n = 75/320) in March and the lowest at 2.7% (n = 8/297) in September. G–I had the highest detection rate of 0.9% (n = 3/316) in February and the lowest at 0.0% (n = 0/405, 0/316, 0/370) in May–July. G–II had the highest detection rate of 28.2% (n = 115/408) in December, the lowest detection rate of 4.1% (n = 13/316) in June and 4.1% (n = 11/267) in October (Fig. 2).
Table 3. Comparison of monthly mean meteorological factors and viral diarrhea infection

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Meteorological factors</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Rotavirus (Gr.A)</td>
<td>Wind chill Temperature (°C)</td>
<td>−0.156</td>
<td>−0.03</td>
</tr>
<tr>
<td></td>
<td>Relative Humidity (%)</td>
<td>−0.27</td>
<td>−0.08</td>
</tr>
<tr>
<td></td>
<td>Rate of sunshine</td>
<td>−0.077</td>
<td>0.063</td>
</tr>
<tr>
<td>Norovirus G-I</td>
<td>Wind chill Temperature (°C)</td>
<td>−0.013</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Relative Humidity (%)</td>
<td>−0.014</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Rate of sunshine</td>
<td>−0.009</td>
<td>0.008</td>
</tr>
<tr>
<td>Norovirus G-II</td>
<td>Wind chill Temperature (°C)</td>
<td>−0.32</td>
<td>−0.147</td>
</tr>
<tr>
<td></td>
<td>Relative Humidity (%)</td>
<td>−0.247</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Rate of sunshine</td>
<td>−0.128</td>
<td>0.066</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001

3.5 Analysis by climatic factors

During the study, the detection rate of Gr.A was the highest at a wind chill temperature of 5.3°C and relative humidity of 59.3% and the detection rate was the lowest at a wind−chill temperature of 23.0°C and relative humidity of 74.6% (Fig. 2).

The detection rates of G-I and G-II were the highest at a wind chill temperature of 1.1°C and relative humidity of 68.3%, and the lowest at a chill temperature of 16.6°C and relative humidity of 73.6%. In addition, Gr.A, G-I, and G-II infections showed increased detection rates in winter and spring (Fig. 2). Student’s t−test analysis showed that the detection rate of Gr.A and relative humidity (p<0.001) were significantly correlated (Table 3). The detection rate of G-II was significantly correlated with the wind−chill temperature (p < 0.001) (Table 3). However, there was no significant correlation between the detection rate of norovirus G-I and relative humidity, atmospheric pressure, rate of sunshine or wind−chill temperature (Table 3). High infection rates were associated with lower temperatures.
4. Discussion

In this study, the detection rates of Gr.A, G-I, G-II were not gender-specific, but were highest in population under the age of 10. Out of the three viruses, Gr.A, G-I, and G-II, the highest detection rate was observed with of G-II, followed by Gr.A and G-I, which was the same as other studies that analyzed norovirus detection rates in temperate climates [9].

Gr.A had the highest detection rate in 2015, but tended to decline over the years, whereas G-I and G-II had the highest detection rate in 2016 and did not show a trend of decline that was different from that of Gr.A. The results show that since the introduction of the rotavirus vaccine in 2007, the rate of rotavirus infection decreased drastically after 2010.

Gr.A was found to develop in winter and spring, from January to April, especially in March, in a large number of cases. Some studies have shown that Gr.A infection occurred in spring, fall, and winter but did not occur in summer [20]; however, the prevalence of rotavirus-related gastritis typically occurs in winter and early spring in temperate climate countries, consistent with previous studies that showed occurrence in January and March, including a Japanese study that the main period of rotavirus as February and March [2, 6, 7, 21]. Although the detection rate of G-I in seasonal distributions remained unchanged and there was no significant change in annual trends, G-II was detected from November to April, showing a trend that was prevalent from winter to early spring. Overall, G-I and G-II were observed to be common in winter. Norovirus-related diseases occur throughout the year, but the norovirus activity, which is the main cause of acute diarrhea, generally occurs from January to March, with the most common winter [12, 19] followed by fall, spring, and continuous high levels of norovirus detection until early summer in June [2, 22]. However, a German study found that norovirus outbreaks occurred from November to October of the year. Similar to studies that have shown that norovirus outbreaks are high in winter and spring in temperate regions and peak year-round in December [13, 20, 23, 24], our results showed a rise from November and marked a significant high in December. In the Northern Hemisphere, norovirus is generally shown to be active in cold winter months [12]; however, in Korea, it occurs almost continuously in spring or summer (March or June) as well as in winter [24]. The detection rates of Gr.A, G-I, and G-II during the study period particularly correlated with temperature changes. The detection rates of the viruses were high even at low relative humidity, and relative humidity was shown to have a negative effect on the detection rates of Gr.A. Sensory temperature was shown to have a negative effect on the detection rate of norovirus G-II. Korea is geographically located in the temperate climate zone of the mid--latitude and has four distinct seasons. Under this climate, past rate of norovirus food poisoning has been nearly half as high in winter as in summer. [24, 25]. This may reflect the important effect of temperature and humidity on the activity of the virus.

