INCIDENCE AND CLINICAL MANIFESTATIONS OF ROTAVIRUS INFECTION AMONG CHILDREN WITH ACUTE DIARRHEA ADMITTED AT BURI RAM HOSPITAL, THAILAND

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Abstract. To study the incidence and clinical manifestations of rotavirus among children at Buri Ram Hospital, admitted with the diagnosis of acute diarrhea between November 2005 and February 2006. In the course of a cross sectional descriptive study, 103 stool samples obtained from inpatients below the age of 5 years were examined for rotavirus by RT- PCR. Data on clinical manifestations, complications, administration of antibiotics, length of admission and hospital cost were obtained by means of questionnaires distributed among physicians. The statistics used were presented as percentage, mean and 95% confidence interval, while chi-square and unpaired *t*-test were used to establish significant differences at p<0.05. Rotavirus was detected in 45 of 103 stool samples (43.68%, 95%CI = 33.93, 53.81). No significant differences were found between clinical manifestations of children with rotavirus infection and non-rotavirus infection as both groups displayed acute watery diarrhea (p=0.33), fever (p =0.80), nausea or vomiting (p=0.08), predominant lymphocytes (p =0.54), absence of red blood cells (p=0.63) or white blood cells (p=0.57) in the stool examination, moderate or severe dehydration (p=0.06), lactose intolerance (p=0.41), hypokalemia (p=0.55), metabolic acidosis (0.18) Administration of antibiotics was significantly reduced for treatment of rotavirus acute diarrhea (31.1% *vs* 63.8%, p=0.001). Hospital cost and length of admission were significantly reduced in rotavirus acute diarrhea (1,845.04 baht *vs* 2,297.00 baht, p<0.01) (2.09 days *vs* 2.81 days, p<0.001). Compared to previous studies, no differences were found in the percentage of rotavirus acute diarrhea. Rotavirus infection is a major cause for hospitalization of children below the age of 5 years with acute diarrhea. Clinical characteristics of rotavirus acute diarrhea were not significantly different from those due to other etiologies. Diagnosis of rotavirus infection should be based on various clinical manifestations and specific laboratory methods. Further studies on the cost benefit of rotavirus vaccine in Thailand ought to be performed before implementing a universal vaccination program.

INTRODUCTION

In 2000, WHO reported that 10.5 million children below the age of 5 years, died annually. Among these deaths, diarrhea was the second most common cause (17.5%) (Mathers *et al,* 2003). Also, Black *et al* (2003) established that diarrhea is responsible for 14.1% deaths during childhood. In developing countries, children below the age of 11 months on average suffer from 3.8 acute diarrhea episodes per child per year and those between 1 and 4 years of age from 2.1 (Kosek *et al*, 2003).

Most episodes of acute diarrhea in chil-

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dren below the age of 5 years are caused by a virus. Globally, the major cause is rotavirus while other viruses such as Adenoviruses, Astroviruses, Norwalk viruses and Noroviruses are more frequently encountered among higher age groups (Bureau of Epidemiology, 2006).

One of 65 children with rotavirus infection requires hospitalization and one of 293 succumbs to the infection (Center of Disease Control, 2006a). Proper symptomatic treatment plays a crucial role in rotavirus gastroenteritis; however, bacterial gastroenteritis calls for administration of antibiotics along with improvements in hygiene and sanitation.

The prevalence reported for rotavirus diarrhea varies. Parashar *et al* (2006). reported 22% worldwide, whereas the Asian Rotavirus Surveillance Network reported 45% in Asia alone (Bresee *et al*, 2004). In Thailand, the incidence of rotavirus infection in young children amounted to 27-50% among inpatients diagnosed with diarrhea and the prevalent genotype varied from year to year. Other known causes of acute diarrhea are Norwalk virus, *E. coli*, *Salmonella, Shigella* and *V. cholerae*, *C. jejuni*, *E. histolytica* (Pinyosamosorn *et al*, 1988; Kaowangkoon *et al*, 1992; Suwatano *et al*, 1997; Maneekarn and Ushijima, 2000; Noppornpanth *et al*, 2001; Jiraphongsa *et al*, 2005; Theamboonlers *et al*, 2005).

In 1998, rotavirus vaccine had been used in the United States until associated serious complications, especially intussusceptions, were observed in infants (Murphy *et al*, 2001). Nowadays, the WHO classifies rotavirus vaccine as an urgency vaccine that should be developed early (Parashar *et al,* 2003). In January 2006, the Human Rotavirus Vaccine Study Group reported that a two-dose course of attenuated G1P[8] human rotavirus oral vaccine effectively reduced severe rotavirus gastroenteritis as well as severe gastroenteritis resulting from any etiology without an increase in the intussusception rate (Ruiz-Palacios *et al,* 2006). At the same time, Vesikari *et al* (2006) reported the same results of pentavalent human-bovine (WC3) reassorted vaccine.

