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Protective effect of rotavirus immunization in acute diarrhea due to rotavirus infection: a prospective cohort study

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ABSTRACT

Background: Diarrhea is a life-threatening disease for children. In Indonesia, the incidence of rotavirus infection was 67% of all cases of acute diarrhea. Immunization is an effort that can be done to prevent acute diarrhea due to rotavirus infection. Rotarix® vaccine is expected to have protective effect against acute diarrhea due to rotavirus infection. This study aims to investigate the protective effect of immunization against rotavirus on the incidence of acute diarrhea in children due to rotavirus infection.

Methods: This was an analytical study using prospective cohort approach in private pediatric clinics in Denpasar, Bali, Indonesia. Participants include one-hundred children aged 6 months was divided into two groups, i.e. vaccinated and non-vaccinated groups, each consisting of 50 children. Incidence of acute diarrhea in both groups were measured and subsequent statistical analyses were conducted to determine contributing factors for the protective effect of rotavirus vaccine against acute diarrhea.

Results: The incidence of rotavirus infection in vaccinated children was 6%, compared to 24% in the non-vaccinated children. There was significant association between vaccination status and the incidence of acute diarrhea due to rotavirus infection (p value=0.012). The probability of acute diarrhea due to rotavirus infection in both groups were 15% and 52% for the vaccinated and non-vaccinated groups, respectively. The relative risk reduction of acute diarrhea due to rotavirus infection is 71% [RR=0.288; 95% CI (52% -82%)].

Conclusions: Immunization against rotavirus infection has a statistically significant protective effect against acute diarrhea due to rotavirus infection.

Keywords: Diarrhea, Rotavirus, Immunization

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INTRODUCTION

Diarrhea is a life-threatening disease for children. Globally, diarrhea had caused 1.5 cases of death, the majority of which were due to infection by pathogens. It is estimated that 29% of death in children under 5 years old is due to diarrhea from rotavirus infection.[1] In South-East Asia, the rate of rotavirus infection was 54.6% in 2000 and 50.7% in 2013. Death due to rotavirus infection was 59,052 cases in 2000 and 21,252 cases in 2013.[2]

In Indonesia, a hospital-based epidemiological study showed that the incidence of rotavirus infection was 67% of all cases of acute diarrhea, with the youngest patient being 2 months old and the oldest being 54 months. The genotypic distribution of variant G found in this study was G1 (35%), G9 (12.5%), G2 (7.5%), and for variant P the distribution was P6 (52.5%), P8 (17.5%), and P4 (10%).[3] Rotavirus infection has become a concern in Indonesia due to a study conducted by Soenarto et al., who found that severe dehydration from acute diarrhea which can lead to death was more commonly found in patients with rotavirus infection compared to those who are not (91% vs 82%; p<0.05).[4]

Immunization is an effort that can be done to prevent acute diarrhea due to rotavirus infection. There are two vaccines currently available, the Rotarix® vaccine and the Rotateq® vaccine. Both have been used extensively worldwide. Rotarix® is a monovalent vaccine consisted of G1, P8, while Rotateq® is a pentavalent vaccine consisted of G1, G2, G3, G4, and P8. Both vaccines have high efficacy when used in developed country, but the numbers are much lower in developing countries.[5]

Rotavirus genotypic distribution data revealed that the largest percentage of rotavirus strain in Indonesia is the G1 variant, and therefore the administration of monovalent vaccine (Rotarix®) is expected to have protective effect against acute diarrhea due to rotavirus infection. This study

aims to investigate the protective effect of rotavirus immunization against acute diarrhea due to rotavirus infection by comparing the incidence of acute diarrhea in children aging 6 months to 2 years who were followed for 1 year after given Rotarix® vaccine as immunization against rotavirus.

METHODS

This was a prospective cohort study conducted in a private clinic in Denpasar, Bali, Indonesia from January 2018 to January 2019. Subjects consisted of children aged 6 months who have been vaccinated against rotavirus and who have no previous history of acute diarrhea.

Samples were collected using consecutive sampling technique. Inclusion criteria include (1) children who have been vaccinated twice with monovalent vaccine against rotavirus at 6 months of age; (2) children who have not been vaccinated; (3) children who have no previous history of acute diarrhea at the time of data collection; and (4) children whose parents/guardian provided informed consent to participate in the study. Exclusion criteria were children with immunodeficiency and children with congenital heart disease.

