Original Article

Genotype Profiles of Rotavirus Strains in Children under 5-year-old Outpatients with Diarrhea in Bandung, West Java, Indonesia

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Abstract

Introduction: Diarrhea is a global leading cause of morbidity and mortality among children under five, with rotaviruses being the most common cause. This study aimed to determine the genotypes of rotavirus in children under 5 years with diarrhea in Bandung, Indonesia. **Methods:** This cross-sectional study was conducted from 2014 to 2018 on 450 children under five with acute diarrhea in primary health centers in Bandung, Indonesia. Fecal samples were examined for rotavirus antigen using an enzyme-linked immunosorbent assay method, and genotype was determined through sequencing using polymerase chain reaction. Results were statistically analyzed using Pearson Chi-square in Epi Info version 3.5.4, with $P < 0.05$ considered statistically significant. **Results:** Rotavirus was identified in 8.9% of the subjects, slightly higher in boys ($n = 24$, 9.8%) than girls ($n = 16$, 7.8%). We found that the most rotavirus positive in age group is >12–24 months and >24–59 months, while the highest percentage is at the age of ≤6 months (11.8%). Moderate malnutrition was observed in more subjects (12.8%). Vomiting was more frequent in patients positive (55%, $P = 0.013$) and fever was seen in 32.5% ($P = 0.645$). No signs of dehydration were seen in most subjects (75%), $P = 0.227$. Rotavirus genotypes identified were G1P[8] (18, 45%), G3P[8] (14, 35%), G3P[6] (4, 10%), G3P[9] (2, 5%), G2P[4] (1, 2.5%), and nontypeable (NT) (1, 2.5%). **Conclusions:** The dominant rotavirus genotype is G1P[8], followed by G3P[8], G3P[6], G3P[9], G2P[4], and NT. The most common rotavirus positive in age group is >12–24 months and >24–59 months, while the highest percentage is at the age of ≤6 months.

Keywords: Children, genotype, outpatient, rotavirus

Introduction

Diarrhea is the second leading cause of death in children under the age of 5 worldwide. About one in five child deaths or 1.5 million annually is related to diarrhea.[1] In developing countries, diarrhea is a major cause of infant morbidity and mortality.[2] Annual mortality due to diarrhea is about 9.9% in children under 5 years of age.[3]

Diarrhea research in Southeast Asia in 2008–2018 found 40.78% caused by rotavirus infection.[4] Rotavirus infection is the leading cause of severe diarrhea in young children worldwide, especially in developing countries including Indonesia. In Indonesia, rotavirus is found year round.[5]

Severe acute gastroenteritis in children <5 years of age that can be fatal is mostly caused by rotavirus, with almost 90% of deaths occurring in low-income countries such as Asia and Africa. Five countries that contribute to more than half of all deaths from rotavirus infection are as follows: the Democratic Republic of the Congo, Ethiopia, India, Nigeria, and Pakistan.[6]

The rotavirus genus belongs to the family Reoviridae and, based on its antigenic and genetic properties, is divided into seven groups (A–G). The main cause of gastroenteritis in humans is rotavirus group A, which consists of two structural

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proteins, VP7 and VP4, and is classified into genotypes G and P.[7,8] Different predominant genotypes of human viruses are observed in different countries and can change over a certain period.[8-10]

In human rotaviruses, the predominant genotypes include G1, G2, G3, G4, and G9, which are combined with P[4], P[6], or P[8].[8,9,11] Recently, G9 and G12 genotypes have been increasingly detected and become one of the important rotavirus genotypes in many countries. G3 is also recognized as the dominant genotype and expands in many Asian countries, including Japan[12] and Hong Kong.[13]

This study aimed to determine profile genotypes of rotavirus in outpatient children under five years of age with diarrhea in Bandung, West Java, Indonesia.

Methods

This study was conducted in two primary health centers (nonhospitalized), Ibrahim Adjie and Garuda public health centers, in Bandung, West Java, Indonesia. The study included 450 children aged 1–59 months who visited the center with diarrhea complaints during the period of 2014–2018. Diarrhea in this study was defined based on the definition of diarrhea and degree of dehydration from the World Health Organization criteria.[14] All samples collected at the two primary health centers were transported using ice boxes to the laboratory, and aliquots were stored at −70°C. Enzyme-linked immunosorbent assay (ELISA) test was performed to quantitatively determine the presence of the virus in stool with a positive or negative result. Genotype of the rotavirus was then confirmed through sequencing using the polymerase chain reaction.[15] Rotaviruses were extracted using the QIAamp Ribonucleic Acid Mini Kit at the Bio Farma Surveillance Laboratory.

Statistical analysis

Data were expressed as sums and percentages. The statistical test used to describe the relationship between characteristics of rotavirus was Pearson Chi-square with Epi Info version 3.5.4. Atlanta, Georgia, US. Significance of the test result was determined using $P < 0.05$.

Results

From a total of 450 children who visited the primary health centers with diarrhea complaints in children aged <5 years, 40 were positive for rotavirus (8.9%) as evaluated by ELISA, consisting of genotypes G1P[8] followed G3P[8], G3P[6], G3P[9], G2P[4], and nontypeable (NT).