In Thailand, 5 million children are below the age of 5 years. The Bureau of Epidemiology (2006) reported that every year more than 300,000 children (6,000 per 100,000 cases) are diagnosed with acute diarrhea, despite the Health Department's attempts at reducing the patients to 3,000 per 100,000 or approximately 150,000 per year. When the number of patients increases in Buri Ram Province during the winter months, the cost of treatment has a great impact on the government budget. Identifying the cause of acute gastroenteritis may better enable us to control, prevent and manage the disease.

Our objectives are to study the frequency, clinical manifestations of rotavirus diarrhea in pediatric patients and the cost of treatment at Buri Ram Hospital, Thailand.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted in order to establish the incidence of rotavirus infection in children below the age of 5 years who had been admitted with a diagnosis of acute diarrhea to the inpatient department at Buri Ram Hospital between November 2005 and February 2006. The study was approved by the institutional ethics committee of Buri Ram Hospital.

Patients

One hundred and three from 256 inpatients under the age of 5 years diagnosed with acute diarrhea between November 2005 and February 2006 were randomly recruited into the study. The sample size was calculated using p=0.43 according to previous rotavirus surveillance study in Thailand (Jiraphongsa *et al*, 2005) and acceptable error of 0.1 (Dobson, 1984). The detail of the study was informed to their parents and written consents were obtained. Acute diarrhea was defined as

loose stool ≥ 3 times/day or mucous bloody stool of less than 7 days duration. Exclusion criteria were any immunosuppressive disorder and recurrent readmission due to diarrhea.

Study procedure

After written informed consent had been provided by the parents, physicians took the history of diarrhea and examined the patients. All patients received the appropriate therapy as tailored per individual by the physician. Physicians provided the information on each patient required to fill in the questionnaire, which was expanded and verified by pediatric experts.

Data collection

The questionnaire completed by the physician comprised demographic data, history, physical examination, examination results, complications and treatment in the hospital.

The total hospital cost included all examinations and treatment during admission. Every indirect cost and the laboratory diagnosis for rotavirus such as RT-PCR for rotavirus detection is considered exceptional. Fecal specimens were collected and transferred to -70ºC until tested.

On every volunteer, CBC, serum electrolyte, stool examination and stool culture were performed at Buri Ram Hospital Laboratory Center while RT-PCR on stool samples was conducted at the Viral Hepatitis Research Unit, Faculty of Medicine, Chulalongkorn University, Bangkok.

Stool culture at Buri Ram Hospital is seeded on four standard media: SS agar, PCDF agar, buffer broth agar and MSRV agar. The detectable pathogens are *Salmonella* group A-I, *Shigella* and *Vibrio*. All specimens of children up to two years of age were serologically tested for organisms of the enteropathogenic *E. coli* groups I, II and III.

Rotavirus detection

Fecal specimens were diluted 1:10 in PBS (phosphate buffered saline), centrifuged, and the supernatant was analyzed for Rotavirus-RNA by RT-PCR. Briefly, RNA was extracted from 50 μl of diluted feces applying the guanidinium-isothiocyanate method as described elsewhere (Theamboonlers *et al,* 2002) and subsequently reverse transcribed into cDNA using primer End 9 5′ GGTCACAT CATACAATTCTAATCTAAG 3′ (nt 1,062-1,036) for the VP7 gene. The resulting cDNA was amplified by PCR using primer Beg9 5′-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3′ (nt 1-28) as the sense primer, and again End 9 as the antisense primer for the VP7 gene (Gentsch *et al,* 1992). The amplification reaction was performed in a thermocycler (9600 Perkin Elmer Cetus, Norwalk, CT) as follows: initial denaturation at 95ºC for 3 minutes, followed by 30 cycles each of 1 minute at 95ºC for denaturation, 1 minute at 60ºC for primer annealing, and 1 minute at 72ºC for extension and concluded by a final extension step at 72ºC for 10 minutes. After electrophoresis in a 2% agarose gel stained with ethidium bromide on preparation, the expected 1,062 bp-band product was visualized on a UV trans-illuminator (Gel Doc 1000, BIO-RAD, CA).

Statistical analysis

Based on the results published in the report by the Rotavirus Surveillance Project Thailand Study Group, the sample size calculated to evaluate the incidence of rotavirus infection should comprise 94 cases. The data was computed as number and percentage for categorical data and mean with 95% CI for continuous data. Employing chi-square and unpaired *t*-test, differences were considered statistically significant at $p<0.05$.