Subjects were then divided into two groups: one group for children who have been vaccinated against rotavirus using monovalent vaccine, and one group for children who have not been vaccinated against rotavirus. The subjects' parents/guardian were then asked to complete a questionnaire regarding demographic characteristics and social-economic background, as well as their rotavirus immunization status.

Subjects were followed until they reached the age of 18 months, and follow up assessment was conducted every 3 days (by phone) to ask for whether the child had diarrhea. If the child had diarrhea, the feces were collected and sent to a laboratory within 48 hours to undergo test for rotavirus. The test was conducted using chromatography technique.

At the end of the data collection period, all data were analyzed using IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY: IBM Corp). Sample characteristics were presented descriptively using narration and tables. Statistical analysis was conducted using Chi-square test. Multivariate analysis using logistic regression test was also conducted.

The study has been approved by the Ethical Committee of the Faculty of Medicine of Universitas Udayana/Sanglah General Hospital through permit number 1381/UN.14.2/Litbang/2018 and has also been approved by the Reseach and Development Division of the Faculty of Medicine of Universitas Udayana/Sanglah General Hospital with a permit number 2018.02.1.0666.

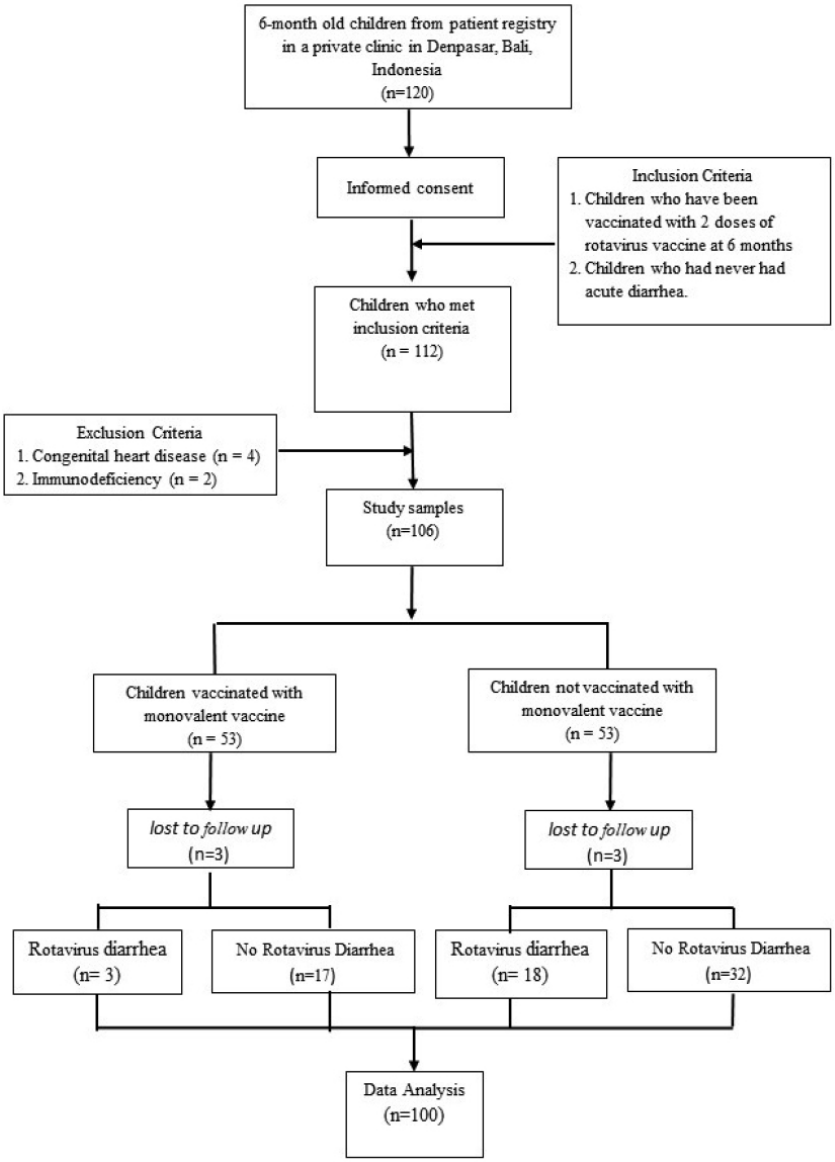

Figure 1. Study Protocol

RESULTS

A total of 120 children were interviewed for this study. Eight children were excluded due to the previous history of acute diarrhea, 6 were excluded due to congenital heart disease, and 2 were excluded due to immunodeficiency. The remaining children were observed until they reached the age of 18 months, and 3 children in each group were lost to follow up: one due to unwillingness to collect feces sample, one due to sample cannot be analyzed by the laboratory, and the last one could not be contacted for follow up. (Figure 1).

