Molecular Characterization of Aichi Virus Circulating in Pediatric Patients with Diarrhea in Chiang Mai, Thailand

การตรวจหาคุณลักษณะเฉพาะในระดับโมเลกุลของเชื้อไวรัสไอชิในผู้ป่วยเด็กที่มีอาการอุจจาระร่วง ในจังหวัดเชียงใหม่ ประเทศไทย

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ABSTRACT

Acute gastroenteritis is one of the major public health problem worldwide. Aichi virus is a newly discovered virus that related to acute gastroenteritis in human. The purpose of this study was to investigate the epidemiology and molecular characteristics of Aichi virus detected in fecal samples collected from pediatric patients with diarrhea in Chiang Mai, Thailand. Total of 302 fecal specimens collected during December, 2010 to December, 2011 were screened for Aichi virus by reverse transcription, semi-nested PCR and nucleotide sequencing methods. As a result, 17 of 302 (5.6%) fecal samples were positive for Aichi virus. The peak incidence of Aichi viruses was detected in winter season i.e. December, January and February. Based on 3C-3D nucleotide sequences, all Aichi virus strains detected in the present study were classified as genotype B. In conclusion, this study provided the prevalence and genotype of Aichi virus circulating in pediatric patients with diarrhea in Chiang Mai, Thailand.

บทคัดย่อ

โรคกระเพาะอาหารและลำไส้อักเสบแบบเฉียบพลันหรือโรคอุจจาระร่วง เป็นปัญหาที่สำคัญทางด้านสาธารณสุขทั่วโลก เมื่อไม่นานมานี้ได้มีการค้นพบไวรัสชนิดใหม่ มีชื่อว่า Aichi virus ซึ่งเกี่ยวข้องกับการก่อโรคอุจจาระร่วงในมนุษย์ วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อศึกษาระบาดวิทยาและคุณลักษณะของเชื้อ Aichi virus ที่ตรวจพบในตัวอย่างอุจจาระจากผู้ป่วยเด็กที่มีอาการอุจจาระร่วงโดยได้ทำการตรวจตัวอย่างอุจจาระทั้งหมดจำนวน 302 ตัวอย่างที่เก็บในช่วงเดือนธันวาคม ปี ค.ศ. 2010 ถึงเดือนธันวาคม ปี ค.ศ. 2011 โดยใช้วิธี reverse transcription, semi-nested PCR และ nucleotide sequencing ผลการตรวจพบ Aichi virus ใน 17 ตัวอย่าง จากทั้งหมด 302 ตัวอย่าง (คิดเป็นร้อยละ 5.6) เมื่อทำการวิเคราะห์ลำดับนิวคลีโอไทด์ของส่วน 3C-3D พบว่า Aichi virus ทั้งหมดเป็น genotype B สรุปการศึกษาในครั้งนี้ทำให้ได้ข้อมูลความชุกและชนิดของ Aichi virus ที่มีการติดเชื้อในผู้ป่วยเด็กที่มีอาการอุจจาระร่วงในจังหวัดเชียงใหม่ ประเทศไทย

Key Words: Aichi virus, Epidemiology, Molecular characterization
คำสำคัญ: ไวรัสไอชิ ระบาดวิทยา คุณลักษณะเฉพาะในระดับโมเลกุล

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Introduction

Acute gastroenteritis is one of the most common cause of morbidity and mortality among children < 5 years old worldwide (Dennehy, 2011). Major aetiological agents of viral gastroenteritis are group A rotaviruses, human caliciviruses, adenoviruses and astroviruses (Marie-Cardine et al., 2002). However, the etiological agents of more than half of acute gastroenteritis patients remain undiagnosed. Recently, Aichi virus has been discovered and identified as a viral agent that related to diarrheal disease in human (Yamashita et al., 1991).

Aichi virus was first discovered in fecal specimens of oyster-associated non bacterial gastroenteritis patients in Aichi prefecture, Japan, in 1989 (Yamashita et al., 1991). The isolated virus was later classified as the first member of a novel genus, Kobuvirus, of the Picornaviridae family (Yamashita et al., 1998). Its genome consists of a single stranded, positive-sense RNA molecule of 8,280 nucleotides and a poly(A) tail. The single large open reading frame encodes a polyprotein of 2,432 amino acids which is cleaved into the typical picornavirus structural proteins VP0, VP3, VP1, and nonstructural proteins 2A, 2B, 2C, 3A, 3B, 3C and 3D as shown in Figure 1 (Reuter et al., 2011).

In 2000, Yamashita et al. (2000) developed a reverse transcription–PCR (RT–PCR) method for amplification of the 519-bp sequence in the junction of non-structural proteins 3C and 3D. Based on the phylogenetic analysis of the 519-bp region, three genotypes have been proposed: genotype A is common in Japan (Yamashita et al., 2000) and Europe (Oh et al., 2006; Ambert-Balay et al., 2008), genotype B has been found in several Asian countries other than Japan (Pham et al., 2007) and in Brazil (Oh et al., 2006), and genotype C has only been detected in Africa (Ambert-Balay et al., 2008). Since then, kobuviruses have been found in other animal species such as cattle (bovine kobuvirus) (Yamashita et al., 2003) and swine (porcine kobuvirus) (Reuter et al., 2008).