RESULTS

A total of 103 patients (60 male and 43 female) were recruited into the study. Mean age was 15.57 months (95%CI = 13.17, 22.96) and mean body weight was 9.57 kilograms (95%CI = 8.91, 10.23). Clinical mani-

festations in the majority of patients comprised acute watery diarrhea (79.6%), fever (81.5%), nausea or vomiting (80.6%), lymphocyte predominance in CBC (63.1%), absence of blood cells in stool (82.5%), and metabolic acidosis (90.3%). Degree of dehydration were mild in 46 (44.7%), moderate in 52 (50.5%), and severe in 5 (4.8%). None of the patients participating in our study had a fatal outcome.

Fig1 depicts authenticated etiologies responsible for acute diarrhea in children below the age of 5 years who had been admitted as inpatients to the Department of Pediatrics at Buri Ram Hospital between November 2005 and February 2006. Rotavirus was detected by RT-PCR in 45 of 103 stool samples (43.68%, 95%CI =33.93, 53.81). Bacteria were detected by stool culture in 5 (4.86%) stool samples. Two were *Salmonella* group C infections and the remaining three were enteropathogenic *E. coli* group I infections. Fifty-three (51.46%) samples, negative for either rotavirus or common bacteria, were classified as unknown cause. Combined infections were found in 2 samples (1.94%).

Based on our study, there were no clinical manifestations significantly predominant in rotavirus infection compared to other causes. Acute watery diarrhea (p=0.33), fever (p=0.80), nausea or vomiting (p=0.08), lymphocyte predominance in CBC (p=0.54), absence of both red (p=0.63) and white blood cells (p=0.57) in stool, moderate to severe dehydration (p=0.06), lactose intolerance (p=0.41), hypokalemia (p=0.55), metabolic acidosis (p=0.18) constituted common symptoms irrespective of the underlying cause. Significantly lower doses of antibiotics were administered in rotavirus acute diarrhea (31.1% *vs* 63.8%, p=0.001). Similarly, the total hospital cost and duration of admission were significantly reduced in rotavirus acute diarrhea. (1,845.04 baht *vs* 2,297.00 baht, p=0.01) (2.09 days *vs* 2.81 days, p<0.001).

A comparison between the clinical characteristics of rotavirus acute diarrhea and those of non-rotavirus acute diarrhea are shown in Table 1.

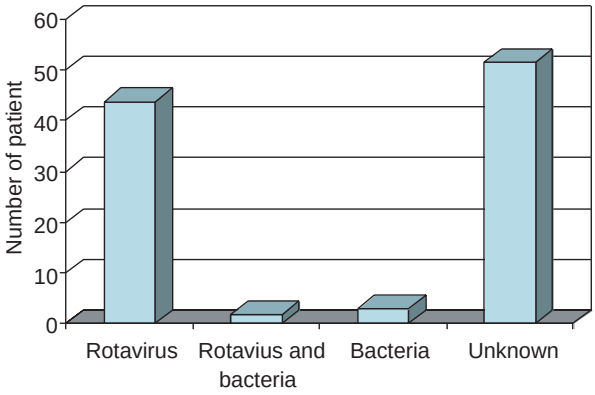

Fig 1–The etiologic causes of acute diarrhea among pediatric patients at Buri Ram Hospital.

DISCUSSION

In the present study, acute rotavirus diarrhea was confirmed by RT-PCR in 45 out of 103 pediatric stool samples. This percentage of infection diagnosed in children below the age of 5 admitted to Buri Ram Hospital between November 2005 and February 2006 agreed with the findings of a previous study by Jiraphongsa *et al* (2005) who reported 43% derived from 6 hospitals in Thailand. As the data for our study had been collected during a short winter season with a rather high frequency of rotavirus infection the percentage arrived at might constitute an overestimate. Our results were characteristic for provincial hospitals. Jiraphongsa *et al* (2005). reported that 12.2% of diarrhea cases were admitted to hospital. Rotavirus is still an important cause of acute diarrhea in this age group requiring hospitalization not only in Buri Ram but worldwide.

The group daubed “unknown causes” could represent various etiologies of diarrhea but we were in no position to subject those samples to detailed investigation.

Clinical manifestations of acute rotavirus diarrhea were not significantly different from

Table 1
Comparison between clinical characteristics of rotavirus acute diarrhea and non-rotavirus acute diarrhea.