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Subjects' Characteristics

The subjects' characteristics based on monovalent immunization status were presented in Table 1. Fifty-six percent of the children in the monovalent vaccine group were male. All samples were 6 months old at the start of data collection. Good nutrition status was observed more frequently in the monovalent vaccinated group (80%) and poor nutrition status was observed more frequently in not vaccinated group (60%). In terms of maternal education level, all mothers in the vaccinated group have graduated from at least an academy (100%). Eighty-percent subjects were breastfed until older than 6 months old in the vaccinated group versus 66% percent in the not vaccinated group.

Bivariate Analyses on the Effect of Immunization Status, Sex, Nutritional Status, Breastfeeding Status, Maternal Education Level to the Incidence of Acute Diarrhea due to Rotavirus Infection

Table 2 showed the result of bivariate analyses between immunization status and confounding variables including sex, nutritional status, maternal education level, breastfeeding status, and the family economic status against the dependent variable (acute diarrhea due to rotavirus infection). All variables with a $p < 0.25$ were included in the subsequent multivariate analysis.

Multivariate Analysis on the Incidence of Rotavirus

Variables included in the logistic regression analysis include: main variable (immunization status), confounding variables with $p < 0.25$ (breastfeeding status, sex, and maternal education level) and interaction variable. Form the analysis on confounding variables, we generated a gold standard model and altenative models as presented in Table 3.

A confounding variable is eliminated from the logistic regression model if said confounding variable causes no more than 10% changes in the OR. These changes are calculated by subtracting the OR of an alternative regression model with the OR of the gold standard model, and the difference is divided by the OR of the gold standard model and multiplied by 100%. Based on Table 3 above, breastfeeding status and maternal education level caused more than 10% changes in the gold standard OR.

The relative risk in this cohort study can be calculated by comparing the probabilities of rotavirus diarrhea in the vaccinated group and the probabilities of rotavirus diarrhea in the not vaccinated group. Based on the result of the logistic regression, the probability of rotavirus diarrhea in the vaccinated group is 15%, versus 52% in the not vaccinated group. This number generated a relative risk (RR) of 0.288 for the occurrence of rotavirus diarrhea in a vaccinated child, compared to non-vaccinated child (95% CI 0.1744 to 0.4771, $p < 0.0001$). The relative risk reduction is 71% (95% CI 52% to 82%), and the absolute risk reduction is 37% (95%CI 24% to 49%).

Table 1. Subjects' Characteristics

Characteristics	Monovalent Vaccine	
	Yes (n=50)	No (n=50)
Sex, n (%)		
Male	28(56)	25(50)
Female	22(44)	25(50)
Breastfeeding status, n (%)		
Not breastfed	4(8)	8(16)
Breastfed < 6 months	6(12)	9(18)
Breastfed ≥ 6 months	40(80)	33(66)
Nutritional status, n (%)		
Poor nutrition	17(34)	30(60)
Good nutrition	33(66)	20(40)
Family Economy Status, n (%)		
< Minimum Wage (Rp. 2.173.000,-)	0 (0)	3(6)
≥ Minimum Wage	50(100)	47(94)
Maternal Education Level, n (%)		
High School	0(0)	18(36)
Academy/University	50(100)	32(64)

DISCUSSION

Our study observed a significant effect of rotavirus immunization against the incidence of acute diarrhea due to rotavirus infection. Multivariate analysis revealed that vaccination against rotavirus reduced the incidence of rotavirus diarrhea from 52% in the non-vaccinated group to 15% in the vaccinated group. Controlling significant confounding variables such as breastfeeding status and maternal education level yielded an absolute risk reduction of 37% with a relative risk of RR 0.288.