Several climate factors, including temperature, humidity and rainfall are related to the occurrence of rotavirus and norovirus. Rotavirus infections are more common in cold and dry seasons, and precipitation can increase norovirus transmission, indicating an influence of temperature and relative humidity on these two viruses [4]. In Europe, gastritis due to rotavirus reaches its peak in late winter [4]. Norovirus activity is generally negatively correlated with the mean temperature [19, 26]. Noroviruses last longer at a temperature range of 15–20°C than at other temperatures [9] and humidity is also an important environmental factor that affects the survival and transmission of norovirus. The
correlation between humidity and norovirus occurrence was statistically confirmed, and most previous studies concluded that low relative humidity is associated with norovirus epidemics [9]. The results of the Korea Centers for Disease Control and Prevention report correlate the detection rate of the causative pathogens causing acute diarrhea with climate change, traffic, overseas travel, and dining out indicating that the pattern of acute diarrhea tends to occur throughout the year, especially as the living environment improves due to the improvement of the standard of living, including the increased use of air conditioners and boilers etc. [24, 25]. The cold and relatively dry conditions of our country in winter may help the activity and spread of norovirus [24].

Studies show that among the pathogens that cause acute diarrhea, viral pathogens are detected intensively in the cold and humid winter months, with some changes in the general pattern [22]. Viral pathogens infections have shown a strong negative correlation between higher temperatures and higher relative humidity, but just before 2017 the general infection pattern of viral pathogens during the cold and humid winter season became weaker [22]. Data from California in the United States reported an association of noroviruses with high humidity environments, and in Germany, noroviruses are constantly detected throughout the year [2, 9]. In Africa, although a high prevalence rate is observed at certain times of the year, rotavirus infections are detected throughout the year.

In general, cool and dry weather conditions lead to a high incidence of rotavirus, while the association between temperature and norovirus infection has not been concluded [28]. At low temperatures, the pathogen is more stable and persists longer on hands, feces, and contaminated objects, promoting transmission. In addition, people living in subtropical or tropical regions are more sensitive to cold weather. During this period, people tend to stay close to each other and spend more time indoors. Therefore, vulnerable people are more likely to interact with infected people and contaminated surfaces, air, food, and water [28].

This study has some limitations. First, conducting surveys for ten years in a single city can be considered relatively short term. Therefore, our results may be biased in terms infections. Based on changes in seasons, sex, and age, we were able to identify differences in positive rates in Gr.A, G-1, and G-II infections. In addition, the rate of rotavirus infection has declined since 2010 in Korea since the introduction of the rotavirus vaccine in 2007; however, unlike that of rotavirus, the rate of infection with norovirus without vaccine has relatively increased [6, 21] of viral dynamics. Second, because sample data were treated anonymously and the patients’ residences were not specified, some of the tested samples may have been provided by patients who did not reside in the study area. Thus, due to differences in geographical location among patients, there may be differences in seasonal distributions of Gr.A, G-1, and G-II observed in this study. Although most previous studies have shown that virus outbreaks are related to low temperatures and low humidity, these findings may not apply to all countries and regions [9]. It is important to identify the regional impact of environmental factors on viral epidemics due to regional and climatic differences, and to investigate these variables in a wide range of climatic conditions, including long-term bacterial and viral pathogen detection and changes in correlation with climatic factors [9, 22]. Finally, the results reflect a trend established after the introduction of the rotavirus vaccine in Korea in 2007 [21]; however, rotavirus vaccination status was not verified in the 4,009 patients with rotavirus that were included in the study.
Despite these limitations, our research provides some insights into changes in positive rates of Gr.A, G-I, and G-II.

Therefore, our results will be very important for assessing and formalizing the potential effects of vaccines as progress continues in the field of norovirus vaccine development [12], and we expect our findings to help with effective prevention strategies against acute diarrhea viral infection, including vaccine development. Since the occurrence of acute diarrhea is expected to increase in the future due to social and environmental changes such as climate change, it is necessary to focus more on the accumulation of long-term analysis data on viral pathogen detection and changes in correlation with climatic factors. Studies on viral infection prevention and management measures to cope with climate and environmental changes [2, 22] in different geographical regions, such as climate factors, age, and gender, and other variables are needed [5].

REFERENCES