Surveillance studies of Aichi virus demonstrated that, Aichi virus has been detected with an incidence of 0.9-4.1%, primarily outbreaks of diarrhea in children or young adults (Ambert-Balay et al., 2008; Kaikkonen et al., 2010; Reuter et al.,2009; Sdiri-Loulizi et al., 2009; Yamashita et al., 1995). Aichi virus seems to circulate worldwide, and detection of Aichi virus have been reported in Asia (Pham et al., 2007), South America (Oh et al., 2006), Europe (Ambert-Balay et al., 2008; Kaikkonen et al., 2010; Reuter et al., 2009; Oh et al., 2006) and Africa

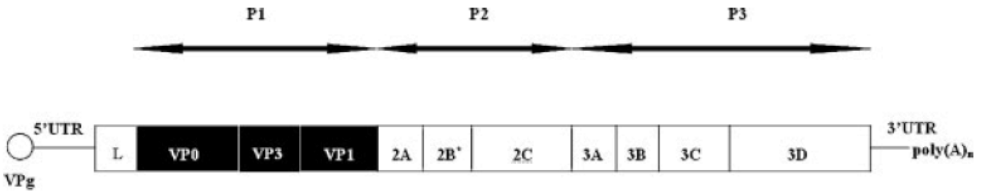

Figure 1 Genome organization of kobuvirus. P1 represents viral structure proteins (black boxes) P2 and P3 represents nonstructural proteins. (Reuter et al., 2011)

In Thailand, the prevalence of Aichi virus was 0.9 % (Pham et al., 2007; Suantai, 2011). However, there has been limited knowledge about the epidemiology of Aichi virus infection in Thailand.

Objectives of the study

1. To investigate the prevalence and genotypic distribution of Aichi virus in pediatric patients with diarrhea in Chiang Mai, Thailand.
2. To analyze nucleotide sequences of Aichi viruses detected in pediatric patients detected in this study.

Methodology

Specimen collection

Total 302 stool samples were collected from pediatric patients under 14 years of age, who were admitted to Maharaj Nakorn Chiang Mai and Nakorn Ping Hospital with a clinical diagnosis of acute gastroenteritis during December,2010 to December, 2011. All specimens were stored at -20 ºC until a batch - testing was performed.

RNA extraction and reverse transcription

The viral RNA genome was extracted from 10% stool suspension using Viral Nucleic Acid Extraction Kit (Geneaid, Taiwan) and reverse transcription (RT) was then performed according to the manufacturer's instruction (Fermentas, Vilnius, Lithuania). Briefly, 10 µl of viral genome were added to 1 µl of 50% di-methyl sulfoxide (DMSO) before heating at 95°C for 5 min. Then, RT reaction using random hexamer primers (Takara, Shiga, Japan) was carried out at 42°C for 1 h, followed by 72°C for 10 minutes and then immediately chilled on ice and stored at -70 ºC.

Polymerase chain reaction

The presence of Aichi virus was detected by semi- nested PCR. The first PCR was conducted with primers 6261 and 6779 to amplify a 519 bp region between the C terminus of 3C and the N terminus of 3D regions (Yamashita et al., 2000). Next, a semi-nested PCR was performed with primers 6261 and AiMP-R (Khamrin et al., 2011) to amplify a 295 bp segment within the 3C region. The primers used in this study are summarized in Table 1.

Electrophoresis

The PCR products were electrophoresed in a 1.5% agarose gel for 30 min, then visualized under ultraviolet (UV) light, and the results were recorded by photography.

Nucleotide sequencing and sequence analysis

PCR products of Aichi virus were purified by Geneaid gel Extraction kit (Geneaid, Taipei, Taiwan) according to the manufacturer's protocol. Firstly, the PCR products were electrophoresed in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. Then, a gel block containing the DNA fragment of expected size was excised under the UV transluminator and purified by column centrifugation. The obtained DNA was quantified and qualified by 1.5% agarose gel electrophoresis. The purified PCR products were sent to First Base laboratory (Selangor Darul Ehsan, Malaysia) for sequencing. The nucleotide sequences were analyzed by NCBI BLAST Sequence Software.

Table 1 Oligonucleotide primers for the detection of Aichi virus

Primer	Sequence 5' – 3'	polar	gene	position	Amplicon size (bp)	Reference
1° PCR						
6261	ACACTCCCACCTCCCGCCAGTA	+	3C	6261-6282	519	Yamashita et al., 2000
6779	GGAAGAGCTGGGTGTCAAGA	-	3CD	6779-6760		Yamashita et al., 2000
2° PCR						
6261	ACACTCCCACCTCCCGCCAGTA	+	3C	6261-6282	295	Yamashita et al., 2000
AiMP-R	GCR GAG AAT CCR CTC GTR CC	-	3C	6576-6556		Khamrin et al., 2011

Results

Total 302 pediatric stool samples were screened for the presence of Aichi virus by semi-nested PCR. Aichi virus RNA was detected in 17 out of 302 (5.6%) samples.