The subjects in this study were mostly boys (54.4%), aged 12–24 months (33.6%), and had a father and a mother with higher than secondary education background (73.6% and 68.7%, respectively) [Table 1]. Most subjects had a father who worked in the private sector (73.3%) and a mother who was unemployed (64.4%) with a combined income of in the low-income category (54.4%) of IDR 1,000,000–3,000,000.

Table 1: Characteristics and sociodemographics of children with diarrhea (*n*=450)

Variable	*n* (%)
Sex	
Boy	245 (54.4)
Girl	205 (45.6)
Age (month)	
≤6	51 (11.3)
>6–12	108 (24.0)
>12–24	151 (33.6)
>24–60	140 (31.1)
Father's education	
Elementary	26 (5.8)
High school	93 (20.7)
University	331 (73.6)
Mother's education	
Elementary	29 (6.4)
High school	112 (24.9)
University	309 (68.7)
Father's occupation	
Unemployed	14 (3.1)
Farmer	1 (0.2)
Private sector	330 (73.3)
Government employee	36 (8.0)
Other	69 (15.3)
Mother's occupation	
Unemployed	290 (64.4)
Farmer	2 (0.4)
Private sector	90 (20.0)
Government employee	11 (2.4)
Other	57 (12.7)
Parent income (Indonesian Rupiah)	
<500,000	12 (2.7)
500,000–1,000,000	91 (20.2)
1,000,000–3,000,000	245 (54.4)
>3,000,000	102 (22.7)

Data on demographic and nutritional status in children with rotavirus diarrhea are presented in Table 2.

The number of boys who came to the primary health center due to diarrhea caused by rotavirus was slightly higher than girls (P = 0.460). The children enrolled in the study were divided into four age groups. The most common rotavirus positive in age group is >12–24 months as much 12 children and >24–60 months as much 12 children, whereas the highest percentage is at the age of ≤6 months (11.8%), followed by the age group of >6–12 months (9.3%) [Table 2].

Apart from diarrhea, vomiting was also more prevalent in patients who were positive for rotavirus diarrhea (55%) with a significant correlation (P = 0.013). Fever was observed in 32.5%. However, the majority of the children in the study showed no signs of dehydration (75%). There was no significant difference in fever and dehydration level between rotavirus diarrhea and nonrotavirus diarrhea [Table 3].

Table 2: Correlation between characteristics of children with rotavirus diarrhea

Variable	Rotavirus			
	n=450, *n* (%)	Positive (*n*=40; 8.9%), *n* (%)	Negative (*n*=410; 91.1%), *n* (%)	*P**
Sex				
Boy	245 (54.4)	24 (9.8)	221 (90.2)	0.460
Girl	205 (45.6)	16 (7.8)	189 (92.2)	
Age (month)				
≤6	51 (11.3)	6 (11.8)	45 (88.2)	0.868
>6–12	108 (24.0)	10 (9.3)	98 (90.7)	
>12–24	151 (33.6)	12 (7.9)	139 (92.1)	
>24–60	140 (31.1)	12 (8.6)	128 (91.4)	
Nutritional status				
Normal	403 (89.6)	34 (8.4)	369 (91.6)	0.324
Moderate malnutrition	47 (10.4)	6 (12.8)	41 (87.2)	

*Pearson Chi-square

Table 3: Correlation between clinical manifestations and rotavirus diarrhea

Clinical manifestation	Rotavirus		*P**
	Positive (*n*=40), *n* (%)	Negative (*n*=410), *n* (%)	
Vomiting	22 (55)	144 (35.1)	0.013
Fever	13 (32.5)	119 (29.0)	0.645
Degree of dehydration			
No signs of dehydration	30 (75.0)	339 (82.7)	0.227
Some dehydration	10 (25.0)	71 (17.3)	

*Pearson Chi-square

Table 4: Summary of genotypes G and P of human rotaviruses

Rotavirus genotype	Total (*n*=40), *n* (%)
G1 P8	18 (45)
G2 P4	1 (2.5)
G3 P6	4 (10)
G3 P8	14 (35)
G3 P9	2 (5)
Nontypeable	1 (2.5)

The genotypes of the rotavirus in these children were, in order of frequency, G1P[8] (*n* = 18, 45%), G3P[8] (*n* = 14, 35%), G3P[6] (*n* = 4, 10%), G3P[9] (*n* = 2, 5%), G2P[4] (*n* = 1, 2.5%), and NT (*n* = 1, 2.5%) [Table 4].