This result is consistent with a meta-analysis conducted by Velasquez et al., in Latin America and the Caribbean, who found that Rotarix® reduced the risk of any severity rotavirus-related gastro-enteritis by 65% (RR 0.35; 95% CI 0.25-0.50) and reduced the risk of severe gastro-enteritis by 82% (RR 0.18; 95% CI 0.12-9.26).[6] This result is also consistent with a meta-analysis of 48 Randomized Control Trials (RCT) which showed that rotavirus vaccine had a 88.4% efficacy (95%CI=67,1-95,9) in preventing the occurrence of acute diarrhea due to rotavirus infection in children under 5 years old in East Asia

Table 2. Bivariate Analyses on the Effect of Immunization Status, Sex, Nutritional Status, Breastfeeding Status, Maternal Education Level to the Incidence of Acute Diarrhea due to Rotavirus Infection

Variables	Rotavirus Diarrhea		P value
	Yes	No	
Immunization status, n (%)			
Yes	3(6)	47(94)	0.012
No	12(24)	38(76)	
Sex, n (%)			
Male	11(20.8)	42(79.2)	0.087
Female	4(8.5)	43(91.5)	
Nutritional Value, n (%)			
Poor	7(14.9)	40(85.1)	0.978
Normal	8(15.1)	45(84.9)	
Maternal education level, n (%)			
High school graduate	1(5.6)	17(94.4)	0.196
Academy graduate	14(17.1)	68(82.9)	
Breastfeeding status, n (%)			
Not breastfed	7(58.3)	5(41.7)	<0.001
Breastfed < 6 months	5(33.3)	10(66.7)	
Breastfed ≥ 6 months	3(4.1)	70(95.9)	
Family Economy Status, n (%)			
< Minimum Wage	1(33.3)	2(66.7)	0.389
≥ Minimum Wage	14(14.4)	83(85.6)	

Table 3. Analysis on Confounding Variables

Models	Variables	OR	95% CI	OR Reduction from gold standard model (%)	Precision
Model 1 (gold standard)	Immunization status controlled with breastfeeding status and maternal education level	0.139	0.028-0.681	-	0.653
Model 2	Immunization status controlled with breastfeeding status	0.215	0.046-0.997	54.68%	0.951
Model 3	Immunization Status controlled with maternal education level	0.122	0.031-0.483	12.23%	0.452
Model 4	Immunization Status	0.202	0.053-0.768	45.32%	0.715

and South East Asia.[7] Another study investigated the effectiveness of monovalent rotavirus vaccine (Rotarix®) and found that vaccination against rotavirus reduced the incidence of acute diarrhea due to rotavirus infection in infants <12 months (p<0.001), where the prevalence of diarrhea decreased along with the increased coverage of vaccination.[8] A meta-analysis of Cochrane database involving 44 RCT studies with a total of 175,944 infants showed that both Rotarix® and Rotateq® are effective in reducing the incidence of acute diarrhea due to rotavirus infection compared to placebo. Rotarix® was found to reduce the incidence of rotavirus acute diarrhea by 72% within the first year of vaccination (RR=0.28; 95%CI=0.17-0.48 in 6 RCTs). However, those studies differ from our study in terms of design, where their meta-analysis included only RCTs and not cohort study. In addition, there is a significant difference in terms of sample size where our study analyzed only 100 samples and the meta-analyses analyzed up to hundred-thousand samples.

Other studies also followed the subjects for longer period. Where the current study followed the subjects until they reach the age of 18 months, other studies have followed their subjects up to 2 or 3 years with varying results. Bar-Zeev et al., followed their subjects until they reached 2 years old with a reduced acute diarrhea incidence of 67% (RR= 0.33; 95%CI=0.21-0.50 in 5 RCTs). On the contrary, Soares-Weiser et al., followed their patients up until they reached 3 years old and found no difference in terms of effectiveness when compared to placebo.[9]

Other studies also investigate the effect of repeat immunization on the incidence of rotavirus diarrhea. A meta-analysis on 67 RCT studies conducted on children showed that rotavirus vaccination reduced the incidence of acute diarrhea due to rotavirus infection by 78% (RR=0.22; 95%CI=0.18-0.28; p<0.001) in a dose-dependent manner.[10] An RCT conducted in South Africa and Malawi involving 4,939 infants, where 1,647 infants received 2 doses of vaccine, 1,651 received 3 doses and 1,641 received placebo showed that the efficacy of rotavirus vaccine against rotavirus diarrhea were 58.7% (95%CI=35.7-74.0) in the 2-doses group and 63.7% (95%CI=42.4-77.8) in the 3-doses group. The reduction of rotavirus diarrhea incidence due to rotavirus vaccination was 30.2% (95%CI=15.0-42.6; p<0.001).[11]

Those studies used different methods than the current study. Therefore, the results cannot necessarily be compared. The main difference lies in the duration of follow up and the dosage of vaccine administered. Additionally, the current study also uses no placebo as in the study by Soares-Weiser

et al. The data collected in the present study may also not be adequate in concluding dose-dependent effectiveness as in the study by Kazimbaya et al.