Characteristics	Rotavirus acute diarrhea (N=45)	Non-rotavirus acute diarrhea (N=58)	p-value
Age (month)			
Mean	15.44	15.66	0.764
95%CI	11.86-19.02	12.35-18.98	
Age group, no.(%)			
Less than 24 months	34(75.6)	43(74.1)	1.00
History, no.(%)			
Watery stool	38(84.4)	44(75.9)	0.33
Fever	36(80.0)	48(82.8)	0.80
Vomiting	40(88.9)	43(74.1)	0.08
Investigation, no.(%)			
CBC : lymphocyte predominate	30(66.7)	35(60.3)	0.54
Stool exam :			
white blood cell negative	40(88.9)	49(84.5)	0.57
red blood cell negative	44(97.8)	55(94.8)	0.63
Complication, no.(%)			
Hypokalemia[a]	7(15.6)	6(10.3)	0.55
Metabolic acidosis[b]	43(95.6)	50(86.2)	0.18
Moderate or severe dehydration[c]	28(62.2)	29(50.0)	0.06
Lactose intolerance[d]	5(11.1)	10(17.2)	0.41
Antibiotic usage, no.(%)			
Antibiotic usage	14(31.1)	37(63.8)	0.001
Total hospital cost, baht (US)	1,845.04 (46.13)	2,297.00 (57.43)	0.009
Mean	1,547.61-2,142.48	2,015.44-2,578.56	
95%CI	(38.69-53.56)	(50.39-64.46)	
Length of admission (day)			
Mean	2.09	2.81	<0.001
95%CI	1.87-2.31	2.41-3.21	1

[a] Hypokalemia; serum potassium ion less than 3.5 mmol/l,
[b] Metabolic acidosis; serum bicarbonate ion less than 20 mmol/l,
[c] Mild dehydration; 3-5% weight loss, mild thirsty, decreasing urine output
Moderate dehydration was defined as 6-9% weight loss, tachycardia, dry lips, urine specific gravity >1.020, capillary refill 2-3 seconds.
Severe dehydration was 10% weight loss or more, PP<20 mmHg, crying without tear, sunken eyeball, urine specific gravity >1.030, capillary refill > 3 seconds.
[d] Lactose intolerance used clinical diagnosis; abdominal distension and perianal inflammation

those due to other causes and neither did they differ from the previous report by the CDC in America nor the previous study in Thailand (Center of Disease Control, 2006b; Kaowangkoon *et al*, 1992). Parashar *et al* (2005) reported that rotavirus infection was significantly more prevalent among younger age groups (3-24 months) (Charles *et al*, 2006) yet, based on our study this difference between the groups is non-existent. Ideally, immunization ought to be ad-

ministered before the age of 12 months yet, prior to the implementation of a universal immunization program its cost effectiveness ought to be thoroughly investigated. Diagnosis of rotavirus infection should be supported by various clinical manifestations and specific laboratory methods. However, this is not practical. As most acute diarrhea during childhood can be caused by a multitude of viruses, identification of rotavirus based solely on clinical manifestations is unreliable. Hence, it is debatable whether administration of antibiotics constitutes sensible treatment.

At Buri Ram Hospital, administration of antibiotics for childhood acute diarrhea is significantly less pronounced with rotavirus infection yet, 1/3 of acute rotavirus diarrhea is still being treated by antibiotics. To reassure anyone concerned about health care budgets, both the duration of admission and the total hospital expenditure were statistically less significant in rotavirus diarrhea. The total expenditure accrued by hospitalization did not include indirect cost. Based on the report by Zimmerman *et al* (2001), rotavirus-associated hospitalization in the US is much more costly (US$ 2,303) than in Thailand (US$ 46.13), undoubtedly due to the difference in Gross National Income. Similar to a study conducted in Argentina, the average duration of admission for rotavirus acute diarrhea amounts to 2.6 days, which is exceeded if the patient's infection is due to other causes (Cuestas *et al*, 2005).

Recently, rotavirus vaccine has been registered in Thailand, but it is still very costly and as yet, its cost-effectiveness has not been satisfactorily established. By now, at least two studies have been conducted on health care cost of diarrhea disease and estimates of the cost-effectiveness of Rota vaccine for children in Asia (Podewils *et al,* 2005). For example, in Vietnam universal vaccination of infants at a cost of US$ 7.26 or less per vaccine dose would be a cost effective public health intervention (Fischer *et al,* 2005).

As for Thailand, further community and genotype based studies conducted over a longer period on samples obtained from all the different regions of the country are imperative in order to establish the true prevalence of rotavirus, sensible administration of antibiotics and cost-effectiveness of rotavirus vaccination, as well as identify the as yet unknown etiologic agents.

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