Discussion

Global rotavirus surveillance network declared the mean percentage of rotavirus detection as 36%.[16] This study describes the prevalence of rotavirus in children with diarrhea in two primary health centers (nonhospitalized) in Bandung, West Java, Indonesia, during the period of 2014–2018. Of the 450 children with diarrhea enrolled in this study, 40 children (8.9%) were detected positive for rotavirus. A previous study on the rotavirus prevalence among hospitalized children with acute watery diarrhea in Indonesia presented a prevalence of 47.5%.[17] Our study shows lower than the prevalence of rotavirus infection in nonhospitalized children in Japan 19.9%[8] and South Africa 26.4%.[18]

The majority of the children in our study were boys (54.4%), with 9.8% being positive for rotavirus. The prevalence of rotavirus in boys is higher than in girls, which is similar to the prevalence in the previous study.[19] In this study, the most diarrhea was found in the age group >12–24 months (33.6%) The most common rotavirus positive in age group is >12–24 months and >24–59 months, while the highest percentage is at the age of ≤6 months (11.8%). This result supports the finding of a study in Nigeria that demonstrated rotavirus diarrheas to be the highest in the age group of <6 months (27.2%).[20] Rotavirus is transmitted by fecal-oral contact, possibly through contaminated surfaces and hands, and is spread by inhalation.[21] A meta-analysis study reported that breastfeeding was not directly associated with rotavirus diarrhea.[22]

This study shows that the highest education of mothers is from the university group (68.7%). Higher levels of education can contribute to increasing awareness of healthy living, good sanitation, as well as the prevention and transmission of diarrhea.[17] Most of the mothers were unemployed (64.4%) and the income of the parents was mostly low (77.3%).

We observed no correlation between nutrition status and rotavirus-positive status with *P* = 0.324, which is not different from the finding from an earlier study in Uganda.[23] The symptoms of acute diarrhea caused by rotavirus are

watery diarrhea, vomiting, fever, and dehydration.[19] The clinical symptom of vomiting was mostly seen in rotavirus diarrhea (55%) with a significant correlation (P = 0.013). This is lower compared to the findings in the hospital studies of 86%–88.8%. Fever was observed in 32.5% of the subjects, which is almost similar to the findings in hospital studies of 43.96%–44%.[24,25] The majority of the children in our study did not show any sign of dehydration (75%). The degree of dehydration was proven to have no correlation with being positive for rotavirus in this study (P = 0.227), which is different from the result of a previous study that demonstrated a significant association between the degree of dehydration and rotavirus diarrhea.[23]

Regarding the G and P genotyping of rotavirus in our study, five common G–P combinations were identified, including G1P[8] as the dominant genotype (45%), followed by G3P[8] (35%), G3P[6] (10%), G3P[9] (5%), G2P[4] (2.5%), and NT (2.5%).

A previous study in Bandung, Indonesia, shows that the prevalence of rotavirus in hospitalized children was 47.8%, with G[1] (37.5%) and P[6] (53.5%) as the identified rotavirus genotypes.[26] In Indonesia, rotavirus immunization has not yet been included in the national immunization program, but it can be obtained at private health services.

In Indonesia, the rate of rotavirus diarrhea in hospitalized children under five is 37–69%, while the same rate in outpatients is 40%. The most commonly detected genotypes are G9 (30%) and P[6] (56%). G1P[6] and G9P[6] account for 34% and 21% of strains, respectively.[23] A recent study declared that the most prevalent G and P genotypes were G1P[8] in 2010 (63.2%), 2011 (64.1%), and 2012 (74.6%) and G3P[8] in 2013 (49.7%), 2014 (82.5%), and 2015 (84.4%).[17]

The five rotavirus genotypes that are considered the most prevalent globally were G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] although regional differences are observed.[16] The 6-year surveillance period in 15 Eastern and Southern African countries demonstrated that 23.8% of the identified strains are G1P[8], followed by G2P[4] (11.8%), G9P[8] (10.4%), G12P[8] (4.9%), G2P[6] (4.2%), and G3P[6] (3.7%).[11]

Our study demonstrated that G1P[8] is the dominant genotype among the subjects (45%), which is similar to the finding of previous studies.[8,11,12] The G3 (50%) and G1 (45%) strains had high sequence similarities to those identified in studies in Asian countries including Japan, Vietnam, China, Hong Kong, and Pakistan.[8,12,13,27-29] In Indonesia, rotavirus immunization has not yet been included in the national immunization program, but it can be obtained at private health services.

The long-term impact of rotavirus vaccine on circulating strains is still unknown and need requires further research. Studies in countries that have been vaccinated against rotavirus show that the highest prevalence is the G2P[4] and G3P[8] genotypes.[30]

The genotypes and variations presented in this study complement the diversity of genotypes and variations from year to year, both in Indonesia and in other countries. It is expected that this information can provide background information to the policymakers for the implementation of rotavirus vaccines in the region.

Conclusions

The predominance of the G1P[8] genotype was observed in this study, followed by G3P[8], G3P[6], G3P[9], G2P[4], and NT. In view of the fact that the most common rotavirus positive in age group is >12–24 months and >24–59 months, while the highest percentage of children with rotavirus-positive status is seen in the age group of ≤6 months. Based on these results, we recommend starting immunization as early as possible to protect infants from rotavirus infection.

Research quality and ethics statement

This study was approved by the Institutional Review Board/Ethics Committee (IRB No. 630/UN6.C2.1.2/KEPK/PN/2014). The author followed applicable equator Network (http://www.equator_network.org/) guidelines during the conduct of the research project.

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Conflicts of interest

There are no conflicts of interest.

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