The efficacy of vaccine has been empirically proven to be varied. The most prominent difference in terms of efficacy can be observed when comparing the high-income population and low-income population. The efficacy of vaccine tends to be higher in high-income population due to the fact that confounding factors such as malnutrition is often found in low-income population.[12] In a 2018 study, Das found that the efficacy of rotavirus vaccine against severe rotavirus diarrhea were 61% and 30% in Africa and Asia, respectively;[13] which was lower than the current finding. This might be due to the difference in terms of the subjects' socioeconomic background and nutritional status.

The current study was conducted on 100 children, the size of which is relatively small compared to other studies. However, several studies with smaller sample size also showed significant protective effect against rotavirus diarrhea. A study conducted in Spain on 467 children under 2 years old showed that the effectiveness of 1 dose of rotavirus vaccination was 91.5% (95%CI= 83.7%-95.6%) against acute diarrhea due to rotavirus infection.[14] A study conducted by Braeckman et al in Belgium on 215 children using 2 doses of monovalent vaccine also showed an overall effectiveness of 90% (95%CI=81%-95%). When broken down into age groups, the effectiveness was 91% and 90% in children aged 3-11 months and in children >12 months, respectively. In 25% of cases, rotavirus infection was found to have protective effect against co-infection by adenovirus, astrovirus and norovirus.[15]

Multivariate analyses revealed that nutritional status did not affect the protective effect of rotavirus vaccine. It is known that nutritional status played an important role in a child's immunity and vulnerability against infections by pathogens. However, a study in Bangladesh supported the result of the current study, where the study found that malnutrition had no effect on the incidence of rotavirus diarrhea. The study found that healthy intestinal epithelial cells are required for the adhesion of virus, and it is harder for the virus to infect intestinal tissues with decreased number of cells and proliferation, as often found in children with malnutrition.[13] The fact that our study excluded children with malnutrition also may play a role in the difference between our findings and that of previous study.

The multivariate analysis conducted in this study also revealed that exclusive breastfeeding affected the protective effect of rotavirus vaccine. This finding differs from that of previous studies. A study in South Africa on the immunogenicity of rotavirus vaccine found no statistically significant difference between children under 5 years old with exclusive breastfeeding and without breastfeeding, after the administration of 1 dose (p=0.859) and 2 doses (p = 0.485) of Rotarix®.[16] Another study in Uganda also found that exclusive breastfeeding had no significant protective effect against rotavirus diarrhea (OR=1.08; p=0.8). Our finding might be due to the fact that the highest level of IgA antibody protection against rotavirus exists in the colostrum from breastmilk produced by the mother in the child's the first few days of life, which then decreased during the first week of life.[17] A meta-analysis on 10,841 samples also showed no significant difference in terms of rotavirus diarrhea incidence in the group with exclusive breastfeeding and the group without breastfeeding (OR=0.86; 95% CI=0.63-1.16). The study went on to explain that breastmilk only had a significant effect on children ≤ 1 year, while its protective effect on children ≥2 years is inadequate to prevent diarrhea, although it may temporarily delay the occurrence of diarrhea due to rotavirus.[18]

A study in India found that breastfeeding may affect rotavirus antigenemia and RNAnemia, where exclusive breastfeeding significantly reduced rotavirus antigenemia (p<0.001). Rotavirus antigenemia is indirectly associated with the severity of acute diarrhea due to rotavirus. This might be due to the fact that maternal serum rotavirus IgG is higher in children who were breastfed, with IgG titre >1:800 may have an 80% protective effect against rotavirus diarrhea. Additionally, the Lactadherine content in breastmilk may also act as an anti-rotavirus antibody.[13]

Our finding related to the significant protective effect of 6-month exclusive breastfeeding may also be affected by our duration of follow up. Shen et al also stated that the protective effect of breastfeeding against rotavirus diarrhea is most effect in children under 1 year old, and may disappear when the child reach 2 years old.[18] Our subjects were followed from the age of 6 months to 18 months; thus, half of the follow up duration lies in the protective period.

The limitation of this study lies in the fact that we only assess the incidence of acute rotavirus diarrhea in one episode of diarrhea, and we did not determine the serotype of rotavirus which caused the diarrhea in our subjects.

CONCLUSION

Vaccination is proven to prevent acute diarrhea due to rotavirus infection after controlling confounding variables such as breastfeeding and maternal education background. Rotavirus vaccination was

found to reduce the relative risk of acute diarrhea due to rotavirus infection.

AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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