Aichi virus genotyping was performed by nucleotide sequencing of 295 bp in the 3C region and compared the sequences with Aichi virus reference strains available in GenBank. It was interesting to observe that all samples were classified as Aichi virus genotype B. These sequences showed approximately 94 - 99% nucleotide sequence homology with those of Aichi virus B in GenBank database (Table 2).

The monthly distribution of Aichi virus detected in Chiang Mai, Thailand from December, 2010 to December, 2011 is shown in Table 3. Aichi viruses were detected with the highest peaked in winter season of Thailand (December, January and February). Infection rate between male:female was 11:6. In addition, Aichi virus was detected in children with the age of 3 months to 13 years old. It should be noted that Aichi virus infection was observed more often in children with the age of more than two years (Table 3).

Discussion and Conclusions

Acute gastroenteritis continues to be a major public health problem worldwide. Children under 5 years old are particularly affected with more than 700 million cases every year (Dennehy, 2011). Aichi virus has been proposed as a causative agent of gastroenteritis after detected initially in gastroenteritis outbreak in Japan (Yamashita, 1993) and Germany (Oh, 2006). Subsequently, there are additional data of the detection of Aichi virus in several countries such as Japan, Bangladesh, Thailand, Vietnam,and Hungary (Pham,2007; Reuter, 2009). In Thailand, epidemiological data of Aichi virus is limited. From the literature search, there are two reports on the detection of Aichi viruses, one was reported the detection of Aichi virus in children with diarrhea at 0.9% (Pham, 2007) and another was the detection of Aichi virus in adults with diarrhea at 0.9% (Suantai, 2011). The data demonstrate that Aichi virus is an uncommon pathogen associated with acute gastroenteritis in both children and adults as it was detected at a very low detection rate of 0.9%.

Table 2 Characteristics of Aichi virus strains detected in this study

	Sample	Blast	genotype	Max identity	Accession
1	S3/11	B175/05	B	95%	EF079157
2	S7/11	Ven-7	B	99%	GU807431
3	S16/11	AIV/8BF/3CD/2012/IT	B	97%	KC693052
4	S34/11	Qld/2008/204/Australia	B	94%	EU715251
5	S35/11	139/96	B	95%	AB092830
6	S37/11	488/97	B	95%	AB092833
7	S47/11	B175/05	B	95%	EF079157
8	S48/11	139/96	B	96%	AB092830
9	S64/11	Chshc3	B	98%	FJ890521
10	S67/11	488/97	B	95%	AB092833
11	N3/11	Ven-7	B	99%	GU807431
12	N4/11	58174	B	98%	GU339099
13	N7/11	AIV/8BF/3CD/2012/IT	B	99%	KC693052
14	N22/11	Chshc3	B	99%	FJ890521
15	N67/11	B-171/05	B	95%	EF079157
16	N77/11	139/96	B	95%	AB092830
17	N121/11	AIV/8BF/3CD/2012/IT	B	98%	KC693052

Y, Year; M, month; D, Day.

In the present study, Aichi viruses were detected in 5.6 % of fecal specimens collected from children admitted to hospital with acute diarrhea. These findings demonstrated the increase of the prevalence of Aichi virus in pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand when compared with previous studies.

It has been reported previously that Aichi virus circulated in pediatric patient with acute diarrhea in 2000-2004 in Chiang Mai is genotypte A (Pham, 2007). In contrast, Aichi virus detected in pediatric patients in 2011 in the present study in genotype B. The data indicate that both genotype A and B circulate in pediatric patients in Chiang Mai and the predominant genotype has changed overtime. In support of this notion, both genotypes A and B have been detected in adult patients with diarrhea in Chiang Mai in 2008. (Suantai, 2011) In conclusion, this study provides the information of epidemiology and genotype of Aichi virus that causes diarrhea in children in Chiang Mai, Thailand.

Table 3 Clinical characteristics of samples positive for Aichi virus

	No.	Collection Date	Sex	Age
1	S3/11	January 2011	Male	9Y7M10D
2	S7/11	January 2011	Female	3M 29D
3	S16/11	February 2011	Female	13Y6M9D
4	S34/11	May, 2011	Female	4Y11M28D
5	S35/11	May, 2011	Female	4M 3D
6	S37/11	April, 2011	Male	8Y5M5D
7	S47/11	December 2010	Male	1Y 3M
8	S48/11	January 2011	Male	3Y
9	S64/11	June 2011	Male	7Y3M13D
10	S67/11	May, 2011	Male	1M 29D
11	N3/11	January 2011	Female	4Y 11M
12	N4/11	January 2011	Male	8Y
13	N17/11	February 2011	Male	8M
14	N22/11	February 2011	Male	7Y
15	N67/11	April 2011	Male	3Y
16	N77/11	May 2011	Male	2Y1M25D
17	N121/11	August 2011	Female	-

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